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SHORT COMMUNICATION

Thysanolaena latifolia (Roxb. ex Hornem.) Honda as natural resource and product for the tribals of Srikakulam District, Andhra Pradesh, India

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Thysanolaena latifolia (Roxb. ex Hornem.) Honda, popularly known as tiger or broom grass is one among the non-timber forest products gathered by the tribals of Srikakulam district, Andhra Pradesh. The study documented the process of making brooms and its economic viability. The brooms made using these plants are an important seasonal livelihood for the local people. *T. latifolia* can be grown in a wide range of agro-climatic conditions and soils up to 2,000 m above the sea level. It grows easily on shady slopes, damp and steep river banks, degraded areas and gravelly soil on weathered rock surfaces. The study recommends cultivation of broom grass on a large scale as an effective source for income generation in tribal areas of Andhra Pradesh.

Keywords: Broom grass, Economic benefit, India, *Thysanolaena latifolia* (Roxb. ex Hornem.) Honda, Tiger grass, Tribals.

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Introduction

The tribal areas of any region are the remaining perennial reserves of natural resources, products and traditional handicraft skills. The documentation of traditional knowledge and skills of ethnic people is necessary for various needs, besides conservation. This knowledge may play a crucial role in achieving sustainable development and establishing intimate relationship between man and nature. The tribal communities depend on nature for their varied needs. Besides agriculture, they also depend considerably on all other forest resources for their sustenance[1]. Therefore, importance should be given to encourage their traditional skills and products. Here non-timber forest products (NTFPs) play a vital role to help millions of forest dwellers globally and provide instant money to meet the basic needs of tribal people.

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Thysanolaena latifolia (Roxb. ex Hornem.) Honda syn. *T. maxima* (Roxb.) Kuntze (Plate 1a) popularly known as Broom grass (*Konda cheepuru gaddi* in Telugu) is a member of family Poaceae. It is one of the important NTFPs of tribal areas of the Eastern Ghats of India. It grows in almost all parts of South and Southeast Asia up to an elevation of 1,600 m. Main economic product of the plant is terminal culms bearing inflorescence. Earlier studies on *T. latifolia* were on exporting brooms to Thailand and to the Northern provinces of Laos[2], bioengineering device for soil erosion control on hill tops and slope stabilization[3], leaves as elephant fodder, growth pattern, production and marketing as fodder in fodder scarce areas[4], in conservation and development[5], non-perishable cash crop[6] and as a major economic activity[7,8]. Besides panicles, other vegetative parts of the broom grass are also economically important: culms are used as raw material in paper, small scale cottage and other industries. It is an important minor forest product of Meghalaya growing wild in almost all parts of the state[9,10]. Conversely, attempt has not been made so far to document the traditional handicraft skill of broom-making from the terminal culms of broom grass and its economic viability. Hence, the present study attempts to document the traditional skill of the ethnic people in preparation of brooms and to identify the commercial viability of broom grass, which is a natural resource and product in natural habitats.

Materials and Methods

Study area

The study area is located in Srikakulam district of Northeastern Andhra Pradesh, India (180° 5'-19° 12' N and 83° 32'-84° 47' E). Although within the State, the district ranks low in terms of area (5837 km^2) and population (27,08,114). It possesses considerable tribal population 1,66,118 (6.15 %)[11] in hilly and forest habitats. The dominant ethnic groups are *Konda Savara*, *Jatapu* and *Kapu Savara*, in which *Konda Savara* is considered as particularly vulnerable tribal group.

Collection of data

Collection of the data was mainly based on interviews, interactions and field surveys undertaken in

seven mandals (viz. Seethampeta, Kothuru, Bamini, Hiramandalam, Pathapatnam, Meliaputti and Mandasa) of the district during January 2014 to April 2015. Interviews were held with different tribal groups regarding gathering of the broom grass and use of fibres in making brooms. The data on procurement quantity and price of the broom was obtained from the Girijan Co-operative Corporation Limited (GCCI, Visakhapatnam) for the last 12 years. Village elders, farmers and womenfolk were also brought into the purview of discussion to obtain firsthand knowledge and information.

Results and Discussion

Method of broom preparation

The different habitats from where ethnic people gather the broom grass are moist, damp, steep, sloppy, sandy, sandy loam soils apart from the banks of ditches, ponds, etc. The culms of broom grass arise centrifugally during the peak growth period and produce inflorescence at the end of vegetative growth. The productive period starts with the flowering. Harvesting of panicles with terminal culms starts from December or January and it continues till March. Integrated Tribal Development Agency (ITDA), Seethampeta usually provides the harvesting tools (Plate 1b). The quality of the brooms depends upon the time of harvesting. The collected panicles with terminal culms are kept in sunlight to dry for a month on the roof or in front of their huts. Drying makes the broom lighter (Plate 1c). A pile of loose culms bearing panicles are taken into hand and tied with the fibre. Generally, the fibers of two important plant species

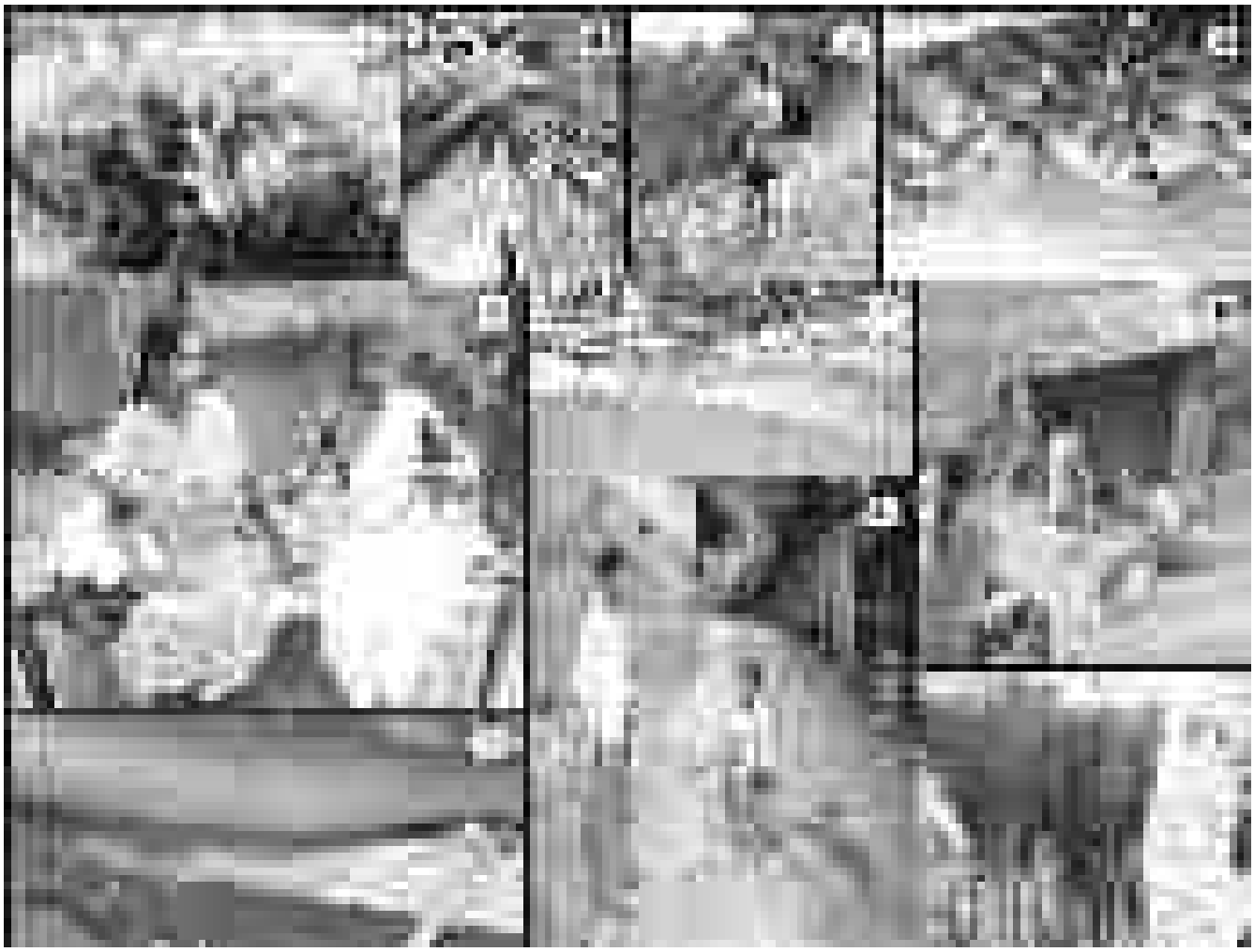

Plate 1—The ethnic process of broom making from broom grass. a) *Thysanolena latifolia* mature plants, b) Traditional tools supplied by ITDA for cutting culms, c) Drying of terminal culms with panicles, d) Fibres of *Grewia tiliifolia*, e,f) Splitting of *Hibiscus sabdariffa* stem into halves to extract fibre as observed by first author and fibre, g) Dipping of fibre in water for tying the broom witnessed by the first author, h) Skill in tying of broom, i) Cutting evenly at the base of broom on *Gmelina arborea* wood and j) Bundles of brooms for sale in a shandy.

namely *Grewia tiliifolia* Vahl and *Hibiscus sabdariffa* L. (Plate 1d, f) are used to tie the brooms. Fibres of the former are removed by cutting the stem transversely and peeling the bark. They are brought home and drooped under the roof of their houses (Plate 1d). Before using the fibre, they are dipped in water for a short period. The fibre becomes flexible to tie within one minute after dipping. If it were soaked for more than one minute, it becomes too soft and difficult to tie the broom. In the case of *H. sabdariffa*, the dried stem is cut into two longitudinal halves (Plate 1e, f) and kept. Later, the fibre is removed and made soft and flexible by applying water with fingers (Plate 1g). After completion of tying process (Plate 1h), the lower parts of the culms are cut with metal sickle by placing on the wood of *Gmelina arborea* Roxb. (Plate 1i).

After harvesting the terminal culms, the basal parts of culms above ground level are burnt, which is an essential step for the growth of new culms of good quality. The underground rhizome remains intact and alive until the next rainy season. The tribal people said that the new culms are produced in one week or ten days after the first rain.

Economic viability

A total of 25,668 tribal households were involved in broom making with ST population of 1,10,331 in the study area[12]. Monthly income due to broom making is about Rs.9000 per month during the season, which varies depending on the individual's skill and seasonal climatic conditions. No attempt has so far been made to cultivate broom grass in the study area, which grows wildly. If it is cultivated, it may enhance the income of the tribal people as it requires minimum investment. On an average, one person can make 20-30 brooms per day. They bundle about 30-40 brooms into a set and carry them on their head to a nearby shandy for sale (Plate 1j). The brooms produced are procured by GCCI of the Srikakulam district for onward transport to local and non-local markets and may even be exported. Singh *et al*[10] mentioned that the price fixation of the broom should be through open auction and also that there is need to develop cooperative marketing. As it is nonperishable, marketing may not be a problem.

The data on procurement quantity and price of single broom by GCCI over the past 12 years is presented in Table 1. The data shows variation in number of brooms procured in 2011-2012, which may have been due to the poor quality of the brooms because of adverse climatic conditions in the season. The private traders reach shandy points early to buy the brooms. If price is not fixed by the GCCI, private traders may procure it for very less price. GCCI is protecting the interest of the tribals by fixing a base price. If they receive better price, they sell it to private traders, which is the main reason for fluctuation in the procurement quantity of the GCCI. The tribals are aware of the base price fixed by the GCCI officials such as shandy inspectors[13]. From the data it is obvious that there is an increase in the quantity of brooms made and the price per broom. The cost of the broom declined during 2011-12 due to poor quality of brooms[13]. The price fixed by the GCCI is considered base price but in practice brooms are often sold at a higher rate in shandies than what was fixed by GCCI.

Table 1—Procurement quantity and price of brooms by GCCI in Srikakulam district (2003-2015)

Procurement Year	No. of brooms procured	Value (in Lakhs)	Procurement cost per broom (in ₹)
2003-04	57128	3.26	6
2004-05	1828	0.3	16
2005-06	59954	8.94	15
2006-07	20916	3.12	15
2007-08	10376	1.56	15
2008-09	6272	1.14	18
2009-10	2307	0.68	29
2010-11	16158	4.85	30
2011-12	12163	3.42	28
2012-13	56712	17.38	30
2013-14	13108	4.33	33
2014-15	17550	6.93	40

About 4,27,440 ha of land is in holding in the district with an operational area of 1,57,500 ha (including 7,960 ha by Scheduled caste, 12,010 ha by Schedule Tribes and 1,37,480 ha by others)[14]. The marginal land is nothing but less fertile land, which is most suitable for the cultivation of the broom grass. It will generate increased income than the income obtained from making the brooms from the grass grown wildly. Broom grass farming can also be a part of agroforestry system to rejuvenate degraded lands.

Conclusion

The present study documented the traditional handicraft skill of making brooms as products from a natural resource namely *T. lalifolia* and utilization of fibers of *Tada/Tella jana – G. tiliifolia* (wild) and *Gogu nara – H. sabdariffa* (cultivated) of family Malvaceae. The present study envisages and recommends the following three- i) broom grass cultivation in tribal areas to increase income with minimum input and

labor, ii) to develop mechanical tools to cut the terminal culms with panicles to make the culms into bundles, tying and packing and iii) minimum support price of GCCI should be increased rationally. Brooms are essential and continuous requirement in every house and hence cultivation of broom grass on marginal lands which are unsuitable for food production will enhance household income.

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GREWIA TILIIFOLIA VAHL A MULTIPURPOSE TREE UTILIZED BY THE TRIBALS OF SRIKAKULAM DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT

The present study investigated the utilization of *Grewia tiliifolia* (Thada chettu) by ethnic communities in Srikakulam district, Andhra Pradesh. They are using *Grewia tiliifolia* for a number of domestic uses. It is also used for its edible fruits, fodder and medicine. Documentation and intergenerational transmission of traditional knowledge on multipurpose use of *Grewia tiliifolia* is helpful for well-being of future generations, gratitude and respect for nature; management, conservation and sustainable use of biodiversity outside formal protected areas; and transfer of the species among the households, villages and larger landscape. The extraction of NTFPs is considered sustainable if it has no long-term deleterious effect on the regeneration of the harvested population. The contribution of NTFPs to improving livelihoods can best be assured through a process of gradual domestication of NTFPs in human-modified (agro) forest types. Documentation of ethnic process of cloth washing by utilizing bark is reported for the first time from the tribals of Srikakulam district.

Key words: NTFPs, Domestic uses, Sustainable use, Domestication, Ethnic cloth washing process.

Introduction

The tribal areas are the reserves of traditional knowledge. The documentation of traditional knowledge plays a crucial role in achieving their sustainable development and establishes intimate relationship between man and nature. The tribal communities depend on nature for their varied needs. Besides agriculture, they also depend considerably on all other forest resources for their sustenance. Tribals are called ecosystem people, because they view themselves and to a degree actually live as a part of an ecosystem, rather than simply users or exploiters of nature. The ecosystem people maintain a proper balance between their economic needs/activities and the ecological requirements of the environment where they live in. Non-Timber Forest Products (NTFPs) play a vital role to help millions of forest dwellers around the world. Every plant in their area either for food, medicine, building materials or for cultural and spiritual practices. Works on *Grewia tiliifolia* Vahl are fruits are reported by Arinathan *et al*. (2007); Bandyopadhyay and Mukherjee (2009), response to pruning in terms of biomass production also varied from species to species. Fifty percent Pruned trees produced comparatively more amount of foliage and branches produced annually than by the un - pruned trees when the pruning was carried out once in two years, studied this aspect on ten locally important tree species, *Grewia tiliifolia* is one of them by Chandrasekhara (2007); most commonly used such products are wild spinaches, fuel wood, wooden utensils, edible fruits, grass, hand-brushes, and twig hand-brushes, used by 85% or more of households. More than half the households investigated also make use of edible insects, wood for construction, bush meat, wild honey and reeds for weaving by Shackleton and Shackleton (2004); root powder is used at night for curing the sprain by Patil and Bhaskar (2006); decoction of stem bark is used for curing cough by Reddy *et al.* (2007); use of bark fibre for hair cleaning, promotion of hair growth by Silja *et al*. (2008) ; crushed bark is used for hair washing by Yesodharan Sujana (2007).

A plant of the moist, lowland tropics and subtropics, where it is found at elevations up to 900 m. It grows best in areas where annual daytime temperatures are within the range 30 - 42°C, but can tolerate 7 - 47°C. Mature plants can be killed by temperatures of -5°C or lower, but young growth will be severely damaged at -1°C. It prefers a mean annual rainfall in the range 1,000 - 2,500mm, but tolerates 750 - 4,000mm. Succeeds in full sun and light shade. Succeeds in a range of well-drained soils, prefers a pH in the range 6 - 6.5, tolerating 5.5 - 7.0 by Ecocrop, FAO (2000).

Objectives

The main objective of the present study is the documentation of NTFPs obtained from *Grewia tiliifolia* Vahl and utilization pattern of plant parts by the tribals of Srikakulam district.

An urgent need of popularizing luxuriant multipurpose trees like *Grewia tiliifolia* with good no of comestible, ecofriendly, biodegradable nature of NTFPs and mainstreaming utilization pattern from different parts of India and at global level.

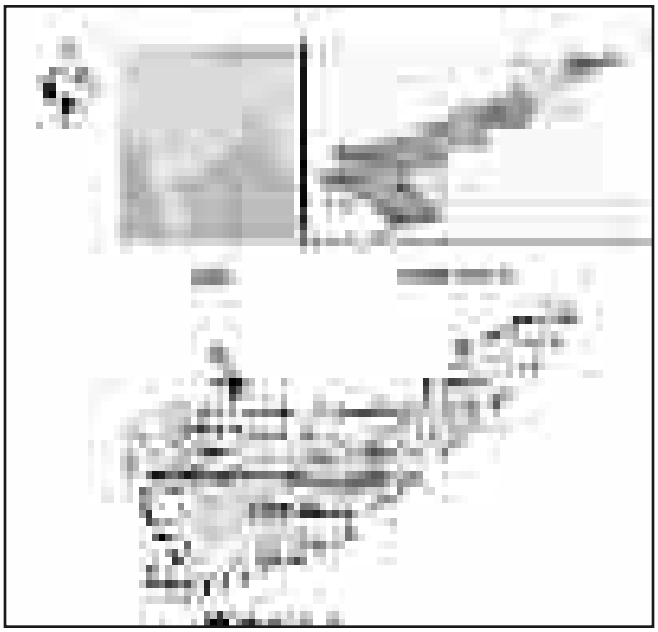

Fig. 1: Geo-graphical locations of the study area

Study area

The study area is located in Srikakulam district which is the extreme North-Eastern District of Andhra Pradesh State situated within the geographical co-ordinates of 180°5'-19°12' of northern latitude and 83°32'-84°47' of eastern longitude and shown in fig. 1. Though the district stands low rank in area (5837 Km^2) and in density of (2708114) among the districts of Andhra Pradesh, possesses considerable percentage of tribal population 1,66,118 (6.15%) in hilly and forest areas. The dominant tribal groups in this area are Savara, Jatapu and Kapu Savara.

Table 1: Utilization pattern of *Grewia tiliifolia* by the tribals of Srikakulam district.

S.No.	Utilization pattern	Parts used
1	Agricultural implements and agriculture	wood
2	Construction	branches, fibres, wood
3	Edible Fruits	ripen fruits
4	Fuel wood	Branches, wood
5	Household articles	wood
6	Leaf Plates	leaves
7	Ropes	fibre
8	Hair cleaning	Leaves, fibre
9	Cloth washing	Bark

Methodology

The study is mainly based on interactions and field surveys undertaken in seven mandals of the district (viz. Seethampeta, Kothuru, Bamini, Hiramandalam, Pathapatnam, Meliaputti and Mandasa) during December 2014-November 2015. Interviews with different tribal groups of people are carried out during the study regarding the utilization of various parts of *Grewia tiliifolia* (Thada chettu). Village elders, farmers and womenfolk are

Fig. 2 : (a)*Grewia tiliifolia* Vahl. Plant in flowering., (b) Producing new branches., (c) Leaves, (d) Traditional agricultural implement Plough., (e) Cooking food with fuel wood., (f) Cattle shed., (g) Fibre extraction from stem bark., (h) Dried extracted fibre at home., (i) Author is observing utilization of fibre in broom making., (j-m) Ethnic process of cloth washing utilizing bark.

also brought into the purview of discussion to obtain firsthand knowledge.

Results and Discussion

Present study reported that six NTFPs such as bark, branches, fibres, fruits, leaves and wood of *Grewia tiliifoila* (Fig. 2a) are utilized for 9 purposes to fulfill their needs (Table 1).

The branches are chopped to collect leaves as cattle feed (Fig. 2b). Leaves are used as fodder and plates to have their food while collecting their NTFPs in the forest (Fig. 2c). They may be used either as single leaf or stitched together instantly into a plate depending upon their requirement. Small wood is used in making traditional agricultural implements like ploughs to plough their land (Fig. 2d). Wild edible fruits are very important to meet their micronutrient requirement. The fruit are rich in potassium and magnesium by Valvi and Rathod (2011). Food and shelter are primary needs of a person. Tribals construct their own huts, cattle shed and hedge (Fig. 2e and 2f). During winter season they extract (Fig. 2g) the required fibre. Six - seven feet long fibre is extracted, sundried (Fig. 2h) for one week and stored for future needs like construction and broom tieing and rope making. Construction requires mainly poles and fibres. Fibre yielding plants are second only to food plants and United Nations declared 2009 as 'International year of Natural Fibres' with the main aim of raising global awareness. These are biodegradable. Broom made from the *Thysanolaena maxima* is an important income generating NTFP for them during Feb – April (Fig. 2i). Its making mainly requires fibres of *Grewia tiliifolia*. Fibres are also sold in the market either as raw or woven ropes. Household article like wooden spoons, bed legs, cot frames are made. *Grewia oppositifolia* Roxb. ex Mast in Central Himalaya is also utilized for leaves as fodder and fibre for making ropes by Bisht *et al.* (2006). For hair cleaning leaves and fresh fibres are used. Fibres are folded and rubbed on hair to clean.

Ethnic process of cloth washing

Washing clothes by utilizing the bark of *Grewia tiliifolia* by the tribals of Srikakulam district is very interesting. Ash (Fig. 2j) from burnt fuel wood is taken into a pot, poured with water, dirty clothes are dipped, bark is cut into pieces and added (Fig. 2k). Now this pot is boiled (Fig. 2l). The ash and bark removes the dirt and stains on clothes. Later these clothes are taken to nearby pond (Fig. 2m) washed with water and dried. The clothes obtained after washing are as equal as those of washed by the chemical detergent soaps and surf.

Present investigation on comestible nature of fruits, hair cleaning properties of bark and fibre are coincided with the previous reports of Arinathan *et al.* (2007); Bandyopadhyay and Mukherjee (2009); Silja *et al*. (2008); Yesodharan and Sujana (2007). Documentation of ethnic process of cloth washing by utilizing bark is reported for the first time from time tribals of Srikakulam district. Utilization of natural products in hair cleaning and cloth washing are eco friendly. With the above uses it can be considered as a multipurpose plant. Every tribal household is maintaining at least 3 trees of *Grewia tiliifolia*. The ethnic knowledge in conservation of this tree for varied needs is highly appreciable. The branches are cut to harvest fibre once in two years. Again it is used for fibre extraction in third year only. Next year fibre is extracted from the second tree. Nearly the fibre extracted from one tree is enough for tieing of 100 brooms. Next year another tree is harvested for fibres. They are maintaining this tree for their edible fruits, fodder and fibre, fuel wood requirement. It is worth mentioning to discuss the study of Chandrasekhara (2007) where 50% Pruned trees produced comparatively more amount of foliage and branches annually than the un pruned trees when the pruning was carried out once in two years. This is one of the important plant described in Valmiki Ramayana.

Conclusions

With the above investigation *Grewia tiliifoila* can be concluded as a multipurpose tree. Tribals are conserving this tree for varied purposes. Documentation of traditional knowledge on utilization pattern of *Grewia tiliifolia* is very helpful in domestication of wild tree species into urban gardens or modified (agro) forest types. It is a fast growing tree species, and its domestication can reduce global warming. When a multipurpose tree is planted, a number of needs and functions can be fulfilled at once. While all trees can be said to serve several purposes, such as providing habitat, shade, or soil improvement. But multipurpose trees have a greater impact on a farmer's well being because they have the ability to provide numerous products and perform a variety of functions in farming or forestry. Domestication of multipurpose trees in farms will be more beneficial to farmer, as he construct his own watching hut in the field, can feed his cattle with leaves and can eat fruits. The contribution of NTFPs to improving livelihoods can best be assured through a process of gradual domestication of NTFPs in human-modified (agro) forest types. There is an urgent need of popularizing multipurpose trees with good no of NTFPs with comestible, ecofriendly, biodegradable nature. Studies in different parts of India and at global level on *Grewia tiliifolia* will benefit the increasing populace for their needs with sustainable utilization.

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श्रीकाकुलम जिला, आन्ध्र प्रदेश, भारत की जनजातियों द्वारा प्रयुक्त एक बहुउद्देशीय वृक्ष *ग्रीविया टिलिफोलिया* वाहल

टी.एम.ए. निवेदिथा

सारांश

वर्तमान अध्ययन में श्रीकाकुलम जिला, आन्ध्र प्रदेश में मानवजातीय समुदायों द्वारा *ग्रीविया टिलिफोलिया* (थाड़ा चेट्टू) के उपयोजन की जांच की गई। ये लोग अनेकों घरेलू उपयोगों के लिए *ग्रीविया टिलिफोलिया* का उपयोग कर रहे हैं। इसका उपयोग इसके खाद्य फलों, चारे व दवाइयों के लिए भी होता है। भावी पीढ़ियों के कल्याण, प्रकृति के प्रति सम्मान एवं कृतज्ञता ; औपचारिक संरक्षित क्षेत्रों के बाहर जैवविविधता के प्रबंधन; संरक्षण और पोषणीय उपयोग तथा परिवारों, गाँवों एवं बड़े भूदृश्य में प्रजाति के हस्तान्तरण के लिए *ग्रीविया टिलिफोलिया* के बहुउद्देशीय उपयोग पर पारम्परिक ज्ञान का प्रलेख पोषण और अन्त: पीढ़ीय हस्तान्तरण लाभदायक है। गैर प्रकाष्ठ वन उपजों के निष्कर्षण को तभी पोषणीय समझा गया है, यदि इसकी काटी गई आबादी के पुनर्जनन पर कोई दीर्घकालीन, हानिकारक प्रभाव न पड़े। आजीविकाओं में सुधार करने हेतु गैर प्रकाष्ठ वन उपजों के सहयोग को मानव परिष्कृत (कृषि) वन किस्मों में गैर प्रकाष्ठ वन उपजों को धीरे-धीरे वातावरण के अनुकूल बनाने की प्रक्रिया के जरिए सर्वोत्तम रूप से सुनिश्चित किया जा सकता है। छाल का उपयोग करके कपड़े धोने की मानवजातीय प्रक्रिया का प्रलेख-पोषण पहली बार श्रीकाकुलम जिले की जनजातियों के लिए सूचित किया गया है।

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Wild edible plants of India – A review.

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Abstract: *Wild edibles are important NTFPs for tribes. According to the India State of Forest Report (ISFR) 2015, the total forest and tree cover is 79.42 million hectare, which is 24.16 percent of the total geographical area. Tribal population of India is 8.6 as per 2011 census. In India, the tribal people depend on forests for their livelihood. The tribal people are very close to nature and have hereditary traditional knowledge of consuming wild plants and plant parts viz., tuber, shoots, leaves, fruits etc. as a source offood. Although, these wild edible plants play an important role in food security, they are ignored. The primitive man through trial and error, has selected many wild edible plants, which are edible and subsequently domesticated them. The present paper reviewed on wild edible plants documented in different parts of India and their utilization by the tribes. Streamlining these wild edible plant species will provide food security. Wild edible are less susceptible to diseases, can be grown easily without application of pesticide. Ironically these plants are still unknown or less known to other parts of the world. The wild edible plant species will be popularized after phytochemical analysis and nutraceutical studies. Present study on review of Wild edible plant species will be helpful in pooling different types of edible plant species utilized by various tribes in different parts of India.*

Key words: NTFPs, Wild edibles, tribes, utilization, nutraceutical studies, popularized.

Introduction:

Wild edibles are important NTFPs for tribes. According to the India State of Forest Report (ISFR) 2015, the total forest and tree cover is 79.42 million hectare, which is 24.16 percent of the total geographical area. Tribal population of India is 8.6 as per 2011 census. In India, the tribal people depend on forests for their livelihood. The tribal people are very close to nature and have hereditary traditional knowledge of consuming wild plants and plant parts viz. tuber, shoots, leaves, fruits etc. as a source of food. Although, these wild edible plants play an important role in food security, they are ignored. Various tribal sects of India are repositories of rich knowledge on various uses of plant genetic resources (Khoshoo, 1991).Wild edible plants play a major role in meeting the nutritional requirement of the tribal population. Among the various kinds of plants, food plants received the earliest attention of mankind and reflect man's search for knowing more and more about the nutrient qualities of food plants. The primitive man through trial and error, has selected many wild edible plants, which are edible and subsequently domesticated them. Modern man neither domesticated the left over nor has he identified any new food plants inrecent times, which are widely acceptable; they have improved only a few crop plants. The present day wild edible plants are particularly useful during famine and similar scarcity situation. Even during normal times, wild plants provide materials of diet to the less advanced section of human community, often referred as tribals/adivasis in India who

generally inhabit hilly and other less accessible tracts in both doveloped and developing countries (Arora and Pandey, 1996). In India, it is estimated that about 800 species are consumed as wild edible plants, chiefly by the tribal people (Singh and Arora, 1978). The present paper reviewed on wild edible plants documented in different parts of India and their utilization by the tribes.

Materials and methods: Various journals and books from internet were used to study. Various publications dealing with wild edibles, ethnography and botany were surveyed. All information summarized in this review refers to use of wild edible plants within the boundaries of India, based on literature sources providing relevant information since 1990 onwards. For each publication, geographical area, number of plant species reported and tribes names where sources are available are given. All data were grouped in chronological order.

Wild edible plants – A review

The term "wild food" is used to describe all plant resources outside of agriculture areas that are harvested and collected for the purpose of human consumption in forests, savannah and other bush land areas. Wild foods are incorporated into the normal livelihood strategies of many rural people, shifting cultivation, continuous croppers or hunter gatherers (Bell, J. 1995). Indigenous knowledge of wild edible plants is important for sustaining utilization of those plant species (Jasmine *et al*, 2007).

Kar and Borthakur(2008) reported 57 species of wild plants used as vegetable by the Khabri tribe of khabri along the district of Assam.Out of 57 plant species 4 as fruits,3 as rhizome,3 as tubers,1 corm, 9 flower vegetables,1 stem pith, 1 stem vegetable ,21 leafy vegetable and 16 are shoot vegetable. 57 wild edible plants belonging to 33 families used by the Gujjar tribe of District Rajouri from Jammu & Kashmir. Mukesh Kumar et al reported 21 plant species belonging to 19 families being used by the tribals and rural communities from Odisha (Rashid *et al.*2008);

Khyade *et al*. 2009 studied a total of 31 plant species belonging to 23 families were reported from AkoleTahasil of Ahmednagar district, Maharashtra utilized by the tribes *viz*., Mahadev-koli, Thakars, Bhils and Ramoshies Amaranthaceae was the dominant family with 4 taxa; 151 species belonging to 86 genera spreading over 49 families in the Khasi tribes of Meghalaya to assess their horticultural importance (Jeeva , 2009). Sharma & Mishra, 2009 reported diversity ,utilization pattern and indigenous uses of 217 plant species belonging to 160 genera of 68 families including medicine(85 species), fuel(54 species),wild edible/food(86 species),fuel (54 species), fodder (71 species) religious(5 species) in and around a cement factory in Bilaspur district of Himachal Pradesh. Bandyopadhyay and Mukherjee (2009) reported 125 plant species belong to 102 genera under 54 families as wild edibles eaten by the ethnic people of Koch Bihar district of West Bengal state, on different occasions.

Prabha *et al.* (2010) enlisted 42 plant species belonging to 23 families consumed by the tribals *viz*., Malappandaram, Urali, Malaarayan, Ulladan, Malavedan, Malakuravaand other locals of Melghat area. Binu (2010)

reported a total of 41 plant species of wild edible plants used by the tribals in Pathanamthtta district,Kerala.Six tribal communities inhabitating the area are Malappandaram, Urali, Malaarayan, Ulladan, Malavedan, Malakurava. Choudhury *et al.*(2010) reported the form of eating 10 wild edible plants, phytochemicals present and their medicinal importance by the tribal people of all communities of Agartala and Khowai sub divisions of west Tripura district of Tripura.

Arinathan *et al.* (2011) reported 41 species of wild edible unripe fruits representing 28 genera distributed over 20 families eaten by Palliyars of Western Ghats, Tamil Nadu. Sasi *et al.* (2011) documented indigenous knowledge on 50 wild edible plant species belonging to 31 families under 43 genera from Kotagiri Hills a part of Nilgiri Biosphere ,Southern India byIrulas -People of the darkness and observed that the tribal communities of the study area fulfill their food deficiency by supplementing wild food plants in their daily diet; 152 plant species belonging to 95 genera and 39 families under three categories such as cultivated crops(72), semi domesticated plants(41) and wild plants(39) from East Siang,ArunachalPradesh,Eastern Himalaya (Yumanm, 2011).

Banik (2012) reported utilization of 107 wild edible plants used by the tribals of Bastar region, Chattissgarh).Among them 25 plants for root/tubers,33 for leaf importance,3 for nuts 7 for stems, 9 for flower. Bastar is a home land of various tribal groups like Abujh, Maria, Muria, Bison horn maria, Dhurwa, Dorla, Bhatra, Halba; ethnobotanical studies on 74 wild edible plants belong to 58 genera and 41 families used by Irula tribes of PillurValley, Coimbatore district, Tamil Nadu, (Rasingam, 2012); 71 wild edible plant species belonging to 42 families consumed by the Garo tribe of Nokrek Biosphere Reserve in Meghalaya including rhizome, corm, tuber 8 species, bark 1 species, stem pith, tender shoots, fronds of 9 species, leaves and twigs of 21 species, flowers, flower buds ,inflorescences of 2 species, fruits, pods of 25 species; seeds, nuts, skin kernals of 3 species and whole part of 2 plant species (Singh *et al*. 2012). Gam & Gam (2012) documented 20 plant species habitually use in their food items particularly in non vegetarian diets by the Mising tribe of Assam and also observed that the use of some of these plant species is pertaining to their religious belief and festivals also.

Esther *et al*.(2013) reported 84 wild edible plants belonging to 36 families are being used by the Zou tribe in Manipur. Out of these 84 species, 70 species are used as vegetables & food, 13 species are used as spices and condiments and 1 species *Dioscorea sativa* is used as famine food. Kumar *et al*. (2013) reviewed 30 edible leafy vegetable available in South India along with their pharmacological benefits. Kumar *et al.* (2013) reported 21 wild edible plant species belonging to 19 families with their parts used by local as well as tribal people inhabitating in rural areas of Odisha. Ramachandran and Vani (2013) reported a total of 123 ethnobotanical species used by Paniyas and Kurumbas of Western Nilgiris, Tamil Nadu in which 72 are wild edible plants belonging to 37 families. Out of 72 plant species, 56 were collected from wild and 16 from semi wild/cultivated species. Rao and Reddi (2013) reported a total of 24 plant species involving 19 genera and 18 families

consumed by the primitive tribal groups *viz*., Gadaba, Khond, Porja and Savara from Visakhapatnam district, Andhra Pradesh. Misra and Misra (2013) reported 106 leafy vegetable plant species belong to 88 genera of 49 families from South Odisha.Major ethnic groups are, Bhumia, Bonda, DangariaKandha, Didayi, Gadaba, Kandha, Koya, KutiKandha, Langia-Saura, Paika, Paraja, Sabara, Saura, and other tribes inhabit these districts. The tribal and rural poor people consume many of the wild leafy vegetables available in their surroundings and sometimes during food scarcity. Vaishali and Jadhav (2013) reported 9 non cultivated greens leafy vegetables being used by the rural people and their medicinal use from various regions of Kolhapur District of Maharashtra.

Chauhan *et al*. (2014)reported 51 leafy vegetables being eaten by the tribal and local people of Chattisgarh. Singh and Kumar (2014) reported 17 wild edible aquatic and marshy plants traditionally used in various forms by the Munda tribe of District Khunti,Jharkhand; 31 wild edible plant species from 19 families being used by the tribals from Kupwara,Jammu 7 Kashmir (Mir, 2014); 105 wild edibles being used by the elder generation of tribal and and rural population for sustenance from old Mysore district and categorized into whole plant (04), root (13), Bark (01), stem(01), leaf(20), flower(07), fruit(55), seed(07),sap (01) and gum (03). Various group of tribals are found in the surroundings are Jenukuruba, Bettakuruba, Paniya, Panjari, Yeravas and Soligas (Nandini and Siddamallayya ,2014). Prasanth Kumar and Siddamallayya (2014) documented 29 wild tuberous plant species belonging to 24 genera of 15 families with their mode of consumption and medicinal uses from other local villagers Hassan district, Karnataka. Sanyasi Rao *e tal*. (2014) reported 55 indigenous food plants viz., 24 species as leafy vegetables, 21 species for fruits, 6 species for tubers,4 species for tender shoots, 2 each for seeds and flowers from Dumbriguda area of Visakhapatnam commonly consumed by the tribal communities . The major tribal communities are Nookadora, Kotiya, Kondakammari, Bagatha, Kondh, Muliya, Kondadora and Valmeeki. Sarvalingam *et al*. (2014) reported 68 wild edible plants belonging to 56 genera and 39 families from Maruthamalai Hills,Coimbathore district consumed by the Irulas. Among them rhizomes,roots and tubers of 14 plant species, fruits of 35 plant species leaves of 11 plant species ,seeds and arils of 7 plant species stem pith of 1 plant species. Satyavathi & Janardhan (2014) reported 30 wild edible fruits used by the Badagas of Nilgiri district. Singh (2014) documented fifty wild leafy vegetables belong to 31 families, 38 genera and 50 species from nine districts of Jharkhand used by the local tribal and other communities. Panda (2014) documented 86 wild edible plants belong to 51 families as livelihood used in the interior of Kendrapara district of Odisha state.

Patale Chandrakumar *et al*.(2015) reported a total 80 wild edible plant species belong to 69 genera and 38 families used by Gond, Halba and Kawartribes of Gondia district, Maharashtra. Pradhan and Tamang (2015) reported 26 species of wild leafy vegetables (WLV) used by Nepali, Bhutia and Lepcha ethnic communities from Sikkim. Saikia (2015) reported 51 wild vegetable plants from Dhemaji District of Assam with their medicinal uses.

Uses of wild and semi-wild *Citrus* species viz., *Citrus hystrix., Citrus latipes* and *Citrus indica* by the Khasi and Garo tribes of Meghalaya (Anamika*et al*.2016); ten countries with the largest wild collection areas in 2014 are Finland. Zambia, India, Namibia, Russian Federation, Romania, Brazil, China, Tajikistan and Bolivia (Frick and Bonn, 2016). Pradeep*et al*. (2016) reported 41 species of wild edible plants (WEPs) used byKonyak tribe in Mon district, Nagaland. Jyotsna and Katewa (2016) reported a total of 46 plant species belongs to 27 families from Southern Rajasthan utilized by tribes *viz*., Bhil, Meena, Damor, Garasia and Kathodi,

Conclusion:

Ethnic man depends on nature and utilizes different plant species for food, medicine and various domestic needs. Present study on review of Wild edible plant species will be helpful in pooling different types of edible plant species utilized by various tribes in different parts of India. This will be very useful for further studies such as photochemical analysis of wild edibles and nutraceutical potentialities. Present review on documentation, preservation of orally transmitted traditional knowledge will be a mother load for future generation. Streamlining these wild edible plant species will provide food security. Wild edible are less susceptible to diseases, can be grown easily without application of pesticide. Ironically these plants are still unknown or less known to other parts of the world. The wild edible plant species will be popularized after phytochemical analysis and nutraceutical studies. Then we can achieve Hippocrates quote i. e "Everyone has a doctor in him or her; we just have to help it in its work. The natural healing force within each one of us is the greatest force in getting well. Our food should be our medicine. Our medicine should be our food. But to eat when you are sick, is to feed your sickness". The FAO recognizes that nutrition and biodiversity converge towards a common goal of food safety and sustainable development and that wild species play a key role in global nutrition safety (FAO 2009). The nutritional potential of the wild edible plants has not hitherto been investigated to the extent it deserves. Therefore the present study on review of wild edibles hopefully useful to study in this regard.

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Efficacy of the Antiproliferative Effect of L-Asparaginase from *Streptomyces enissocaesilis* on Different Tumor Cell Lines

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Abstract

The antiproliferative effect of the purified L-Asparaginase enzyme on different tumor human cell lines have been investigated using human leukemic cells. These included MCF 7 (breast cancer cell line), MDA-MB435S (Breast Metastatic cancer), HeLa cell line, Human small cell lung cancer cell line H69PR (ATCC® CRL-11350™) and COLO 205 (ATCC® CCL-222™). The dose- and time-dependent antitumor and cytotoxic effects of L-asparaginase from *Streptomyces enissocaesilis* have been studied. We investigated the sensitivity of tumor cells towards L-asparaginase, as well the effect of L-asparaginase on cell growth rate, protein and DNA synthesis in the presence of various cytostatics. Cell cycle analysis by flow cytofluorimetry and detection of apoptotic cells before and after treatment with L-asparaginase were studied. Experiments have been conducted to investigate whether administration of L-asparaginase, which results in hydrolysis of extracellular asparagine, arrests asparagine dependent protein synthesis and causes subsequent inhibition of cell growth followed by decreased proliferation of leukemic cells.

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Keywords

L-asparaginase,
Tumor cells, Cytotoxicity,
DNA and protein synthesis,
Apoptosis.

Introduction

L-asparaginase is the first studied enzyme possessing anticancer activity (Pasut *et al.,* 2007). Tumour cells, more specifically lymphatic cells and certain other tumour cells require huge amount of L-asparagine to keep up with their rapid malignant growth but they lack or have a very low level of asparagine synthetase hence do not synthesis L-asparagine denovo. These cells use L-asparagine from the diet (blood stream) to satisfy their large L-asparagine demand (Narta *et al.,* 2007). L-asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonia thereby selectively kills tumour cells that rely on asparagine supplied by the serum for survival (Uyttebroeck *et al.,* 2008).

Asselin *et al.,* (1989) have studied the cytotoxic action of L-asparaginase both in vitro and in vivo in patients with ALL undergoing treatment with L-asparaginase as a single agent. This study explored the L-asparaginase induced cell cycle arrest in G2/S phase and apoptotic cell death of leukemic cells. It has been reported that there is a requirement of a functional p53 protein for L-asparaginase to induce apoptosis as observed in studies on JURKAT and HL 60 cell lines (Fu *et al.,* 1989, Nandy *et al.,* 1989). Subsequent studies revealed the action of

L-asparaginase on the various leukemic cells. Human acute lymphoblastic leukemia cell line is markedly inhibited by L-asparaginase, in the G2/S phase (Shimizu *et al.,* 1992). The recent research conventional showed out for the production of L-asparaginase from *Penicillium sp*. had shown cytotoxic activity (Patro *et al.,* 2012) and the cytotoxic activity of L-asparaginase from the marine actinomycete *Streptomyces acrimycini* NGP (Selvam and Vishnupriya, 2013).

Materials and Methods

Cancer Cell Lines

Human cancer cell lines - MCF-7 Cell line (Breast Cancer), MDA-MB435S Cell line (Breast Metastatic cancer), Human cervical cancer cell line (HeLa), Human small cell lung cancer cell line H69PR (ATCC® CRL-11350™) and COLO 205 (ATCC® CCL-222™) used in this study were procured from National Centre for Cell Science, Pune.

Culturing of cells

Tumor cells at the logarithmic growth phase with a cell density of 5×10^8/L were inoculated into 25 ml culture media containing the purified *L*-asparaginase at a concentration of 200 mg/L. Cells were grown in 5 % CO_2 incubator at 37°C for 24 h. The morphology of cells was monitored with an inverse microscope. Cells were then isolated by centrifugation at 200×*g* for 10 min. After discarding the supernatant, cells were fixed with 4 % pentadialdehyde for 24 h followed by a thorough wash with double-distilled water. After dehydration with gradient alcohol washing, cells were embedded with EPON-812 resin. Dissection was accomplished using an LKB-V model microtome. Cell sections were stained with sodium acetate and lead citrate, and subjected to morphology investigation with an H-300 transmission electron microscope.

Cells were cultured in a humid atmosphere (5% CO2, 37°C) using DMEM and RPMI 1640 media, heat inactivated Embryonic Calf Serum (ECS) and Horse Serum (HS) obtained from Gibco (USA). MCF-7 and MDA-MB435S were cultivated in the DMEM medium, HeLa were cultured in the RPMI 1640, Lung cancer cell lines and Colon cancer cell lines were cultivated in the mixture of DMEM and RPMI 1640 (1:1). All media contained 10% fetal bovine serum (FBS). The media contained 2 mM glutamine, antibiotics penicillin and streptomycin (10 U/mL and 10 µg/mL, respectively).

Determination of cytotoxic activity of asparaginases

For detection of cytotoxic antitumor activity of L-asparaginase, the cells were seeded into 96 well plates in 100µl of respective medium containing 10% FBS at plating density of 10,000 cells/well and incubated at 37˚C, 5% CO_2, 95% air and 100% relative humidity for 24 hours prior to addition of extract. The extract was solubilised in Dimethyl Sulfoxide and diluted in respective serum free medium. After 24 hours, 100ml of the medium containing the extract at various concentration (6.25, 12.5, 25, 50,100 & 200 µg/ml) was added and incubated at 37˚C, 5% CO_2, 95% air and 100% relative humidity for 48 hours. Triplicate was maintained and medium containing without extract were served as control. After 48 hours, 15µl of MTT (5mg/ml) in PBS was added to each well and incubated at 37˚C for 4 hours. The medium with MTT was flicked off and the formed formazan crystals were solubilised in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % cell inhibition was determined using following formula.

Effect of L-asparaginase on protein and cell growth in cancer cells

The combined effect of asparaginase with cytostatics on protein and cell growth was analyzed using the cytostatic methotrexate (5µg/mL). *Streptomyces enissocaesilis* AUBT1404 L-asparaginase concentration was 5.0 IU/ml. The experiments were performed using human leukemia cell lines MCF-7 Cell line (Breast Cancer) and MDA-MB435S Cell line (Breast Metastatic cancer). The cell growth and protein synthesis was evaluated by adding 1 µ Ci/well of [14C] amino acids and [14C] thymidine, respectively 5 h before the end of experiments performed in 96 well plates. After this incubation the growth medium was carefully removed and the cell sediment or monolayer was treated overnight at –10°C with 0.1 ml of the ice cold fixing solution ethanol: acetic acid (9: 1) to remove radioactivity of the acid soluble pool (Hashimoto *et al.,* 2009) were then washed with ice cold Hanks solution and after addition of 50 µL 0.3 M KOH were placed into a thermostat at 37°C for 10–12 h. The resultant cell hydrolyzate was neutralized with 1.0 M $HClO_4$ up to pH 7.0 and its radioactivity was counted using a scintillation counter Tri Carb 2800 TR (Perkin Elmer) in Bray liquid. After blank deduction results were calculated as cpm per 106 cells and as % of control. The

number of cells was evaluated in the MTT test using data obtained during cell counting in the Goryaev chamber and the extraction value in the MTT test. Statistical treatment of results (including calculation of mean and SD values) and their graphic presentation were performed by the Excel program. Cell cycle analysis by flow cytofluorimetry and detection of apoptotic cells before and after treatment of tumor cells with L-asparaginases were performed as described by Walker *et al.,* (1993). Tumor cells (0.5 × 106) were cultivated in 25 cm^2 flasks containing 5 mL of the growth medium under standard conditions. After cultivation for 24 h asparaginase preparation (5 IU/mL) was added and cultivation continued for 72 h. After this cultivation cell sediment was collected, treated with propidium iodide (Sigma, USA) for analysis in a cytofluorimeter.

Results and Discussion

The antiproliferative effect of the purified L-Asparaginase enzyme on different tumor human cell lines; MCF 7 (breast cancer cell line), MDA-MB435S (Breast Metastatic cancer), HeLa cell line, Human small cell lung cancer cell line H69PR (ATCC® CRL-11350™) and COLO 205 (ATCC® CCL-222™) was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2- yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan (Mosmann, 1983). The *in vitro* cytotoxic effect of *S. enissocaesilis* L-Asparaginase enzyme on the growth of five human tumor cell lines showed that the enzyme had significant inhibitory effects in different cell lines (Tables 1 and 2). However, the highest antitumor activity was recorded towards MCF-7(82.3%), MDA-MB435S (71.7%), HeLa and H69PR (ATCC® CRL-11350™) were less sensitive (%CI of 64.2 and 60 respectively), while the least activity was obtained towards COLO 205 (52.1%) when compared with the growth of untreated control cells. The incubation of MCF 7 with gradual doses of *S. enissocaesilis* L-asparaginase lead to a gradual inhibition in the cell growth as depicted by the % CI values. The inhibitory efficiency to all tumor cells was directly proportional to the dosage applied.

Determination of dose response studies using standard drugs

Figures 1 and 2 show results of comparative study of the cytotoxic antitumor activities of L-asparaginase and Tamoxifen on MCF 7 cell line and L-asparaginase and Cisplatin on MDA-MB435S, respectively. The results demonstrate that the incubation of MCF 7 (breast cancer cell line) and MDA-MB435S (Breast Metastatic cancer) with increasing amounts of L-asparaginases resulted in a significant dose-dependent decrease in the number of viable (metabolically active) cells as compared with standard drugs.

Table.1 Antitumor activity of purified L-asparaginase on human carcinoma cell lines

CELL LINES	%CI	%CS
MC-7	82.3	17.7
MDA-MB435	71.7	28.3
HeLa	64.2	35.8
H69PR (ATCC® CRL-11350™)	60	40
COLO 205 (ATCC® CCL-222™)	52.1	47.9

Table.2 The effect of L-asparaginase on the number of tumor cells as shown by results of the MTT test performed at 24, 48, and 72 h and shown as absorption at 540 nm

Leukemia cell line	24 h	48 h	72h
MCF-7, Control	0.230	0.595	1.110
MCF-7, Experiment	0.133	0.468	0.470
MDA-MB435S, Control	0.160	0.196	0.312
MDA-MB435S, Experiment	0.125	0.156	0.166

Table.3 Cell cycle analysis by flow cytofluorimetry and detection of apoptotic tumor cells after cultivation with L-asparaginases

Groups	MCF-7	MDA-MB435S
Control		
Apoptosis	**21.4**	**24.7**
G0/G1	**40.5**	**37.8**
S	**17.2**	**26.1**
G2/M	**22.5**	**31.2**
S.enissocaesilis L-Asparaginase		
Apoptosis	**90.0**	**87.1**
G0/G1	**49.5**	**40.5**
S	**26.7**	**15.2**
G2/M	**13.6**	**10.5**

Fig.1 Dose-response curve of L-asparaginase on MCF-7 Cell line

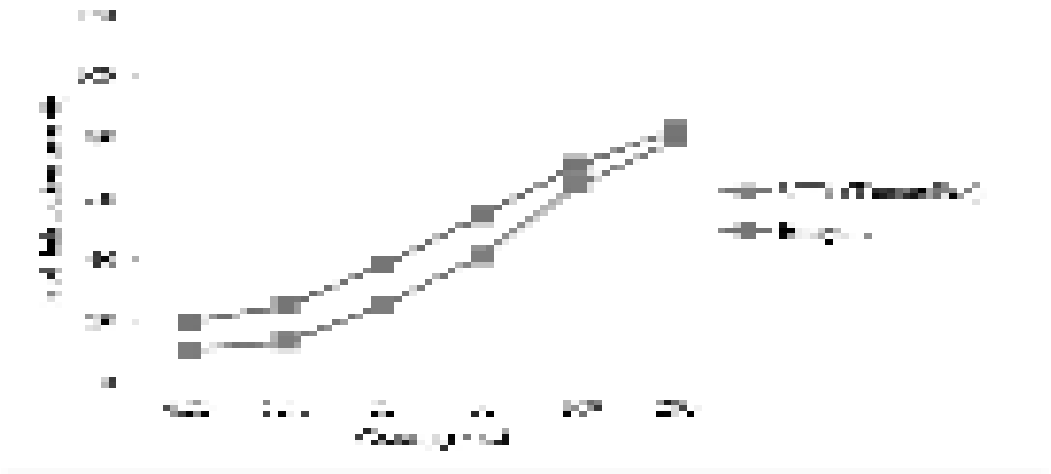


Fig.2 Dose-response curve of L-asparaginase on MDA-MB435S cell line

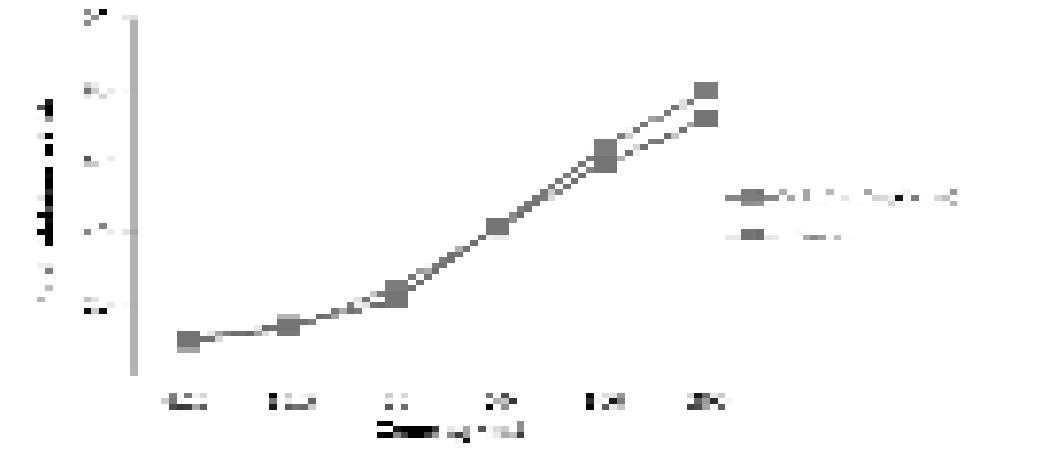
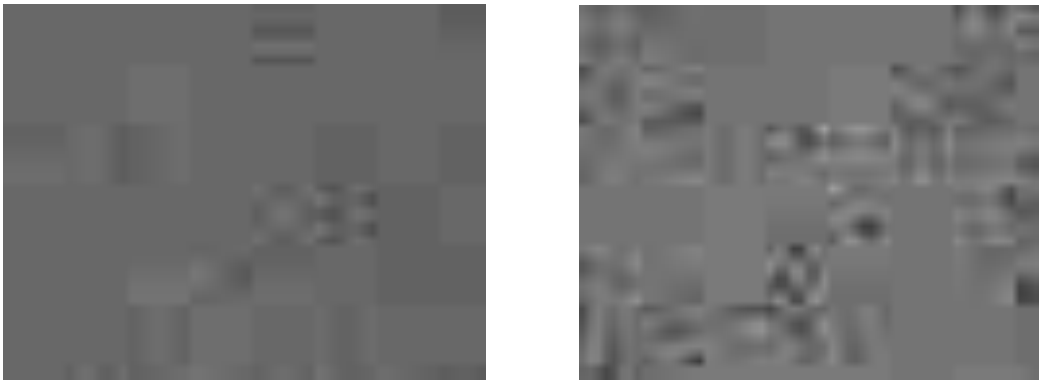


Fig.3 Effect of L-asparaginase on the morphology of the MCF-7cells



MCF-7 before treatment with L-asparaginase enzyme

MCF-7 after treatment with L-Asparaginase Enzyme

Fig.4 Effect of L-asparaginase on the morphology of MDA-MB435S cell

MDA-MB435S before treatment with L-Asparaginase enzyme

MDA-MB435S after treatment with L-Asparaginase enzyme

Fig.5 The effect of the combination of L-Asparaginase with cytostatic methotrexate on protein synthesis of MCF-7

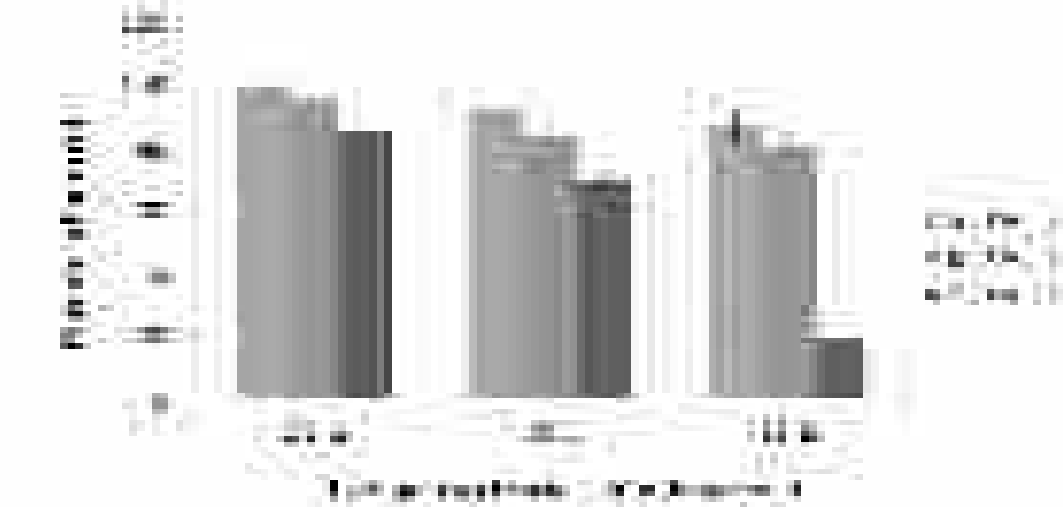


Fig.6 The effect of the combination of L-asparaginase with cytostatic methotrexate on cell growth of MCF-7

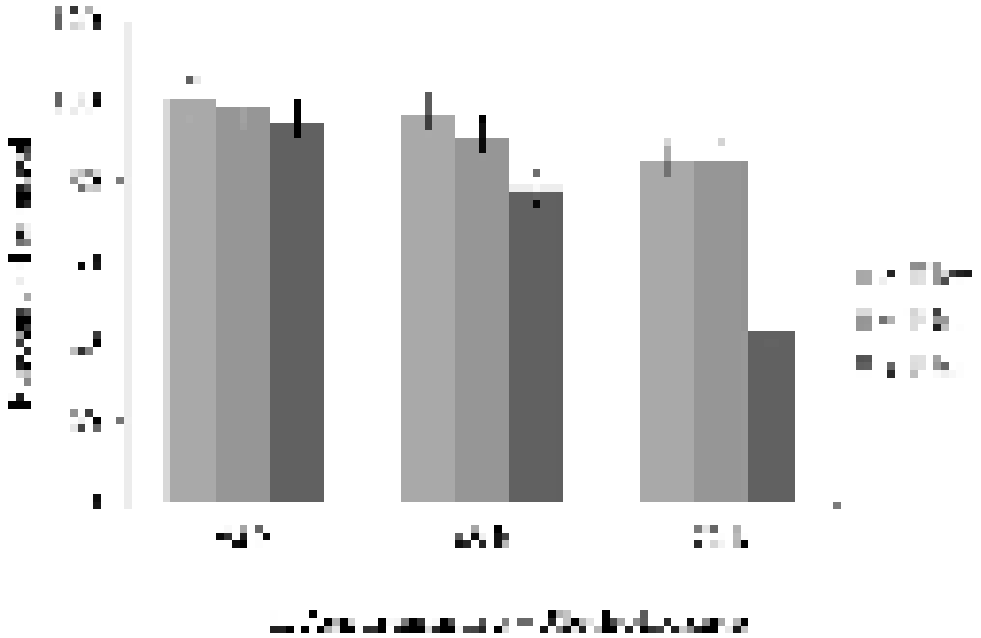
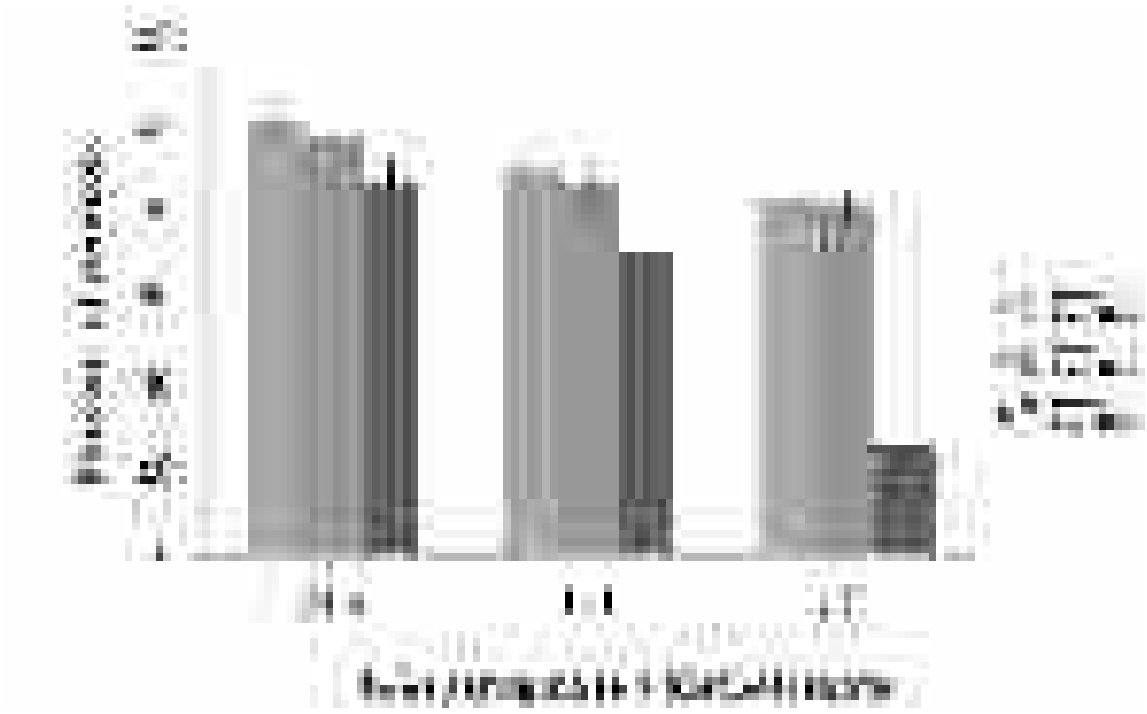


Fig.7 The effect of the combination of L-asparaginase with cytostatic methotrexate on protein synthesis of MDA-MB4352



These results suggest that the isolated L-Asparaginase suppressed cell growth of both the tumor cell lines at certain doses starting from 6.25 µg/ml. Our results on the antitumor cytotoxic toxicity of *S. enissocaesilis* L-asparaginase *in vitro* are rather optimistic. Asselin *et al.,* (1989) demonstrated linear correlation between death of leukemia cells treated with asparaginases *in vitro* and *in vivo.* Thus, results of *in vitro* tests on asparaginase cytotoxic activity may be used for prediction of the enzyme activity or *in vivo* sensitivity to chemotherapy of

acute lymphoblast leukemia and possibly solid tumors in humans.

Flowcytometry

It was demonstrated that treatments of MCF-7 and MDA-MB435S cell lines with *S. enissocaesilis L*-asparaginase had profound effect on the morphology of the cells (Figures 3 AND 4). At the same time cell cycle analysis by flow cytofluorimetry of MCF-7 and MDA-MB435S cells treated with asparaginase and determination of the number of leukemic cells 24, 48, and 72 h after onset of asparaginase treatment suggest that under asparagine deficit these cells stop normal division (compared with corresponding control) and die. The number of these cells decreased within the time interval of this experiment (24–72 h). This is consistent with observation by Asselin *et al.,* (1989).

Table 3 shows that treatment with L-asparaginases was effective in increasing the number of MCF-7 and MDA-MB435S apoptotic cells. MCF-7 and MDA-MB435S cells containing almost 21.4% and 24.7.7% of apoptotic cells in control demonstrated sensitivity: the number of apoptotic cells significantly increased after L-asparaginase treatment. The increase in the number of apoptotic cells up to 90% and 87% was observed in MCF-7 and MDA-MB435S cell lines, respectively after their combined treatment with L-asparaginase and methotrexate (5 μg/mL).

Effect of L-asparaginase on cell growth and protien synthesis

Experiments have been conducted to investigate whether administration of L-asparaginase, which results in hydrolysis of extracellular asparagine, arrests asparagine dependent protein synthesis and causes subsequent inhibition of cell growth followed by decreased proliferation of leukemic cells.

Figure 5 & 6 show the effect of combined treatment of L-asparaginase and cytostatic methotrexate (5 μg/mL) on the protein synthesis by MCF-7 and Figures 7 and 8 show the effect of combined treatment of L-asparaginase and cytostatic methotrexate (5 μg/mL) on MDA-MB435S cells. Treatment of MCF-7 cells with L-asparaginase and methotrexate for 24–48 h insignificantly decreased protein synthesis, while the protein synthesis significantly decreased (20% of control) after 72 h only at L-asparaginase of 7 IU/mL; at asparaginase concentrations 2 and 5 IU/mL it remained elevated. The level of secretion demonstrated dependence to the asparaginase concentrations. Cell growth slightly differed from control cell growth at 24 and 48h. Treatment of cells for 72 h was accompanied by a sharp decrease of cell growth (42% of control).

Protein synthesis in MDA-MB435S cells treated with of L-asparaginase and cytostatic methotrexate did not decrease below control values 24 and 48 h after asparaginase addition, but decreased at 72 h at asparaginase concentration of 7 IU/mL (26% of control). The combined treatment for 72 h the cell growth represented 50% of control.

In conclusion, this study implies that *S. enissocaesilis* L-asparaginase may be a potent drug for treatment of MCF-7 and MDA-MB435S leukemia. The Cell cycle analysis by flow cytofluorimetry demonstrated that the L-asparaginase had profound effect on the morphology of the MCF-7 and MDA-MB435S cells and resulted in increased number of apoptotic cells. A linear correlation was observed between the number of the tumor cells and the dose- and time-dependent antitumor and cytotoxic effects of L-asparaginase from *Streptomyces enissocaesilis.* The administration of L-asparaginase resulted in hydrolysis of extracellular asparagines along with arrested asparagine dependent protein synthesis and caused subsequent inhibition of cell growth followed by decreased proliferation of leukemic cells. From the results, it is concluded that this L-asparaginase can be used for the development of new preparations for the therapy of tumors.

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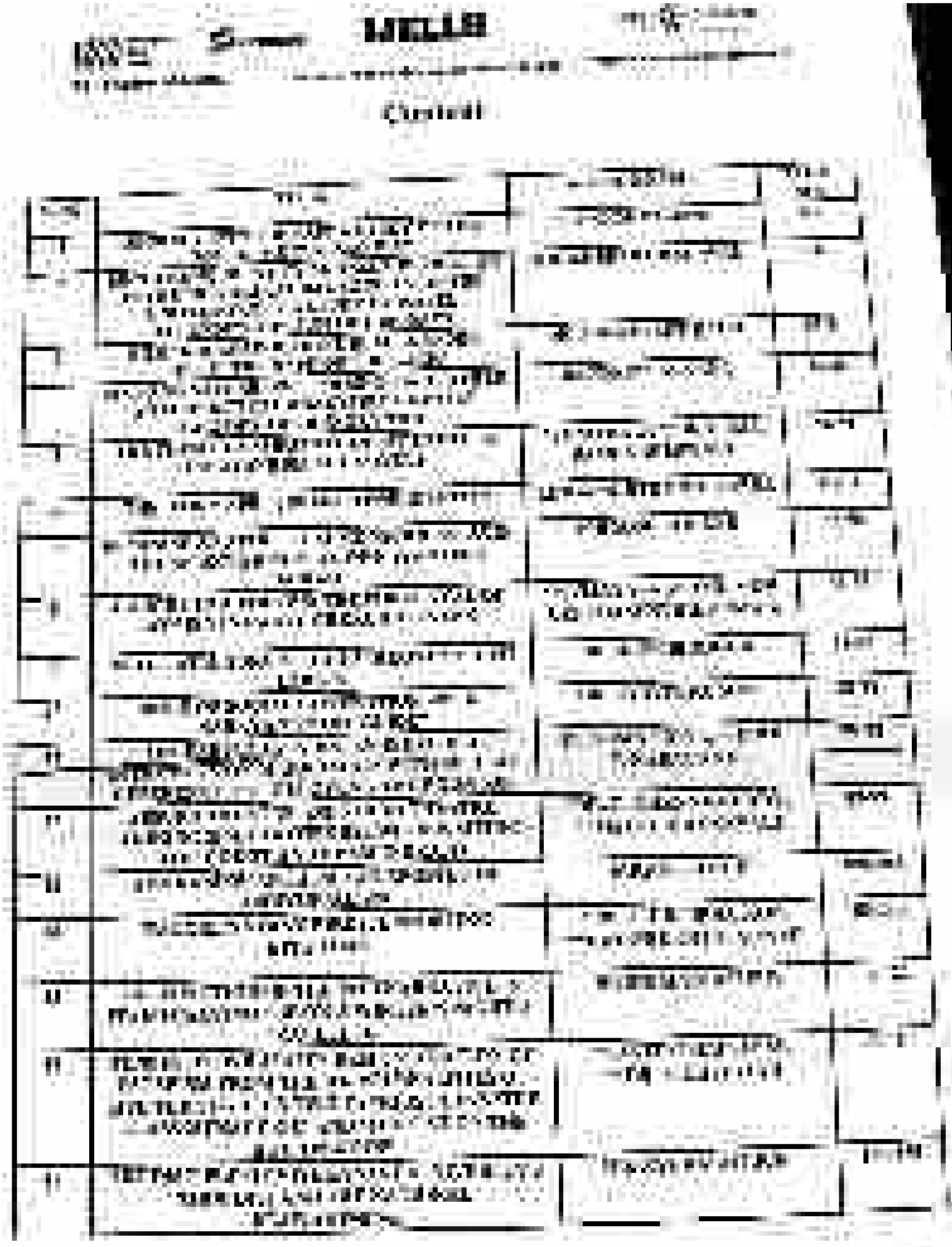

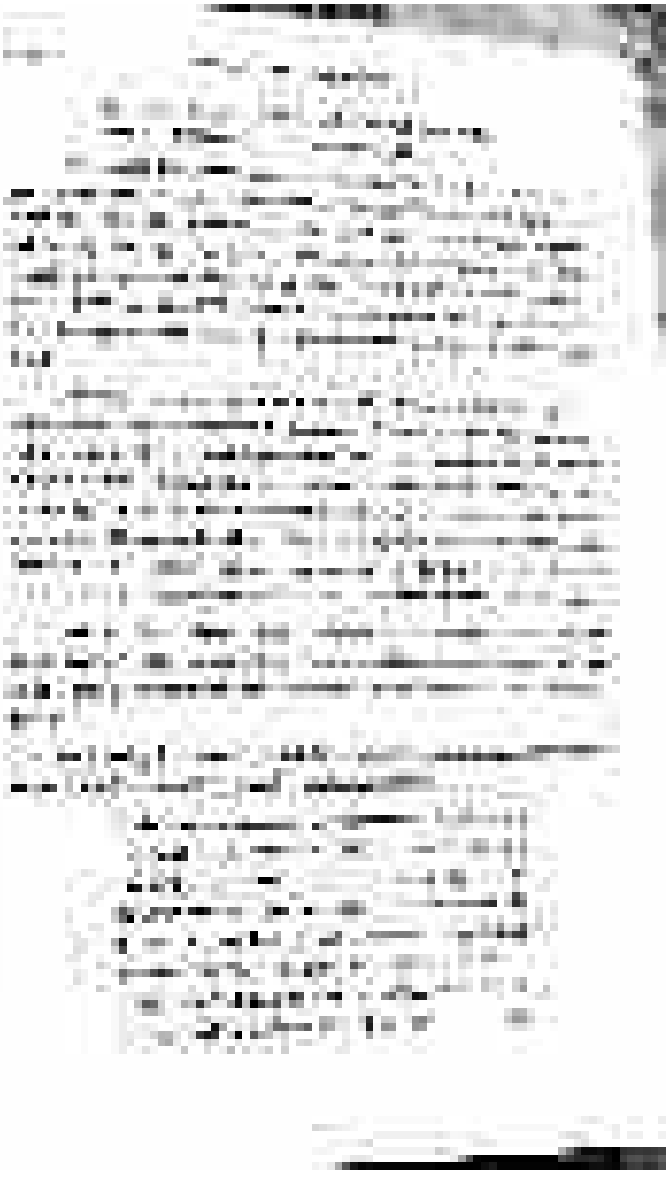

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Microbiological Analysis of Drinking Water Sources in Chilakala Gedda Panchayt Ananthagri Mandal, Visakha district, Andhra Pradesh, India

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Abstract:

The quality of potable water and treatment of waterborne diseases are critical public health issues. Microbial contamination of drinking water sources in the most common health risk. The research determines microbiological quality of drinking water sources in Chilakala gedda panchayat, Ananthagiri mandal, Visakhapatnam, India during the period of 2011-2012. A Total of 10 drinking water samples were randomly collected from Bore well, Well, Tap and stream in different places of Chilakala Gedda Panchayat. The water samples were tested using the MPN multiple tube technique on Macconkey broth for Presumptive coliform count followed by Escherichia coli confirmation, Total Plate count and Coliform count were conducted (APHA, 2005). According to the results the MPN count high in well water sample 2400/100ml and Total plate count range between1.28 x 10^2 to 3.85 x 10^2 cfu / 100ml and Fecal coliform count 0.25 to 1.35 x 10^2cfu/100ml observed in the study area. The isolates were characterized and identified as E.coli, Enterobacter, Klebsiella, Salmonella and Shigella. The result indicates that microbial values are high according to WHO guidelines. The use of contaminated water in drinking can exposes human body to many water borne diseases hence water treatment and improving quality of water before drinking is required.

Key words: Drinking water, MPN, Colifrom count, WHO

INTRODUCTION

Water is one of the most important elements for all forms of life. It is indispensable in the maintenance of life on earth. It is also essential for the composition a renewal of cells. Despite of this, human beings are containing tap pollute water sources resulting in provoking water related illnesses [1].

The public health significance of water quality cannot be over emphasized. Many infectious diseases are transmitted by water through the fecal-oral route. Diseases contacted through drinking water kill about 5 million children annually and make 1/6th of the world population sick [2]. Water is vital to our existence in life and its importance in our daily life makes it imperative that thorough microbiological and physico-chemical examinations be conducted on water. Potable water is the water that is free from disease producing microorganisms and chemical substances that are dangerous to health [3]. In India, majority of the rural populace do not have access to potable water and therefore, depend on well, stream and river water for domestic use. The bacterial qualities of ground water, pipe borne water and other natural water supplies in India, have been reported to be unsatisfactory, with coliform counts far exceeding the level recommendation elucidation of important parameters in water quality assessment may be attributed to the fact that in the overall potability of water such parameters should not be ignored [4].

The aim of the study was to determine the microbiological contamination of drinking water sources in Chilakala Gedda panchayat Ananthagiri Visakhapatnam district, A.P. India and compare with studies of BIS and WHO.

MATERIAL AND METHODS:

A survey was conducted for water samples at Chilakala Gedda village of Visakhapatnam district; Andhra Pradesh, India for this water samples were collected every fortnight intervals and method of sample collection and water analysis as follows.

1. Sample Collection:

Sampling was done according to the procedure recommended by American Public Health Association [5]. Water samples were collected for Bacteriological analysis. Samples were collected for container, which is immediately covered tightly after collection of water samples and transported to the laboratory. In Chilakala gedda panchayat, Bore well, well, tap are present as sources of water in the villages and eight samples were collected that are given in the table number 1.

Figure A. Water in the open well

Figure B. A women collecting from the Bore well

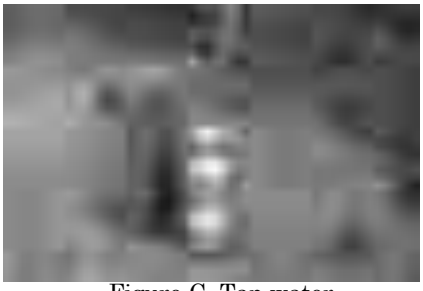

Figure C. Tap water

Table 1. Sampling stations

Name of the Village	Source of water	Sampling Code
Chilakala Geeda	Bore	S1
Chilakala Geeda	Tap	S2
Dasavthota	Bore	S3
Dabbala padu	Bore	S4
Venkayya palem	Bore	S5
Vara goddu	Bore	S6
Julaga padu	Well	S7
Settayyathota	Well	S8

2. Microbiological Analysis:

The most probable number (MPN) technique was used to determine the fecal coliform counts of the water samples. This involved the presumptive test using lactose broth and Total coliforms were test using eosin methylene blue (EMB) agar. The total plate count was conducted by pour plate technique on plate count agar (PCA) and counting the colonies developed after the incubation at 37^{o}C for 24 for 24 hours [6]. All colonies with different characteristics on Endo agar, Manital salt agar, SS agar, MacConkey agar, Thiosulphate Citrate Bile salt sucrose agar (TCBS) were sub-cultured onto Nutrient agar (NA) for purification. Enteric bacteria isolated on respective selective or differential media were identified on the basis of their colonial, morphological and Biochemical properties following Bergey's Manual of determinative Bacteriology, 1994.

RESULTS AND DISCUSSION:

A total of eight drinking water samples were collected from different villages of Chilakala gedda Panchatyat, of which 5 were Bore wells, 2 from wells, 1 from tap waters. The test values were then compared with the standard methods for the examination of water [6] and Bureau of Indian Standards (BIS), World Health Organization (WHO).

Table 2. Water analysis of different samples from villages of Chilakala gedda panchayat

Name of the sample water	MPN count/ 100ml	Total Plate Count cfu/100ml	Fecal coliforms cfu /100ml
Bore-1	210	2.51×10^2	0.56×10^2
Tap	64	1.28×10^2	0.25×10^2
Bore-2	150	1.99×10^2	0.52×10^2
Bore-3	120	1.92×10^2	0.55×10^2
Bore-4	93	1.75×10^2	0.48×10^2
Bore-5	240	2.85×10^2	0.61×10^2
Well-1	2400	3.85×10^2	1.35×10^2
Well-2	1100	3.51×10^2	1.28×10^2

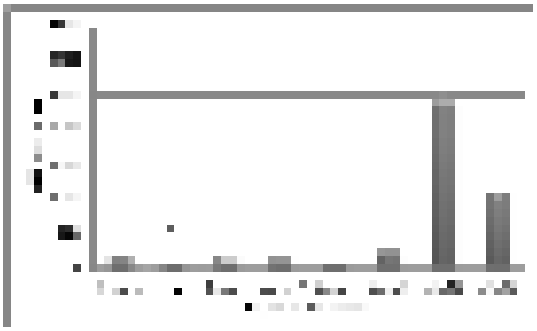

Figure 1. MPN counts of fecal coliform in different water samples

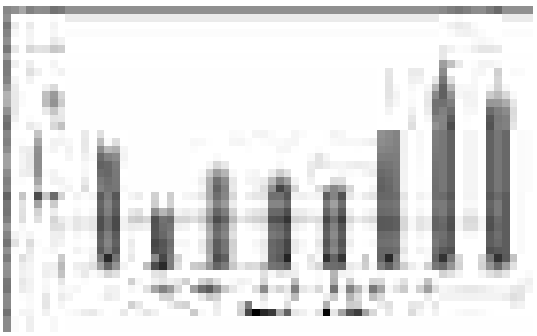

Figure 2. Total bacterial counts from different water samples

Figure 3. Fecal coliform contents from different water samples

Presence of Coliform group of microbes as a whole is recognized as a suitable indicator for drinking water contamination. Total Coliform counts in Well waters were found to be maximum at 2400 MPN/ 100ml in the well-1 sample (Table 2 and Figure 1). The minimum was in the range 64 MPN/100ml in tap water. Compared with the different Well samples form the above observation that the open well waters had high load of coliforms when compared with that of bore well waters and tap water. It might be due to per collation of water from sanitary land filled areas and leachates from septic tanks (Table-1). The present findings on par with the observation of [7], who have reported high load of coliform counts for open well water. Presence of TC in different drinking water sources indicates inadequate treatment and poor sanitation [8]. Adequate treatment and sanitation is necessary for drinking water.

The total bacterial counts (TVC) for all the water samples were generally high exceeding the limit of $1.0X10^2$cfu/ml which is the standard limit of heterotrophic count for drinking water [9]. A high total heterotrophic count is indicative of the presence of high organic and dissolved salts in the water. The Total Plate Count ranged between 1.28×10^2 to 3.85×10^2 cfu / 100ml (Table 2 and Figure 2).

The Fecal coliform count in any region should be below 10 CFU to consider the water to be safe. The results seen in the

table indicates highly unsafe drinking water, inadequate water treatment, seepage of industrial and domestic pollutants and an overall lacking of infrastructure. The highest counts noticed in S7 open well (well-1) waters were staggeringly high (Table 2 and Figure 3).

In the study area different bacterial species were identified based upon the morphological characteristics of isolates which are obtained from the water samples on Nutrient Agar (NA) and different selective media as shown in table 3. The biochemical characteristics of the isolates which are obtained from these water samples were shown in Table. The isolated enteric bacterial species were identified to be the same as those commonly encountered in water which were also reported in study on river water sources of rural Venda region, South Africa [10] and reviewed by [11].

Table 3. Morphological characteristics of isolates

Isolate	Morphological Characteristics	Organism
1	Non- spore forming and non- motile, gram positive cocci, circular, low convex with entire margin, smooth, medium, opaque colony on Nutrient Agar, Yellow colure colonies on Mannitol Salt Agra Media grown at pH 7 and 37^0 C	*Staphlococcus sp.*
2	Gram positive cocci, thin, even, growth on Nutrient Agar, black or brown colure colonies on Bile esilin Agar.	*Group D Streptococcus,*
3	Gram positive rod, spore forming, abundant, opapue, white waxy growth on Nutrient Agar .	*Bacillus sp.*
4	Gram negative rod, circular, low convex, with entire margin, mucoid, opaque, growth on Nutrient Agar, green metallic sheen colony on Eosin Methlene Blue (EMB) Agar.	*E. coil*
5	Gram negative rod, Slimy, white somewhat translucent, raised growth on Nutrient Agar, Dark pink colure colonies on MacConkey Agar.	*Klebsiella sp*
6	Gram negative rod, thin, blue gray, spreading growth on Nutrient Agar.	*Proteus sp.,*
7	Gram negative rod, abundant, thin, white medium turns green on Nutrient Agar. pink Colure colonies on Phenothalin diphospate Agar.	*Pseudomonas sp.,*
8	Gram negative curved rod, abundant, thick, mucous white colure colonies on Nutrient Agar. Yellow colure colonies on TCBS agar	*Vibrio cholera*
9	Gram negative curved rod abundant, thick, mucous white colure colonies on Nutrient Agar. Green colure colonies on TCBS agar	*Vibrio parahaemolytics*
10	Gram negative rod, thin even grayish growth on Nutrient	*Salmonella sp.,*

	Agar	
11	Gram negative rod, thin even grayish growth on Nutrient Agar	*Shigella*
12	Gram negative rod, abundant thick, white glistening growth on Nutrient Agar	*Enterobacter aerogenes*

Table 4. Biochemical Characteristics of isolates

Test	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
Catalase	+	-	+	+	+	+	+	+	+	+	-	-
Oxidase	-	-	-	-	-	-	+	+	-	-	-	-
Motility	-	-	+	+	-	-	+	+	-		+	-
Indole	-	-	-	+	-	+	-	+	-	+	-	-
Methyl-red	-	+	-	+	-	+	-	-	+	+	(+)	-
Voge-Proskauer	+	-	+	-	+-	-	-	+	-	-	+	-
Citrate Utilization	-	-	-	-	+	-	+	+	+	-	+	+
Urease	+	-	-	-	+	+	-	-	-	-	+	+
Hydrogen sulphide	-	-	+	-	-	+	-		+	-	-	-
Starch hydrolysis	-	-	+	-	-	-	-	-	-	-	-	-
Nitrate Utilization	-	-	+	+	+	+	+	+	+	+	+	+-
Gelatin liquefication	-	-	+	-	-	+	-	+	-	-	(+)	+
Lactose fermentation	-	A	-	AG	AG	-	-	AG	-	-	AG	-
Glucose fermentation	A	A	A	AG	AG	AG	-	AG	AG	A	AG	-
Sucrose fermentation	A	A	A	A(+)	AG	AG+-	-	AG	AG	A+-	-	-

W1-Staphylococcus, W2-Streptococcus,W3- Bacillus Sp.,W4- E. coil,W5-Klebsiella SpW6-, Proteus Sp.,W7- Pseudomonas sp., W8- Vibrio sp., W9- Salmonella sp.,W10- Shigella, W11- Enterobacter A-Acid production only; AG - Acid and gas production; +- = Variable reaction; + - Positive;- = Negative ;(+) – Late Positive

Other bacteria isolated from all water samples such as *Staphylococcus, Pseudomonas sp., Enterobacter aerogenes, Micrococcus sp.,* and *Proteus sp.,* also have public health significance. *Staphylococcus aureus* is known to produce enterotoxin. *Proteus Sp.,* belong to the intestinal flora but is also widely distributed in soil and water [12]. *Enterobacter aerogenes* isolated from water samples is an example of non-

fecal coliforms and can be found in vegetation and soil by which the pathogens enter the water [3].

The presence of opportunistic *Pseudomonas* in the water carries the potential for problems in an immuno compromised population. Shallow groundwater samples commonly contain *Pseudomonas sp., Bacillus sp.,* which occur in both soil and fecal material, and may not be indicative of livestock manure [13].

The presence of *Salmonella* in water samples indicates that the public water supply system is poor and chances of outbreak of water-borne *Salmonella* infection is higher among people consuming the water without proper disinfection. Occurrence of *Salmonella, Shigella, and Vibrio in* urban water supply. Water distribution systems have been reported to provide unique condition for the development of biofilm [14] water borne pathogens was found only in those samples which were positive for *coliforms*. Similar result was reported by [15].

CONCLUSION:

This study concluded that water quality distributed at tribal area need more effort in limiting the number of microbial organisms present in drinking water sources. The majority of the water sources had unacceptable total coliform count and all the water sources which were positive for presumptive coliform count had *E.coli* showing fecal contamination of water sources, and the present study revealed and recommend regular disinfection of drinking water sources, periodic bacteriological appraisal of drinking water sources, and construction and distribution of piped water, this research also demonstrated the importance of education for the people who use drinking water. Much needs to be done to increase awareness of the hazard of drinking.

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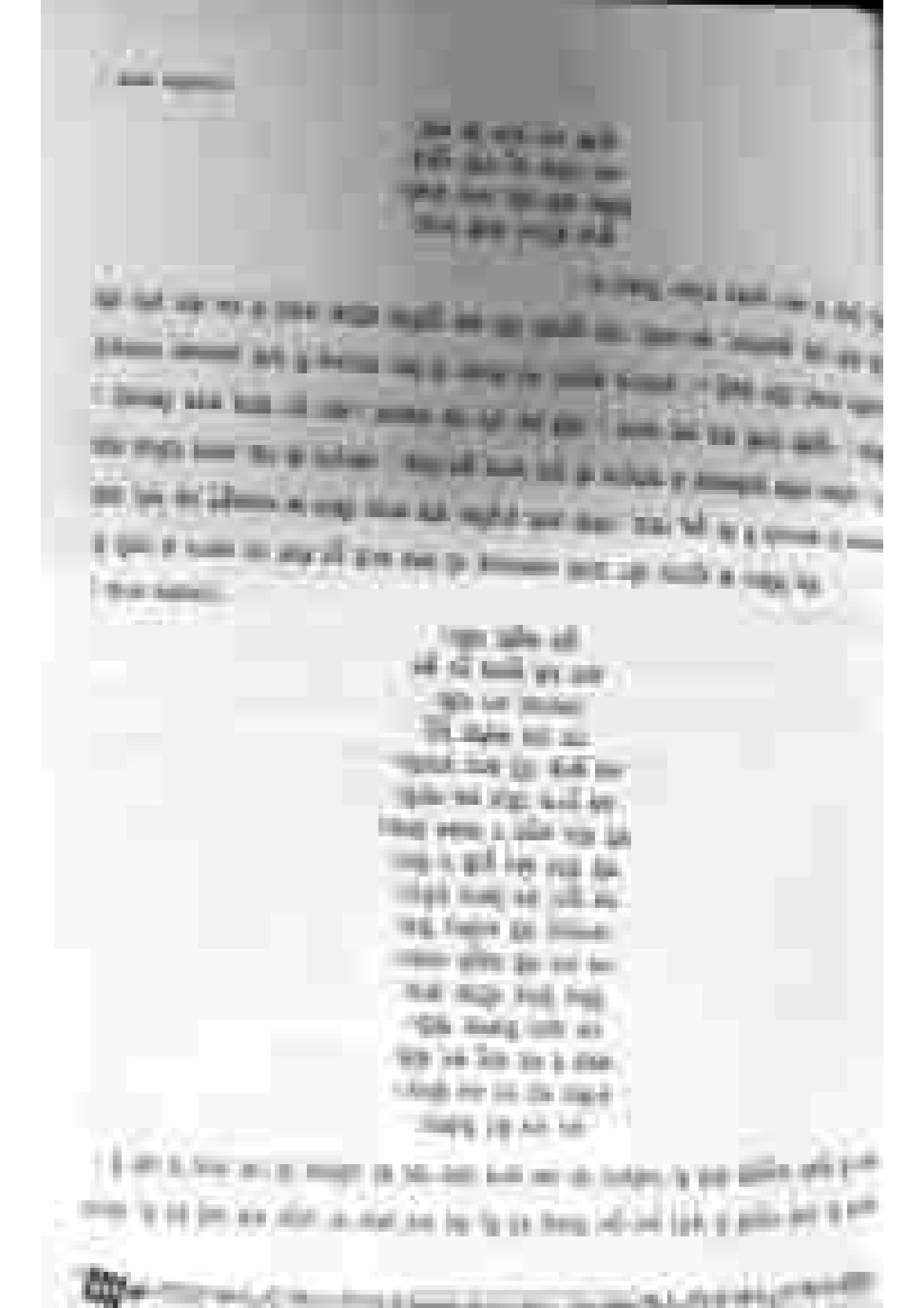

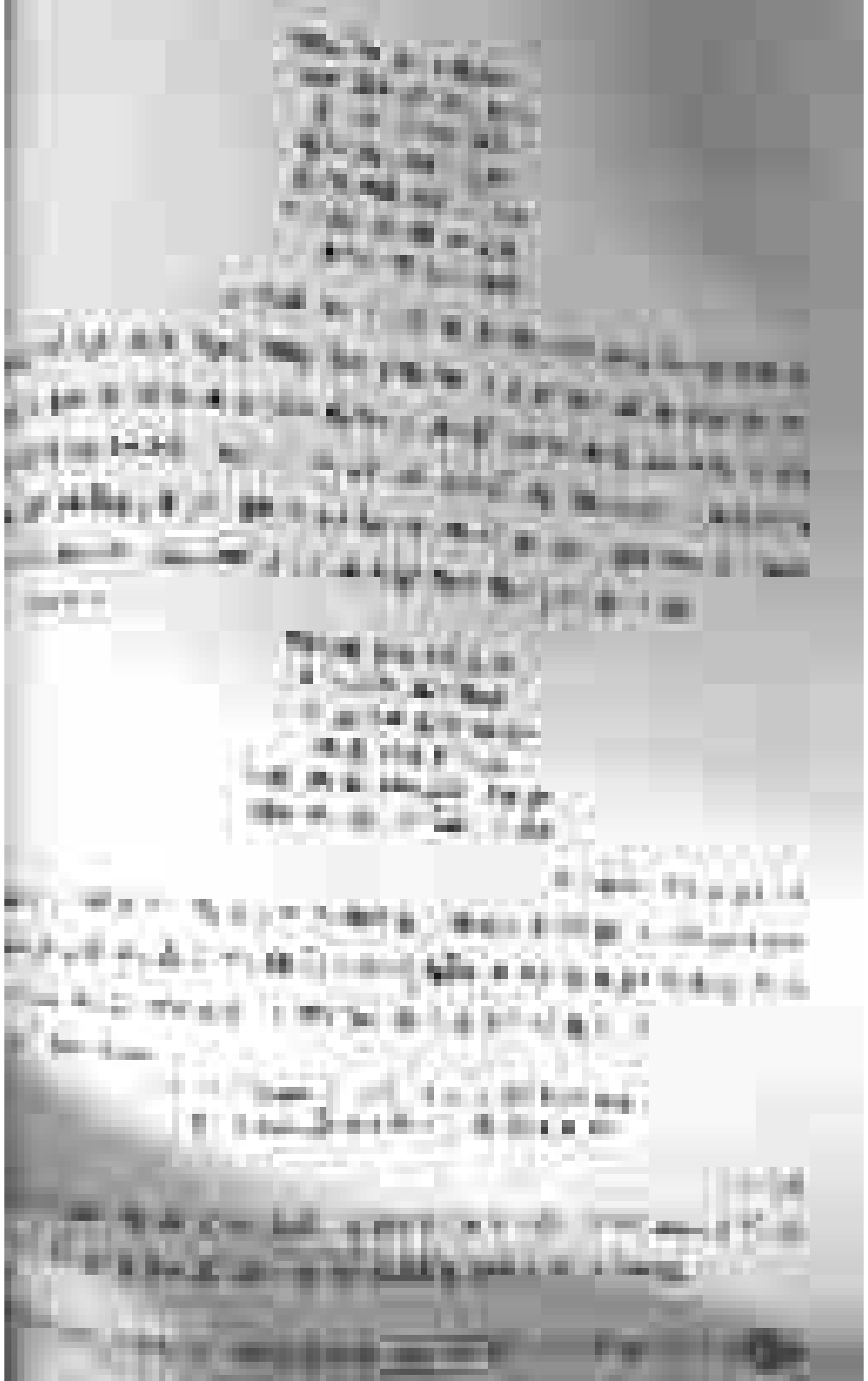

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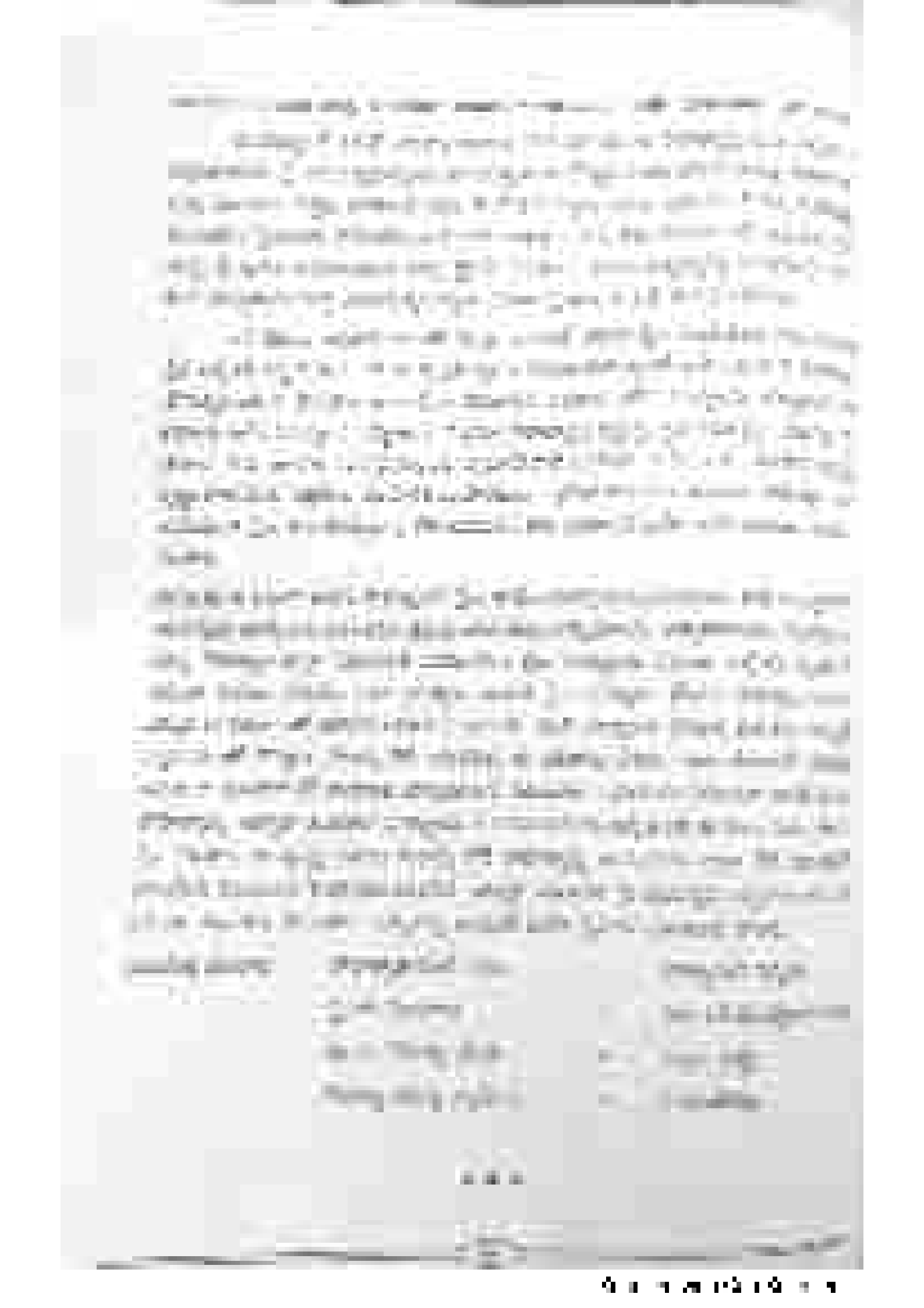

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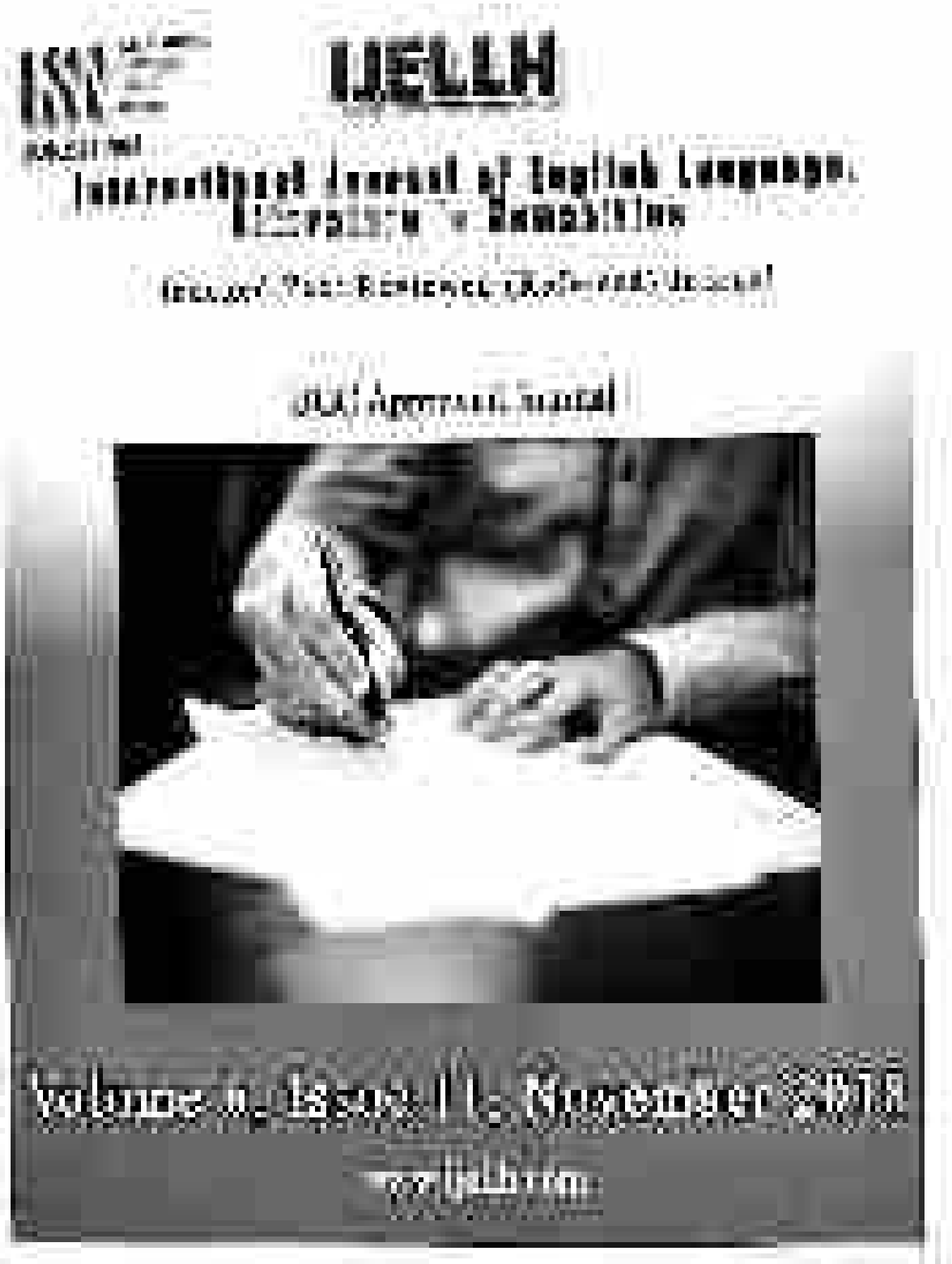

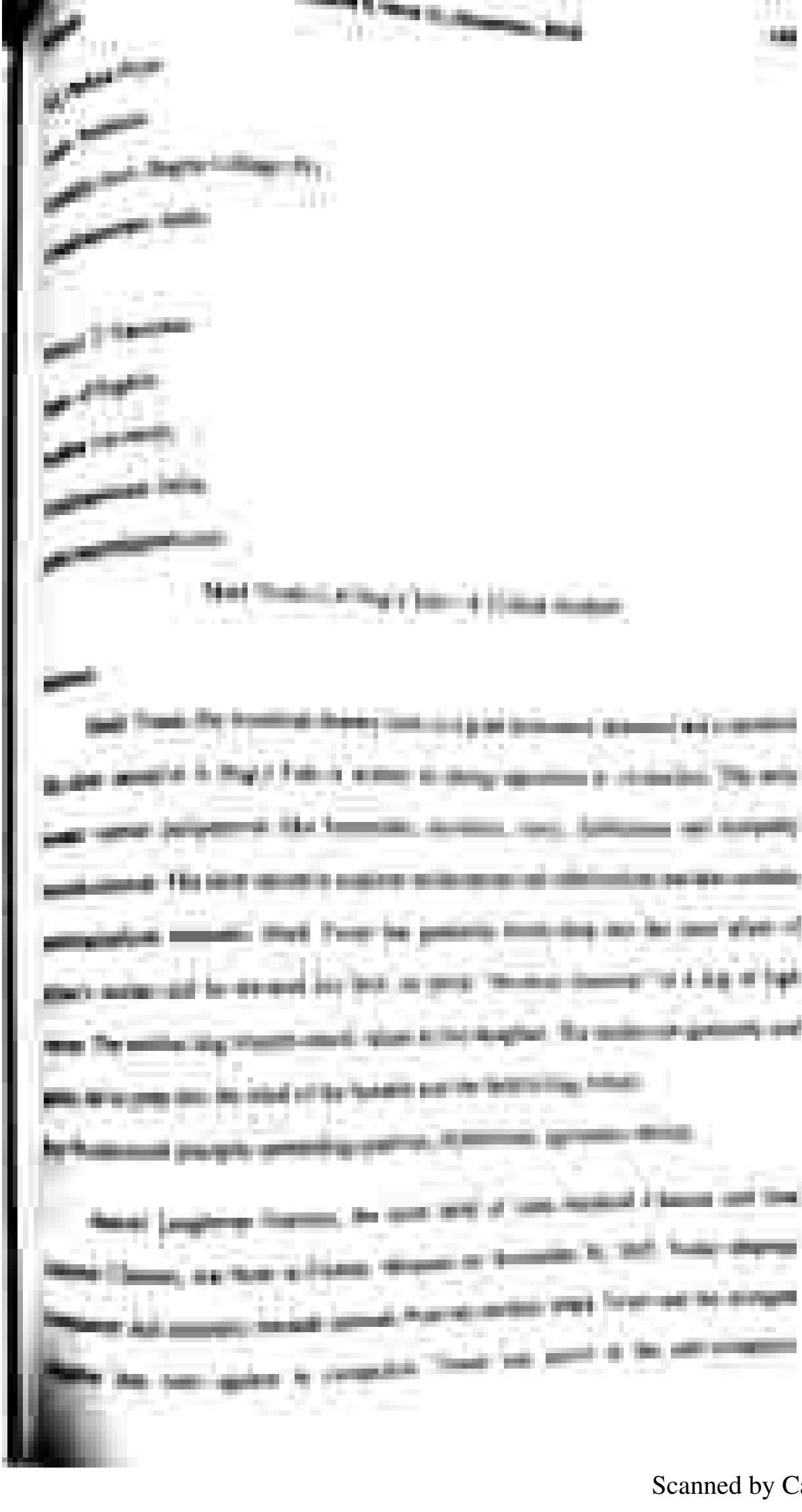

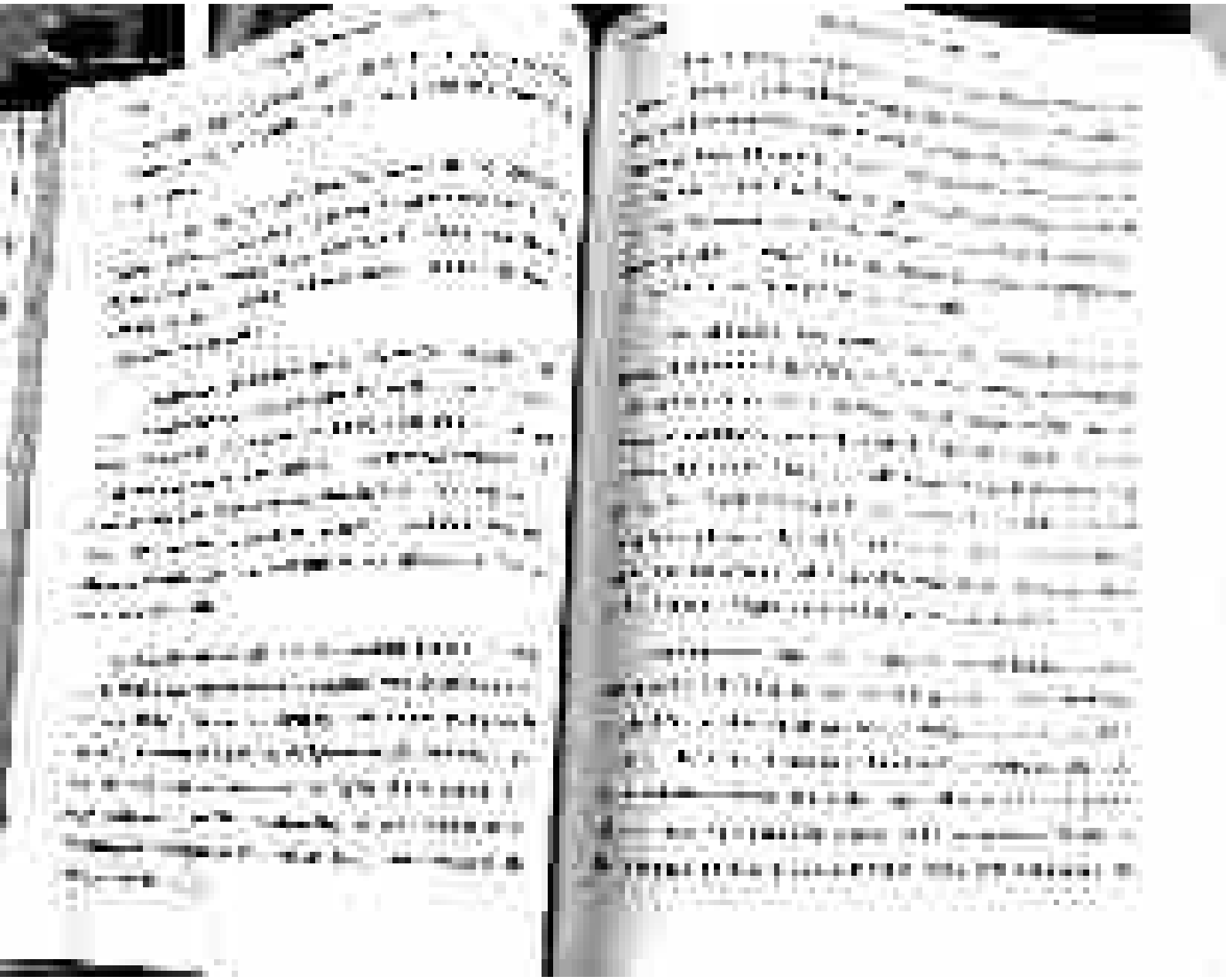

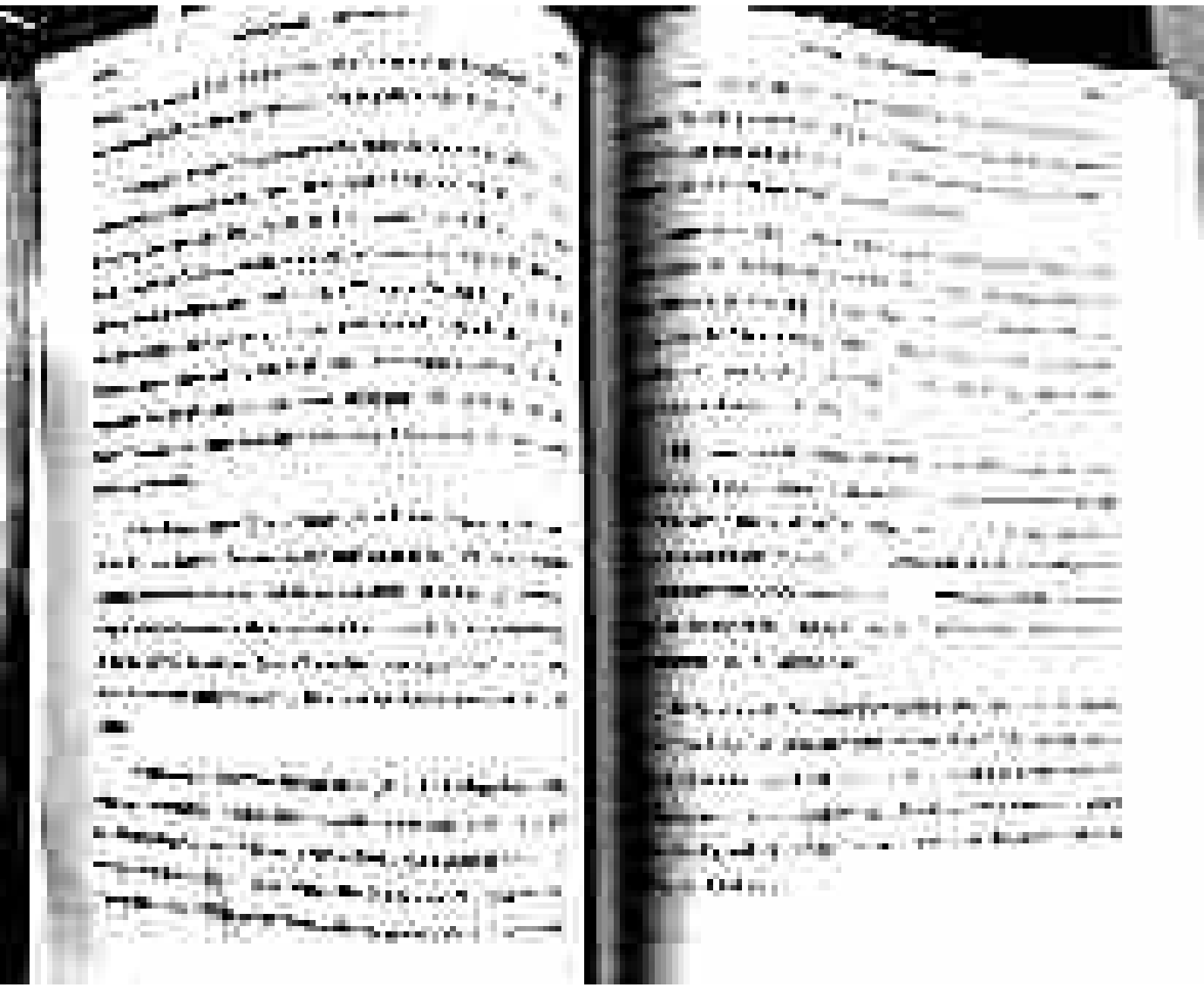

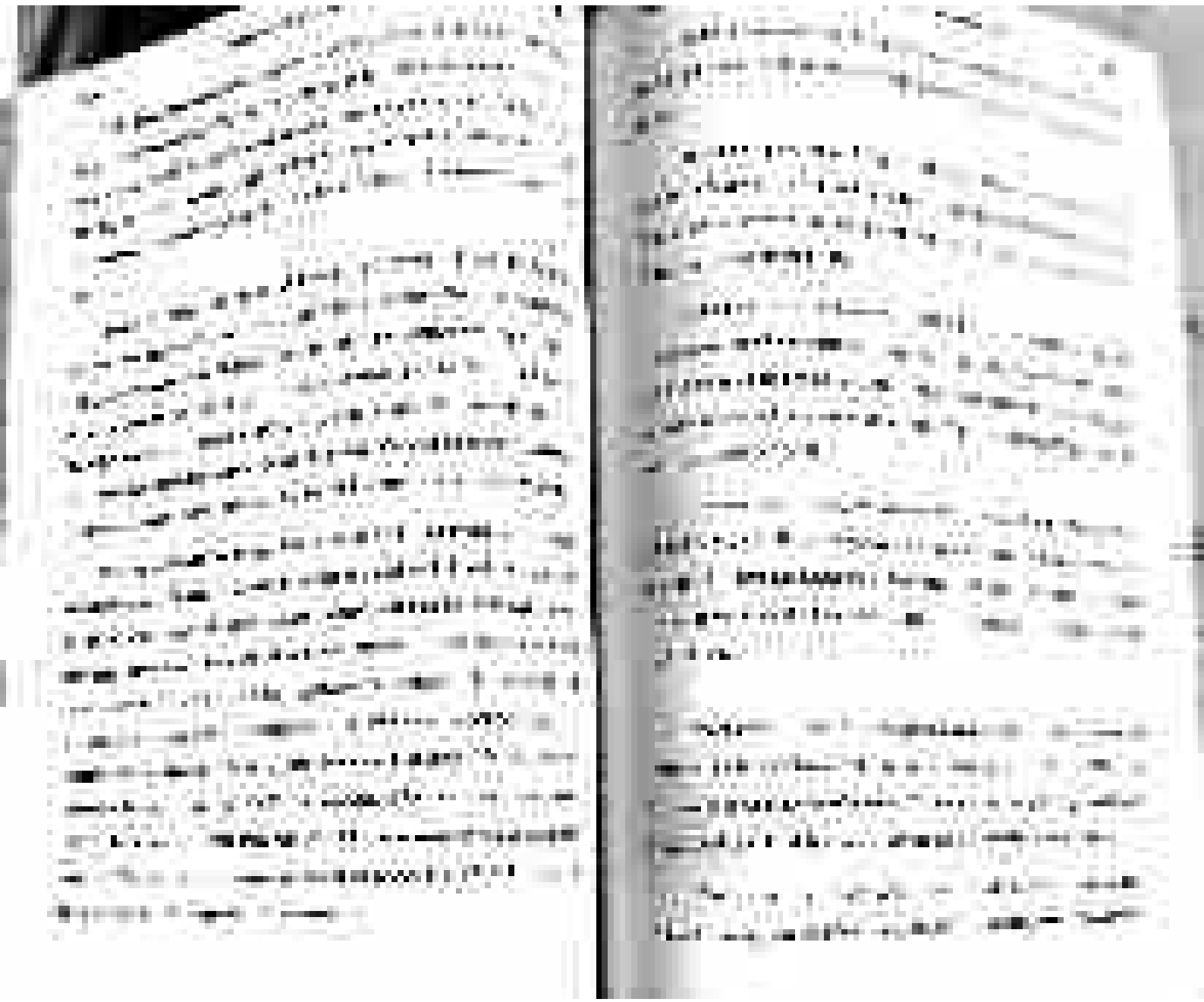

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Multi Component One Pot Synthesis of 3-((1, 3-Bis (Phenyl)-1H-Pyrazol-4-Yl) (1H-Indol-3-Yl) Methyl)-1H-Indole and Their Derivatives by Using Zro_2/SBA-15 Nano Catalyst

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Abstract : The synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole and their derivatives has been prepared through the reaction of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde and1H-indole using low cost, easily seperable and recyclable nano ZrO2/SBA-15 catalyst through the simple one pot synthesis. This method is very efficient and eco friendly. The synthesized 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole derivative's characteristics are assigned by NMR, C^{13} NMR, IR and Mass spectroscopy.

Keywords: one pot synthesis, 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole, recyclable nano ZrO2/SBA-15 catalyst.

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I. INTRODUCTION

The multi component reactions clearly have evolved from being a chemical curiosity towards a powerful tool for synthesis in organic chemistry in the past few years. Most of the multi component reactions are proceeds through three or more components those are reacted in a single reactor to form a product retaining all the reactants in this method the desired product with high yield and completed in short time[1&2]. The multi component reactions play an important role in synthesis of heterocyclic compounds [3]. And also the MCRs have been known for over 150 years with the strecker synthesis of α- amino cyanides[4]. Its importance lies mainly in medicinally potent compounds and its convenient preparation[5-7].

In recent times, the heterocyclic compounds continue to drive the field of synthesis in organic chemistry. Organic chemists have been engaged in extension of produce heterocyclic compounds by developing new and efficient synthetic transformation. Pyrazolyl compounds and their derivatives have shown interesting biological activities such as estrogenic activity[8], antipyretic[9], anti-inflommatory[10,11], anticancer[12, 13], antiviral[14, 15] antibiotic[16],anti microbial [17] and analgesic[18]. 1,3-diphenyl-1H-pyrazole-4-carbaldehyde , one of the starting materials is synthesized in our lab as per the procedure[19].

From the literature survey, pyrazolyl methylenebis indoles provide novel lead structures for drug discovery. However, only a few synthetic strategies have been reported for the synthesis of pyrazolyl methelene bis indoles. Farhanullah *et al* reported Amberlyst 15 catalyzed synthesis of indole-pyrazole based tri(hetero) arylmethanes[20]. Sivaprasad G at al using phosphotungestic acid [21]. From the mesoporous Lewis acidic ZrTUD-1 Kandasamy Karthikeyan et al reported[22]. After several methods have been reported to synthesis of bis indoles indole and its derivatives being a key moiety of physiological properties[23-25]. Bis indolyl metabolites affect the central nervous system [26&27]. Various indolyl derivatives display diverse pharmacological activities and are useful in treatment of fibromyalgia, chronic fatigue and irritable bowel syndrome [28-30]. Vibrindole a bisindolylmethane was known to exhibit anti-bacterial activity [31].

Herein, in order to achieve a more efficient synthetic process, minimize by-products, decrease the number of separate reaction steps, improving the yields and reaction times and also in extending our research on the application of nanocatalysts in MCRs, in this we report a clean and environmentally friendly approach to the synthesis of Bis phenyl pyrazolyl methyl bis indoles, via multi-component reaction in the presence of silica coated zerconia nano catalyst (ZrO2/SBA-15). SBA-15 mesoporous silica was prepared according to literature described elsewhere [32-31]. ZrO2/SBA-15 catalyst was prepared by wet impregnation method with zirconium acetyl acetonate as zirconia precursor and SBA-15 [38]

II. EXPERIMENTAL

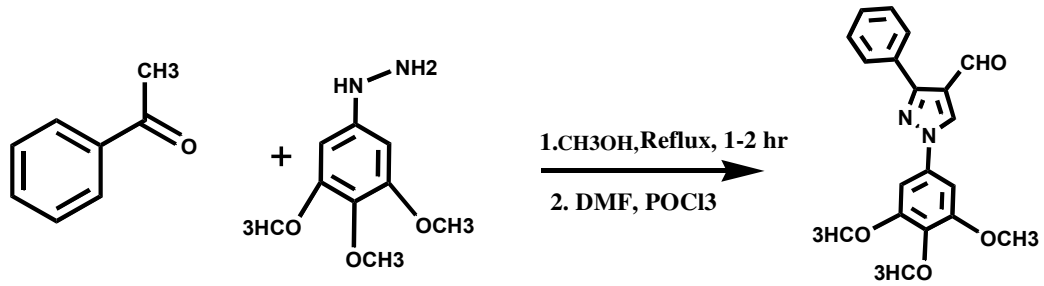
Materials and methods

All the chemicals were used in sigma Aldrich aldehedes and indoles purified by distillation prior. 1HNMR and 13C NMR spectra were recorded on Bruker 300 MHz spectrometer, and 75 MHz, using TMS as an internal standard (chemical shifts in d). Peak multiplicitiesof NMR signals were designated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet) etc. The HRMS spectra were recorded as ESI-HRMS on a Q-TOF LC-MS/MS mass spectrometer. All experiments were monitored by thin layer chromatography (TLC) performed on pre-coated silica gel plates. After elution, plate was visualized under UV illumination at 254nm for UV active materials. Further visualization was achieved by staining with KMnO4 and charring on a hot plate. Column chromatography was performed on silica gel (100-200 mesh) by standard techniques.

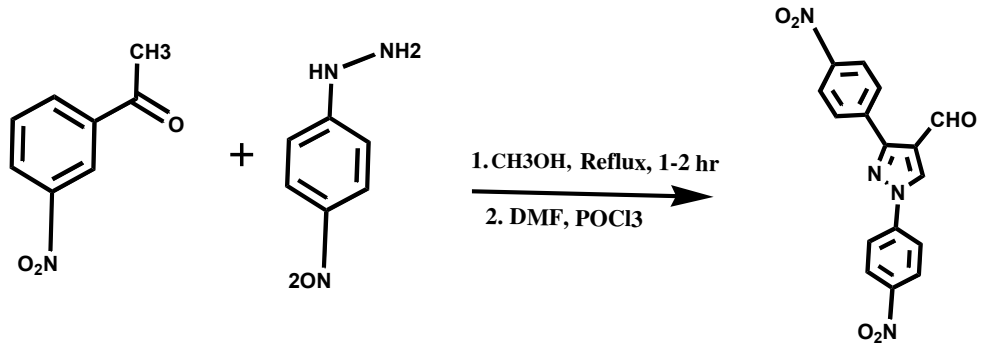
Scheme-1&2 Synthesis of 3-phenyl-1-(3,4,5-trimethoxyphenyl)-1H-pyrazole-4-carbaldehyde and 1,3-bis(4-nitrophenyl)-1H-pyrazole-4-carbaldehyde[19]

Substituted phenyl hadrazones were prepared by heating substituted acetophenone with different hydrazines in methanol under for 1-2hrs. phosphorous oxychloride (0.02 mol) was added drop wise to a mixture of dmf (0.1mol) and phenyl hydrazone (0.01mol) under cold condition.

After the addition, the reaction mixture was stirred at 60-70^{0}C for 1-2 hrs. Solution was cooled and poured into ice cubes and neutralize the solid obtained under the suction and recrystalized from methanol.



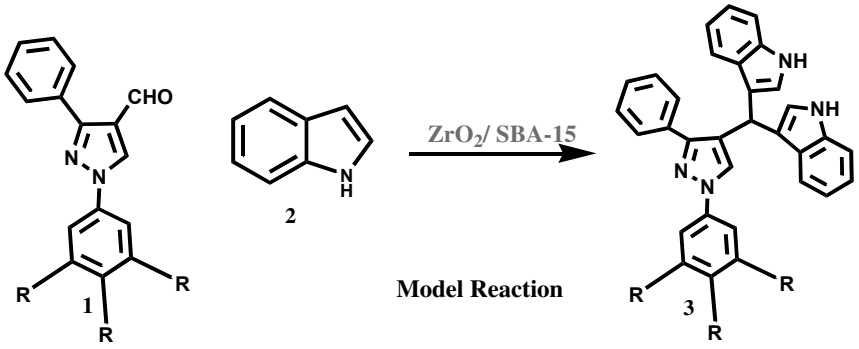
Scheme -1



Scheme -2

Scheme- 3 General experimental procedure for synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole

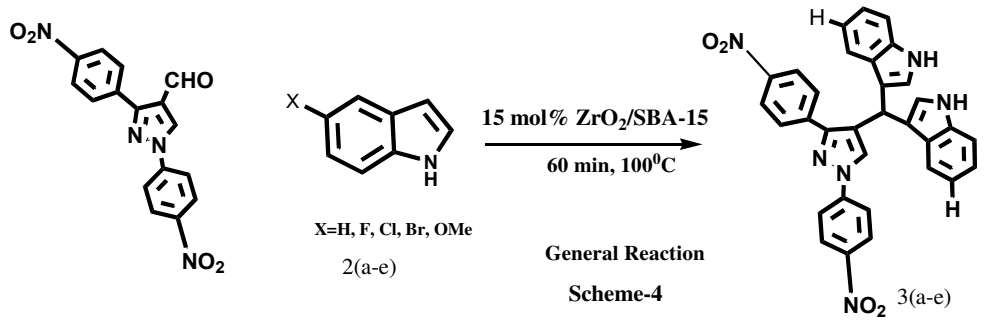
The one pot synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole And derivatives carried out in 100ml round bottomed flask taking 1,3-diphenyl-1H-pyrazole-4-carbaldehyde(1 mmol), indole (2mmol), 15mol% ZrO2/SBA-15 and water as solvent the reaction was stirred for 1hr at 100^{0}C. the progress of the reaction was monitored by TLC. After the completion of the reaction, the catalyst was separated and mixture was cooled, filtered. The obtained solid product is washed with ethyl acetate the desired 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole were obtained with high yields. The identify and purity of the products were confirmed by H NMR, C NMR and mass spectra recorded on a perkin elmer spectra-880 spectrophotometer by using KBr pellets in the Region 400-4500 recorded on a (Perkin Elmer Spectra-880) spectrophotomet cm−1 and 1H NMR spectra was characterized by 400 MHz-(Bruker Avance) in CDCl3 solvent and MASS spectra was recorded at 70 eV (MASPEC low resolution mass spectrometer).



R= NO2, OCH3

From this model reaction procedure new methodology for one pot synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole taking into consideration avoiding Solvents, long reaction time the usage of a new and efficient catalyst with high catalytic activity and easy work up reo the synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole and their derivatives.

Scheme – 4&5 Synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole and their derivatives



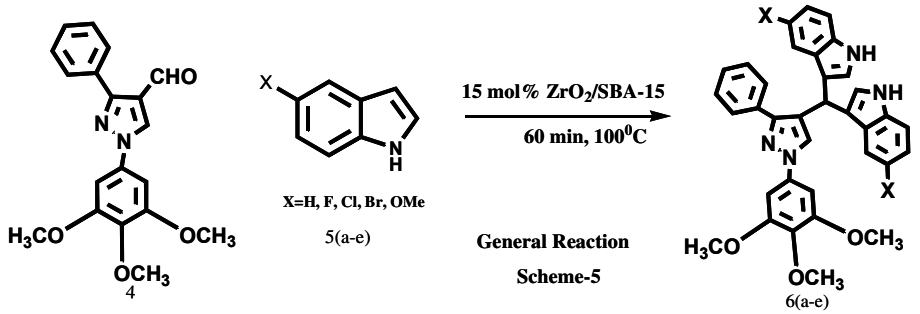


Table:1 Optimised conditions of synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole

Entry	Solvent	Tempertature (^{0}C)	Catalyst (mol %)	Time (min)	Yield %
1	--	--	--	120	--
2	Water	RT	--	120	---
3	Methanol	RT	--	120	--
4	DMSO	RT	--	120	--
5	CH3CN	RT	--	120	--
6	Water	50	5	60	<10
7	Methanol	50	5	60	<12
8	DMSO	50	5	60	--
9	CH3CN	50	5	60	<05
10	Water	90	10	40	42
11	Methanol	90	10	40	<10

12	DMSO	90	10	40	<15
13	CH3CN	90	10	40	<20
14	Water	100	15	60	98
15	Water	100	20	60	98
16	Water	100	25	60	98

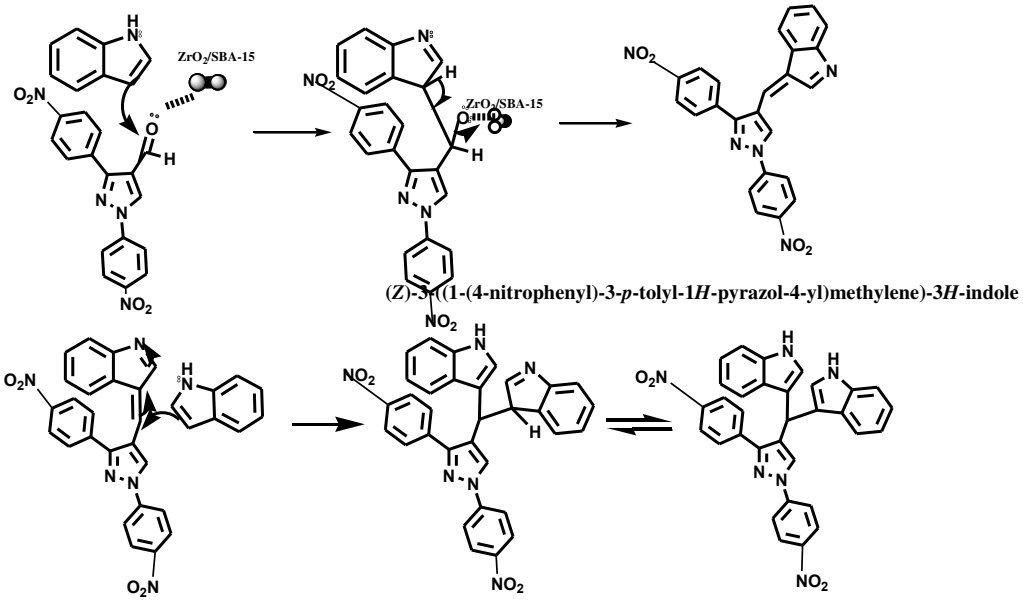
Table- 2 Table 2: Synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole and their derivatives

Code	Aldehydes (R=)	Indoles (X=)	Time (min)	Yield (%)
3a	NO_2	H	60	98
3b	NO_2	F	45	86
3c	NO_2	Cl	60	91
3d	NO_2	Br	55	93
3e	NO_2	OMe	60	98
6a	OCH_3	H	60	87
6b	OCH_3	F	55	79
6c	OCH_3	Cl	45	83
6d	OCH_3	Br	40	84
6e	OCH_3	OMe	50	87

III. RESULT AND DISCUSSION

in present work involves the multi components in one reactor to synthesize 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole and their derivatives the reaction between 1,3-diphenyl-1H-pyrazole-4-carbaldehyde(1) and indole (2) is taken as model substrates in presence of reusable nano ZrO_2/SBA-15 catalyst is shown in scheme 3. In this investigation the effects of catalytic amount, temperature changes and solvents on the yield of model reaction were shown in table.1. Obviously it was clearly observed that no yield of product was obtained without solvent and catalyst at room temperature even after 2hrs(table .1 entry .1) we have investigated the same reaction by using various solvents without catalyst at room temperature even after 2 hrs no new spots observes on TLC plate (table.1 entry 2-5). When added 5 mol % of ZrO_2/SBA-15 catalyst in water and methanol at 50 ^{0} C after 1hr trace amount of yields observed (table.1 entry 6&7). No products observed with the same catalyst loading and temperature in solvents of DMSO and CH_3CN (table. 1 entry 8&9). The reaction proceeds with 10 mol % of ZrO_2/SBA-15 at 100^0 C greater yield was observed in the solvent of water (table.1 entry .10) water than other solvents low yield observed (table.1entries 11-13). Greater percentage of yield observed with 15 mol % of catalyst at 100^0 C in water (table. 1 entry 14). No increase of yield observed in increasing of ZrO_2/SBA-15(table .1 entries 15 & 16).

Successfully optimize the model reaction condition follows the synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole in presence of 15 mol% of ZrO_2/SBA-15 at 100^0 C in water. After finding the reaction condition the model reaction is performed with various aldehydes and indoles were observed yields are summarized in table. 2. Greater amount of yields obtained from nitro substituted aldehydes than methoxy substituted aldehydes (3a-3e) and also observed better yields in without electron releasing grouped indoles (3a, 6a, 3e&6e). These synthesized 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole derivatives were analyzed through proton nuclear magnetic resonance, carbon nuclear magnetic resonance and mass spectral analysis. The plausible mechanism for the synthesized 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indoleby using reusable ZrO_2/SBA-15 is shown in figure .1. the reaction proceeds by the formation of highly reactive , not isolated Z)-3-((1, 3-diphenyl-1H-pyrazol-4-yl) methylene)-3H-indole.



Plausible Mechanism

We need to examine the reusability of the ZrO_2/SBA-15 catalyst by the synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole. From this investigation the catalyst can be re used upto 5 cycles (table.3) the catalyst was seperated by simple filtration after the reaction, washed with pure double distilled water followed by ethylacetate, dried at 100^0 and reused for the next cycle.

Table -3 Reusability of nanocatalyst

Reaction cycle	1	2	3	4	5
Yield (%)	98	96	95	92	90

Spectral analysis data

3a. 3-(1,3-bis (4-nitrophenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-H-indole

Pale red solid. M.P: 175- 177 ^{0}C; HNMR (300MH$_z$, DMSO-d$_6$) : δ 5.95(1H, s), 6.86- 6.91(2H,m), 7.05-7.09(2H,t, J= 7.742), 7.20-7.43(10H, m) , 7.67-7.75(5H,m) 7.83(1H,s),10.4(2H,s).^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 30.0; 111.3; 117.9; 118.1; 118.1; 120.8; 123.4; 125.4; 127.5; 128.1; 129.1; 129.1; 133.3; 133.3; 136.7; 139.5; 150.0.HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{32}H_{22}N_6O_4$: 555.55; found: 555.98

3b. 3-(1,3-bis (4-nitrophenyl)-1h-pyrazol-4-yl)(5-fluoro 1H-indol-3-yl)methyl)-5-fluoro-1H-indole

Pale yellow Solid . M.P: 267- 269 ^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : δ 5.87(1H,s) , 6.90(2H,d,J=1.88),7.057.08(1H,dd,J$_1$=1.88&J$_2$=6.798),7.247.46(9H,m),7.647.74(5H,m),10.49(2H,s) ; ^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 27.6; 110.2; 115.3; 115.8; 118.8; 121.3; 122.2; 122.7; 123.4; 124.8; 125.1; 125.8; 126.7; 130.7; 132.9; 137.2; 147.9.HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{32}H_{20}F_2N_6O_4$: 591.54; found: 591.89

3c. 3-(1,3-bis (4-nitrophenyl)-1h-pyrazol-4-yl)(5-chloro1H-indol-3-yl)methyl)-5-chloro -1H-indole

Pale red Solid . M.P: 203- 206^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : δ 5.87(1H,s) , 6.85(2H,d,J= 1.88), 7.20-7.48(11H,m), 7.68-7.75(4H, m), 1.015(2H,s); ^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 29.0; 110.6; 112.0; 116.8; 117.4; 120.4; 123.0; 123.6; 126.6; 127.0; 127.3; 128.1; 132.2; 134.6; 138.7; 149.4.HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{32}H_{20}Cl_2N_6O_4$: 624.45; found: 624.83

3d. 3-(1,3-bis (4-nitrophenyl)-1h-pyrazol-4-yl)(5-bromo 1H-indol-3-yl)methyl)-5-bromo -1H-indole

Pale pink Solid . M.P: 254- 258 ^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : δ 5.85(1H,s) 6.83-6.91(4H,m) 7.23-7.45 (7H,m), 7.54(1H,d,J=3.021), 7.66-7.75(5H,m) 1.15(2H, broad singlet) ; ^{13}C NMR (75MH$_z$, DMSO-d$_6$) : 31.2; 104.6; 104.9; 110.2; 110.5; 112.9; 113.0; 119.3; 125.7; 126.7; 127.4; 127.6; 128.5; 129.2; 130.0; 134.2; 134.4; 140.7; 151.4; 156.3; 159.4.HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{32}H_{20}Br_2N_6O_4$: 713.35; found: 713.76

3e. 3-(1,3-bis (4-nitrophenyl)-1h-pyrazol-4-yl)(5-methoxy 1H-indol-3-yl)methyl)-5-methoxy -1H-indole

Pale pink Solid . M.P: 194- 198 ^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : δ 2.48(6H,s-OCH$_3$), 4.72(1H,s) 5.56-5.64(4H,m), 5.91(2H, d, J=2.077), 6.18(3H,t,J= 7.931) 6.29-6.40(5H,m), 6.61(2H, dd, J$_1$= 1.700,J$_2$= 6.421) 6.75(2H, d, J= 7.742) ,7.11(1H,s), 9.62(2H,s).; ^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 30.0; 55.5; 101.1; 111.0; 112.5; 118.; 118.4; 124.7; 126.0; 126.3; 127.0; 128.1; 128.3; 128.8; 129.8; 132.2; 133.7; 139.9; 150.5; 153.1.HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{34}H_{26}N_6O_6$ 615.61; found: 615.92

6a. 3-((1H-indol-3yl)(3-phenyl-1-(3,4,5-trimethoxyphenyl)-1H-pyrazol-4-yi)methyl)-1Hindole
White solid. M.P: 236-238^{0}C. ^{1}H NMR (300MH$_z$, DMSO-d$_6$): δ 2.60(6H,t,J= 1.70), 3.75(3H,s), 5.96(1H,s), 6.92-696(8H,m), 7.072-7.11(3H,t,J= 70176), 7.36-7.45(6H,m), 10.54(2H,s).; ^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 30.5; 55.4; 60.3; 111.8; 105.0; 118.3; 118.6; 119.1; 121.3; 124.1; 125.5; 126.1; 126.5; 128.2; 129.0; 129.6; 137.2; 137.5; 139.8; 150.0; 153.0; HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{35}H_{28}N_4O_3F_2$: 555.64; found: 555.55

6b. 3-fluoro-3-((5-fluoro-1H-indole-3-yl)(3-phenyl-1-(3,4,5-trimethoxyphenyl)-1H-pyrazol-4-yl) methyl)-1H-indole
Pale pink Solid. M.P: 286 - 288^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : δ 3.44(6H,s), 3.83(3H,s) 5.87(1H,s), 6.91-6.96(4H,m), 7.08-7.11(2H,dd,J$_1$=1.88,J$_2$=6.60) 7.23-7.47(7H,m) 7.57(1H,s), 7.70(2H,d,J=7.742), 10.1892H,s).; ^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 28.6; 53.6; 55.5; 58.8; 103.1; 111.2; 116.2; 116.7; 119.8; 122.3; 122.9; 123.9; 124.3; 125.7; 126.1; 127.1; 127.6; 133.9; 135.7; 138.1; 148.4; 151.2; HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{35}H_{28}F_2N_4O_3$: 591.54; found: 591.62

6c. 5-chloro-3-((5-chloro-1H-indole-3-yl)(3-phenyl-1-(3,4,5-trimethoxyphenyl)-1H-pyrazol-4-yl) methyl)-1H-indole
White Solid. M.P: 216- 288^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : δ 3.45(6H,s), 3.82(3H,s), 5.86(1H,s), 6.93(4H,m), 7.19-7.35(5H,m), 7.41-747(4H,s). 7.58(1H,s), 7.70(2H,d,J=7.742), 10.46(2H,s).; ^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 29.3; 54.4; 59.5; 103.9; 110.7; 112.6; 116.8; 117.5; 120.2; 123.1; 123.7; 124.6; 125.1; 126.9; 127.2; 127.9; 128.4; 134.9; 136.5; 138.8; 149.1; 152.0; HRMS (ESI) *m/z*: calc. for [M+H$^+$] $C_{35}H_{28}N_4O_3Cl_2$: 624.53; found: 624.62.

6d. 5-bromo-3-((5-bromo -1H-indole-3-yl)(3-phenyl-1-(3,4,5-trimethoxyphenyl)-1H-pyrazol-4-yl) methyl)-1H-indole
Pale pink Solid. M.P: 154- 156^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : 53.43(6H,s), 3.81(3H,s), 5.84(1H,s), 6.84-7.01(8H,m), 7.22-7.45(5H,m) 7.54(1H,s), 7.61(1H,s) 7.69(2H,d,J=7.742) 10.20(2H,s).;^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ30.2; 54.9; 60.1; 103.2; 103.5; 104.3; 109.0; 109.4; 111.8; 117.9; 124.1; 125.4; 127.4; 128.4; 128.8; 133.3; 136.9; 139.4; 149.8; 152.5; 155.1; 158.2. HRMS (ESI) *m/z*: calc. for [M+H$^+$] . $C_{35}H_{28}N_4O_3Br_2$: 713.43; found: 713.53.

6e.5-methoxy-3-((5-methoxy-1H-indole-3-yl)(3-phenyl-1(3,4,5trimethoxyphenyl)1Hpyrazol-4-yl) methyl)-1H-indole
WhiteSolid. M.P: 204- 208 ^{0}C; ^{1}H NMR (300MH$_z$, DMSO-d$_6$) : 3.42(6H,s), 3.76(6H,s), 3.80(3H,s)5.85(1H,s), 6.77-6.90(6H,m), 7.01(2H,s), 7.22-7.34(3H,m), 7.42(2H,t,J=7.554) 7.60-7.72(3H,m), 10.01(2H,s).;^{13}C NMR (75MH$_z$, DMSO-d$_6$) : δ 284; 53.1; 53.6; 58.4; 99.4; 102.6;108.9; 109.9; 115.9; 116.2; 122.5; 122.9; 123.7; 124.7; 125.8; 126.9; 127.0; 130.2; 135.1; 137.7; 148.1; 150.7; 151.0; HRMS (ESI) *m/z*: calc. for [M+H$^+$]. $C_{37}H_{34}N_4O_5$: 615.69; found: 615.86.

IV. SUMMARY & CONCLUSION

In this present study, we concluded that the synthesis of 3-((1,3-bis(phenyl)-1H-pyrazol-4-yl)(1H-indol-3-yl)methyl)-1H-indole and their derivatives by using easily separable and reusable ZrO_2/SBA-15 nano catalyst. This method offers so many advantages including simplicity of operation, low cost, cleaner and short time reaction and being good to excellent yields.

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Bhavani Atyam*1" Multi Component One Pot Synthesis of 3-((1, 3-Bis (Phenyl)-1H-Pyrazol-4-Yl) (1H-Indol-3-Yl) Methyl)-1H-Indole and Their Derivatives by Using Zro2/SBA-15 Nano Catalyst." IOSR Journal of Engineering (IOSRJEN), vol. 08, no. 8, 2018, pp. 01-07.

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Synthesis of 3, 3-Di Indolin-2-One's in Presence of Zro_2/Sba-15 as an Efficient, Reusable Nano Catalyst

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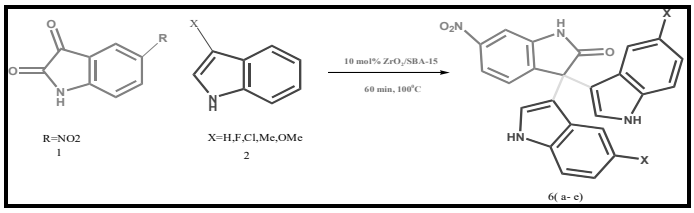
ABSTRACT

A simple and an efficient, multi component, one pot synthesis has been developed for the synthesis of 3,3-di indolin-2-one's and their derivatives by using reusable, easily separable ZrO_2/SBA-15 nano catalyst. The reaction, with these catalyst was carried out under mild and eco friendly conditions, with good yields of 3,3-di indolin-2-one's. The synthesized 3,3-di indolin-2-one's and their derivatives are characterized by ^1H NMR, ^{13}C NMR and Mass spectroscopy.

Graphical Abstract



Model reaction

Keywords: 3,3-di indolin-2-one's, ZrO_2/SBA-15 as an efficient catalyst.

INTRODUCTION

Multicomponent reaction plays a major role in organic synthesis. The main advantages of MCRs shorten the reaction time, minimize the byproducts, the most of the multi component reactions proceeds through convergent reaction way in which three or more starting materials in single reactant to form without any intermediates [1, 2]. And also these multi component reactions have broad range applications in heterocyclic compound synthesis and combinational synthesis [3-6]. Isatine is the major molecule in the designing of bio active agents they are anti fungal [7], anti HIV [8], anti tumor [9], anti viral [10] and anti convulsants [11]. Indoles and their derivatives found in nature they exhibit physiochemical properties [12-14]. In certain, 3,3-diaryloxindole is normally found in biologically

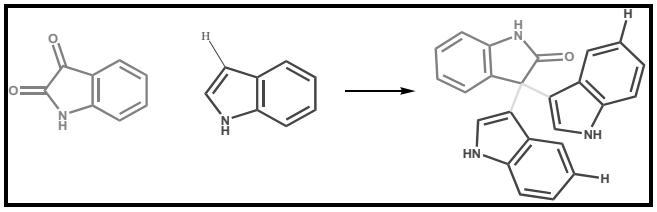
active compounds and also clinical drugs. Predominantly, bis indole methanes segregate from natural sources like vibrindole. 3,3-di (indolyl) indolin-2-one also called as bis indoles are prepared by coupling of isatin with indoles under acidic conditions [15-16]. B. V Reddy et al synthesize the bis indoles by using iodine catalyst [17]. Although, some methodologies' might have been described synthesis of 3, 3-di (indolyl) indolin-2-ones [18-23]. Accordingly the development of elementary and an efficient method reported for the synthesis of [3, 3':3', 3"-terindolin]-2'-one derivatives.

Herein, in order to achieve a more efficient synthetic process, minimize by-products, decrease the number of separate reaction steps, improving the yields and reaction times and also in extending our research on the application of nanocatalyst in MCRs, in this we report a clean and environmentally friendly approach to the synthesis of [3, 3':3', 3"-terindolin]-2'-one derivatives, via multi-component reaction in the presence of silica coated zerconia nano catalyst (ZrO2/SBA-15). SBA-15 mesoporous silica was prepared according to literature described elsewhere [24-29]. ZrO2/SBA-15 catalyst was prepared by wet impregnation method with zirconium acetyl acetonate as zirconia precursor and SBA-15 [30].

MATERIALS AND METHODS

Entire reagents and chemicals were adequate from Sigma- Aldrich St. Louis, MO, USA), Lancaster (Alfa Aeser, Johnson Matthey Company, Ward Hill, MA, USA), or Spectrochem Pvt. Ltd. (Mumbai, India) and were used an absence of any further purification tests. Progress of each reaction was examined by TLC on silica gel glass plate containing GF-254, and visualization was accomplished with UV lamp of 12 stains. All the products were characterized by their NMR and Mass spectra. ^{1}H NMR and ^{13}C NMR were recorded on 300 MHz and 75 MHz, in CDCl3/DMSO, and the chemical shifts were reported in parts per million (ppm, δ) downfield from the Tetramethyl silane (TMS)

General Experimental Procedure For Synthesis 3,3-Di Indolin-2-One's: In this reaction isatin (1 mmol), indole (2 mmol), 10 mol% ZrO_2/SBA-15 and water as solvent were taken in a 100 ml round bottom flask. The reaction mixture was agitated for 2 h, at 100°C. The progress of the reaction was observed by TLC. After the accomplishment of the reaction the catalyst was isolated by using simple filtration and cooled the reaction mixture was filtered and the obtained solid product is cleanse with ethyl acetate before drying over Na_2SO_4. After transferring the solvent in vacuous, the rest of the part was purified by silica-gel chromatography to give the requisite 3,3-di indolin-2-one's in excellent yields. The identity and purity of the products were confirmed by 1H, 13C NMR, and mass spectra.



Scheme 1 General Reaction.

RESULTS AND DISCUSSION

Synthesis of 3,3-di indolin-2-one's, we began our investigations by screening the reaction of isatine and indole were taken in different solvents (Ethanol, CH_3CN, DMSO, Water) in absence of ZrO_2/ SBA-15 catalyst and results were captured in table.1. It was clearly observed that low yield of products were obtained with ethanol, CH_3CN, DMSO, (Table1, entry 1-5) respectively even after 4 h stirring. From table 1, it is evident that low product yields. There was a slight increase in yield (Table 1, entry 6-10), when the reaction mixture was added with 5 mol% ZrO_2/SBA-15 catalyst even on stirring for just 2 h at 50°C. It was observed that yield (46%) obtained was much better in water as

solvent at the same reaction conditions. On increasing the catalyst to 10 mol%, the yield increased up to 97% respectively at 100°C. (Table 1 entry 10). All the results were summarized in table 1. No change was observed on further enhancing the catalyst mol% or time of stirring. The model reaction may be summarized as follows 3,3-di indolin-2-one'ssynthesized by reacting isatine and indole using 10 mol% ZrO_2/SBA-15 catalyst in water and obtained 97% yields at 100°C. Continuing the success, different isatines and were tested in our attempt to synthesis 3,3-di indolin-2-one's derivatives at the same reaction conditions and the results are summarized in table 2. From the results as is evident from table 2, we can conclude that the indols with strong electron releasing groups such gave excellent yields. (Table 2) 3d, 3e, 6d and 6e are formed in 94-97% yields. Indoles with weak electron releasing groups also gave good yield, but less than former case (Table 2) 3a, 3b, 3c, 6a, 6b and 6c. The structures of synthesized 3,3-di indolin-2-one's are confirmed by ^{1}HNMR, ^{13}CNMR and Mass spectra analysis.

Table 1. Optimization of synthesis of 3,3-di indolin-2-one'sin presence of ZrO_2/SBA-15 as a catalyst in different solvents

S.No	Solvent	Catalyst (mol%)	Time (h)	Temp. (°C)	Yield (%)
1	-	-	4	RT	-
2	Ethanol	-	4	RT	10
3	CH_3CN	-	4	RT	10
4	DMSO	-	4	RT	10
5	Water	-	4	RT	10
6	Ethanol	5	2	50	16
7	CH_3CN	5	2	50	21
8	DMSO	5	2	50	23
9	Water	5	2	50	46
10	Water	10	2	100	97
11	Water	15	2	100	97
12	Water	20	2	100	97

The plausible mechanism for the formation of 3,3-di indolin-2-one'sfrom 6- chloroindoline-2,3-dione and 1H-indole using ZrO_2/SBA-15 is shown in figure 1. The reaction may proceed through the formation of highly reactive (Z)-6-chloro 3-(3H-indol-3-ylidene) indolin-2-one. The efforts to isolate intermediates were not successful.

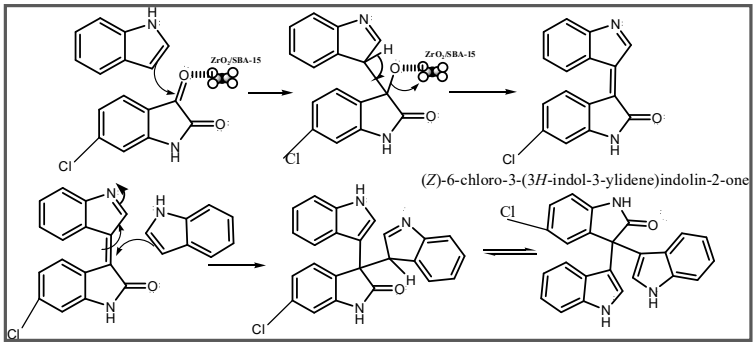
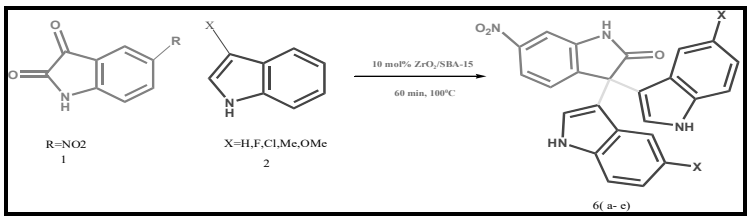
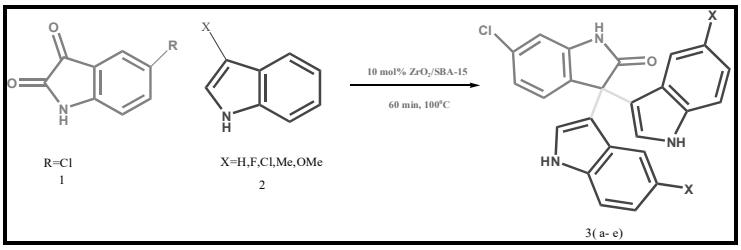


Figure 1. Plausible Mechanism



Scheme 2. Model reaction.



Scheme 3. Model reaction.

Reusability of the catalyst: The reusability of ZrO_2/SBA-15 is one of the most important advantages of this protocol that makes it useful for practical commercial applications. We have investigated the reusability of ZrO_2/SBA-15 catalyst for the model reaction. Interestingly, the retained catalyst could be reused for up to five cycles which is evident from table 3. The catalyst was isolated by using simple filtration after a completion of the reaction, cleansed with water followed by chloroform, dried in oven and reused for the next cycle.

Table 3. Productivity with re-cycle catalyst

Entry	Catalyst	Yield
1	1st cycle	94
2	2nd cycle	92
3	3rd cycle	90
4	4th cycle	87
5	5th cycle	85

Spectral analysis data of synthesized compounds

3a) 6-Chloro-3,3-di(1h-indol-3-yl)indolin-2-one: White Solid; Mp: 332-335°C: ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.06-7.32(7H,m), 7.40–7.47 (2H,m), 7.54–7.62(4H, q J= 8.120), 10.69(1H, s), 10.82 (2H,s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 52.5; 109.9; 111.8; 112.9; 113.9; 121.8; 122.9; 123.9; 124.8; 125.7; 127.2; 127.8; 133.6; 135.6; 140.6; 179.2. ESI-MS: m/z = 397.86 $(M+H)^+$.

3b) 6-chloro-3,3-bis(5-fluoro-H-indol-3-yl)indolin-2-one: White solid; Mp: 298-300°C: ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.9-7.02(2H, d t, J_1= 2.26, J_2 = 6.79) 7.11-7.22(5H,m), 7.38-7.49(4H,m), 7.80(1H,s), 10.39(1H,s), 10.62(2H,s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.7; 109.1; 111.9; 113.3; 119.2; 120.5; 120.9; 122.9; 124.0; 125.0; 125.5; 127.1; 133.0; 134.7; 140.3; 178.3. ESI-MS: m/z = 433.08 $(M+H)^+$.

3c) 6-chloro-3,3-bis(5-chloro-H-indol-3-yl)indolin-2-one: Light brown solid; Mp: >350°C: ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.13-7.24 (6H,m), 7.39-7.53(5H,m) 10.35(1H,s), 10.61(2H,s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.3; 104.1; 104.4; 108.0; 108.3; 108.7; 111.0; 111.1; 113.2; 120.4; 123.6; 124.7; 125.0; 126.7; 132.5; 132.9; 140.1; 153.8; 156.9; 177.8. ESI-MS: m/z = 466.75 $(M+H)^+$.

3d) 6-chloro-3,3-bis(5-methyl-H-indol-3-yl)indolin-2-one: Pale pink solid; Mp: 335-337°C: ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.23(3H,s) 2.37(3H,s) 6.85-6.91(2H,m) 7.01-7.20(5H,m) 7.37-7.47(4H,m), 10.18-1.24(3H,s,brs). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.2; 108.0; 108.8; 109.1; 116.5; 119.7; 118.2; 124.1; 125.8; 126.4; 130.6; 132.6; 133.6; 134.2; 139.7; 178.3. ESI-MS: m/z = 425.91 $(M+H)^+$.

3e) 6-chloro-3,3-bis(5-methoxy-H-indol-3-yl)indolin-2-one: Light yellow solid; Mp: >350°C: ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.79(6H,s), 6.91-7.01(4H,m), 7.11-7.21(4H,m) 7.37–7.53 (3H,m) 9.78(2H,s) 10.01(1H,s). ^{13}C NMR (75 MHz, DMSO-d_6): 52.1; 54.6; 102.6; 108.8; 110.0; 111.1; 113.1; 120.7; 124.2; 124.5; 125.5; 126.8; 131.5; 133.9; 140.5; 151.9; 178.7. ESI-MS: m/z = 457.91$(M+H)^+$.

6a) 3,3-di(1H-indo1-3-yl)-6-nitroindolin-2-one: Pale pink solid; Mp: 325-327°C: ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.8-7.08(7H,m), 7.17-7.40(6H,m), 10.46(1H,s), 10.58(2H,s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.5; 109.3; 110.0; 112.1; 116.8; 119.1; 119.4; 122.8; 123.2; 124.0; 124.4; 125.9; 134.9; 135.5; 138.5; 177.2. ESI-MS: m/z = 408.41 $(M+H)^+$.

6b) 3,3bis(5-floro-1H-indol-3-yl)6-nitroindolin-2-one: White color solid. ;Mp: >350°C:^{1}H NMR (300 MHz, DMSO-d_6): δ 6.79-6.86 (2H,dt J_1= 2.26, J_2 = 6.79)) 6.94-7.02(5H,m) 7.15-7.23(2H,m), 7.31-7.36(2H,q J= 8.120), 10.52(1H,s), 10.75 (1H,s).^{13}CNMR (75MHz,DMSOd_6): δ51.93, 124.87, 124.48,110.42,109.72,112.57,119.91,117.28,123.71,126.54,135.99,135.41,138.98,177.70. ESI-MS: m/z = 444.39 $(M+H)^+$.

6c) 3,3bis(5-chloro-1H-indol-3-yl)6-nitroindolin-2-one: Grey color solid; Mp: 250-252°C:^{1}H NMR (300 MHz, DMSO-d_6): δ 6.93(2H,d), 7.00-7.50(2H ,d, J=8.120), 7.13(1H,s), 7.21- 7.24(1H, d J= 2.26) 7.33-7.36(4H,d J= 6.79), 7.66(1H,s), 10.48(1H,s), 10.78(2H,s).^{13}C NMR (75 MHz, DMSO-d_6): δ 50.8; 103.6; 103.9; 107.5; 107.8; 108.2; 110.6; 112.7; 119.9; 123.1; 124.2; 124.5; 126.2; 132.0; 132.4; 139.6; 153.3; 156.4; 177.3. ESI-MS: m/z = 477.30 $(M+H)^+$.

6d) 3,3bis(5-mrthyl-1H-indol-3-yl)6-nitroindolin-2-one: Light yellow solid. ; Mp: 345-347°C:^{1}H NMR (300 MHz, DMSO-d_6): δ 1.99(3H,s), 2.215(3H,s), 6.46(1H,d, J= 8.120), 6.67 (2H,t, J= 7.36), 683- 6.94(4H,m), 7.12-7.25(4H,m), 9.96(2H), 10.09(1H,s).^{13}C NMR (75 MHz, DMSO-d_6): δ 50.5; 107.2; 108.0; 108.3; 115.7; 117.3; 119.0; 123.3; 125.1; 125.3; 125.7; 129.8; 131.9; 132.8; 133.5; 138.9; 177.6. ESI-MS: m/z = 436.46$(M+H)^+$.

6e) 3,3bis(5-methoxy-1H-indol-3-yl)6-nitroindolin-2-one: Pale pink solid; Mp: >350°C:^{1}H NMR (300 MHz, DMSO-d_6): δ 3.8(6H,s) 6.93-7.00(4H,m) 7.14-7.19(2H,d, J=8.876), 7.36-7.48(4H,m) 7.67 (1H,s), 10.02(2H,s), 10.29(1H,s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 54.9; 55.1; 100.3; 110.8; 111.5; 111.9; 113.6; 114.0; 118.6; 123.4; 123.8; 126.5; 128.8; 127.1; 131.7; 142.4; 147.8; 153.0; 158.5. ESI-MS: m/z = 468.46$(M+H)^+$.

APPLICATION

It has many advantages like less reaction time, minimize the byproducts and better yield of the products.

CONCLUSION

In this present investigation, we report a simple and eco friendly method for the synthesis of 3,3-di indolin-2-one's and their derivatives in presence of reusable ZrO_2/ SBA-15 nano catalyst. This method has many advantages like less reaction time, minimize the byproducts and better yield of the products.

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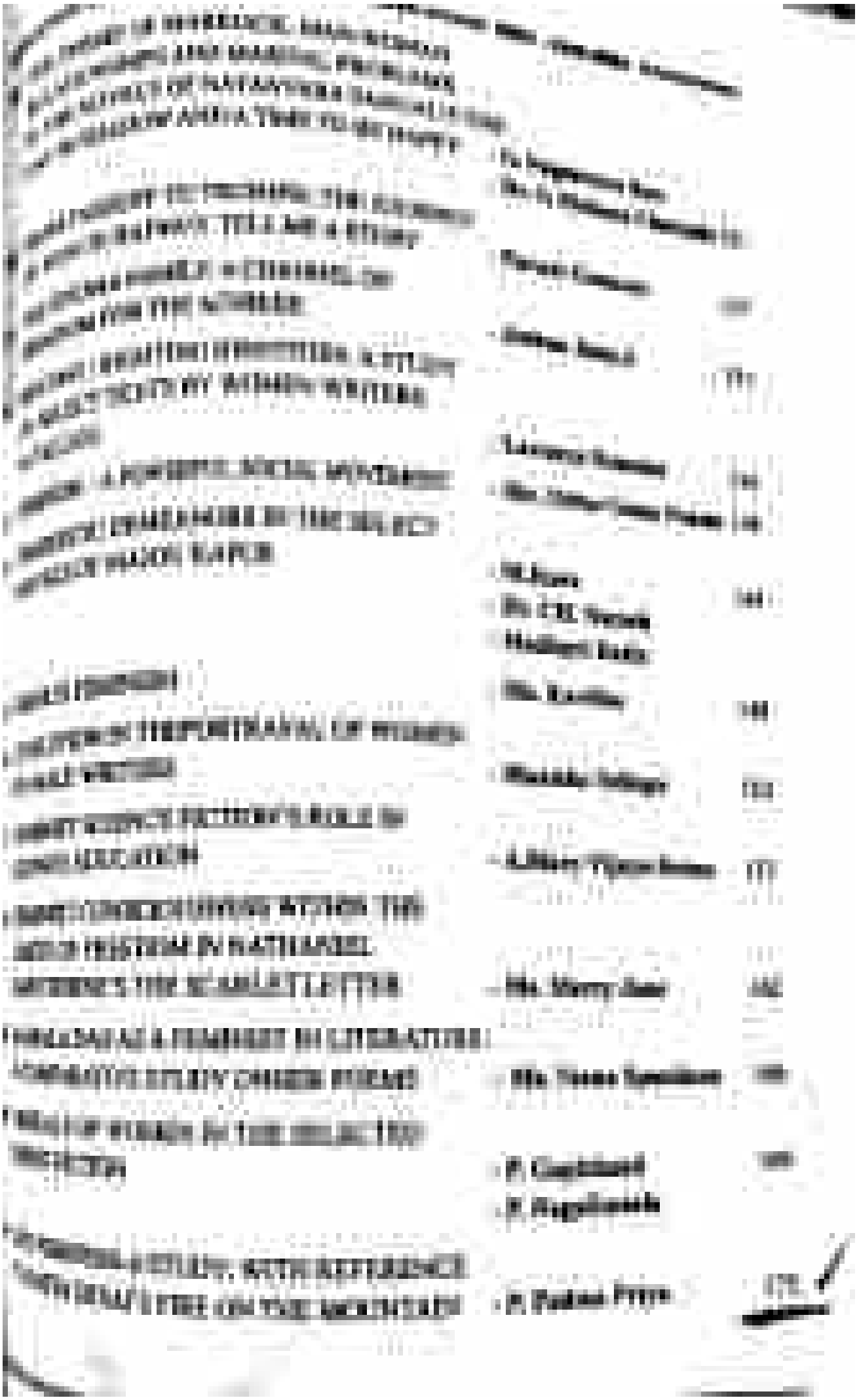

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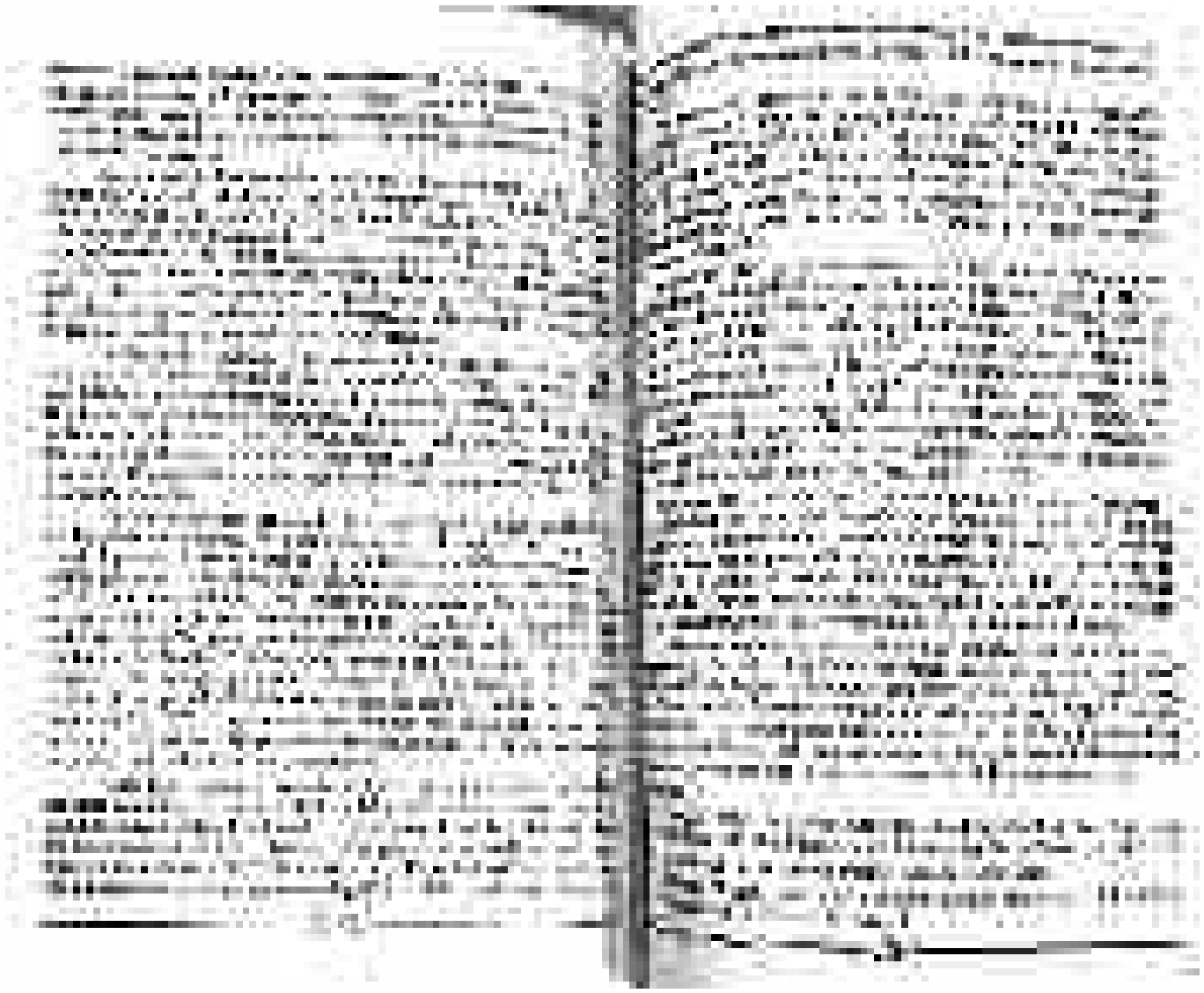

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SYNTHESIS, CHARACTERIZATION OF *SARGASSUM MUTICUM* AQUEOUS EXTRACT MEDIATED SILVER DOPED ZINC NANOPARTICLES FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Biological synthesis of nanoparticles is a relatively new emerging field of nanotechnology which has economic and eco-friendly benefits over chemical and physical processes of synthesis. In the present work, for the first time, the brown marine algae Sargassum muticum (S. muticum) aqueous extract was used as a reducing agent for the synthesis of nanostructure silver doped zinc nanoparticles (Ag-ZnNPs). The surface plasmon resonance (SPR) of the formed AgNPs was recorded at 453 nm using UV-Visible spectrophotometer. The molecules involved in the formation of Ag-ZnNPs were identified by Fourier transform infrared spectroscopy (FT-IR) confirms the presence of aldehydes, alkyls, alkenes, surface morphology was studied by using scanning electron microscopy (SEM), SEM micrograph clearly revealed the size of the Ag-ZnNPs was in the range of 2mm with spherical, hexagonal in shape and poly-dispersed nature while in EDX confirms the composition of nanoparticles present in *Sargassum muticum* aqueous extract, and X-ray diffraction spectroscopy (XRD) was used to determine the crystalline structure. High positive Zeta potential (36.3mV) and particle size was (102nm) of formed Ag-ZnNPs indicates the stability and XRD pattern revealed the crystal structure of the AgNPs by showing the Bragg's peaks corresponding to (111), (200), (220), (311) and (222) planes of face centered cubic crystal phase of silver doped zinc nanoparticles, The synthesized Ag-ZnNPs exhibited

effective antimicrobial activity at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0).

KEYWORDS: Sargassum Muticum; Silver Doped Zinc Nanoparticles; Antimicrobial Activity.

1. INTRODUCTION

Nano-biotechnology is an evolving field that has made its contribution to all domains of human life.[1, 2] Several physico-chemical approaches have been illustrated for the synthesis of metal nanoparticles.[3, 4, 5] A variety of green methods for synthesis of nanoparticles were needed because most of these means are inexpensive, non-toxic, eco-friendly and have easy production technology. The synthesis of new approaches for green nanoparticles from natural sources like plants[6], and algae.[7] For their easy availability, natural occurring, non-toxicity, the macroalgae have several potentials in the biosynthesis of silver nanoparticles such as eco-friendly, easy production technology and low costs[8] unexpensive, green easily amenable approach for biosynthesis of silver nanoparticles by reducing silver nitrate solution using marine macroalgae extract. Several macroalgae have been used for biosynthesis of Ag-NPs, particularly different *Sargassum spp.*[9] It appears to be exceptionally sensible to trust silver nanoparticles biosynthesis as environmentally and ecologically safe as well as to enhance their antibacterial properties. For metal nanoparticles, silver nanoparticles (Ag-NPs) have great efficacy as ideal antimicrobial agents. Because of their antiseptic properties, the silver nanoparticles are widely used in the health-care sector and industrial applications.[10, 11] The use of environmentally benign materials like plant extract[12], bacteria[13], fungi[14] and marine algae[15] for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Green synthesis are found to be superior over physical and chemical method as it is economically feasible, environmental friendly, scaled up for mass-scale production without any complexity.

Historically, seaweed is a readily available food source that has been consumed by coastal communities likely since the dawn of time. Seaweed is consumed habitually in many countries in South-East Asia. Marine algae refer to a wide variety of different species with different medicinal behaviour, which are divided into two groups, namely microalgae and macroalgae. Marine macroalgae or seaweed, are plant-like organisms classified according to their pigmentation into green (chlorophytes), red (rhodophytes) and brown (phaeophytes).

Seaweeds are well-known as functional food for their richness in lipids, minerals and certain vitamins, and also several bioactive substances like polysaccharides, proteins and polyphones, with potential medicinal uses against cancer. Green synthesis of nanoparticles is an emerging branch of nanotechnology.[16] Marine algae are well-known as functional food for their richness in lipids, minerals and certain vitamins, and also several bioactive substances like polysaccharides, proteins and polyphones, with potential medicinal uses against cancer, oxidative stress, inflammation, allergy, thrombosis, lipidemia, hypertensive and other degenerative diseases. Thus, their phyto-chemicals include hydroxyl, carboxyl, and amino functional groups, which can serve both as effective metal-reducing agents and as capping agents to provide a robust coating on the metal nanoparticles in a single step

The current work describes a green and rapid method using brown seaweed (*S. Muticum* belongs to *Sargassaceae* family) aqueous extract solution for the biosynthesis of silver doped zinc nanoparticles in ambient conditions. The current simple synthetic green method using rapid precursors of *S. muticum* aqueous extract provides high-yield nano-sized materials with good anti microbial properties.

2. Experimental Methods

2.1 Materials

Zinc nitrate ($ZnNO_3$), Silver nitrate ($AgNO_3$) were used as received without further purification and double distilled water (DDW) as solvent. *Sargassum muticum* was collected as mat from the sea shores of Nellore district, A.P., India.

2.2 Preparation of Sargassum muticum aqueous extract

Shade dried *Sargassum muticum* mat was ground to and 10g of fine algal powder and was mixed with 100 ml of distilled water (DW) and heated to 80^{o}C for 30min. Then the solution was filtered using filter paper (whatman no.1) and aliquot (10%) was collected for further experimentation and the rest was stored at -4^{o} C.

2.3 Preparation of Sargassum muticum extract mediated silver (Ag) doped Zinc (Zn) nanoparticles

To prepare the silver doped zinc nanoparticles 1M concentration solutions of each element ($AgNO_3$ & $ZnNO_3$) was prepared in double distilled water and mixed at 10 % volume to 90 ml of *Sargassum muticum* extract followed by $AgNO_3$ solution in a series of concentrations (0.1 % to 1.0 % in the 10 % proportion of $ZnNO_3$) and was thoroughly mixed. The mixture

was heated to 60^{o}C and cooled to room temperature, further incubated at room temperature for 24 hrs. The precipitated Ag-ZnNPs were separated from the mixture through ultra filtration and dried. The Ag-ZnNPs as powder were further stabilized by sintering at 500^{o}C for 1hr. The Ag-ZnNPs formed were stored for further characterization and bioactive assays.

2.4 Characterization of the prepared nanoscale AgZnNPs

2.4.1 UV - Visible spectrum for synthesized nanoparticles

The nanoparticles were monitored by UV-visible spectrum at various time intervals. The UV -Visible spectra of this solution was recorded in spectra 50 ANALYTIKJENA Spectrophotometer, from 200 to 600 nm.

2.4.2 Fourier transform infrared spectroscopy (FT-IR)

The Ag-ZnNPs were investigated by Fourier transform infrared (FT-IR) spectroscopy (Perkin–Elmer) in the frequency range of 400–4000 cm^{-1} by pelletizing a homogenized powder of the synthesized particles and KBr. The spectra were corrected by subtracting the spectrum of a KBr blank pellet and were presented in the transmittance mode.

2.4.3 X-ray diffraction (XRD)

The XRD pattern of freshly prepared sample was taken using a (Rigaku,) X-ray diffractometer operating in the reflection mode with CuKα radiation (k=0.1542nm). The scanning range was between 2h = 20–80 with a step size of 0.04 and a dwell time of 6s.

2.4.4 Dynamic light scattering spectroscopy (DLS for size and zeta potential measurements)

Dynamic light scattering (DLS) was performed using a particle sizing system, Horiba Nanopartica at a wavelength of 633nm from a 4.0mW, solid-state He-Ne laser at a scattering angle of 170°. Intensity average, volume average, and number average diameters were calculated from the autocorrelation function using Malvern's Zetasizer Nano 4.2 software utilizing a version of the CONTIN algorithm.

2.4.5 Scanning Electron Microscopy (SEM) and Energy Dispersion Spectroscopy (EDS)

Sample preparation for the SEM was as follows. The ribbon samples were hand grinded using a mortar and pestle to produce fresh fracture surfaces and were placed on CU-tape and loaded into the SEM. The powder samples were also placed on CU-tape but the excess powder was removed with an air duster before analysis.

2.5 Antimicrobial activity

The antibacterial activity of Ag-ZnNPs was evaluated against the following pathogenic strains *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescence*, *Bacillus subtilis*, these cultures were grown on appropriate medium at 37°C for overnight incubation and maintained at 4°C in a refrigerator. Disc diffusion method disc of 5 Mm was made for nutrient agar medium and each disc was dipped at different concentration (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0) efficiency of prepared Ag-ZnNPs. The pure cultures of bacterial pathogens were sub-cultured on an appropriate medium. After incubation at 37°C for 24h the zones of bacterial inhibition were measured. The assays were performed triplicate.

The antifungal activity of Ag-ZnNPs was evaluated against the following pathogenic strains fungal species *viz*., *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus oligosporus*, and *Fusarium oxysporium* and these cultures were grown on appropriate medium at 25-28°C for overnight incubation and maintained at 4°C in a refrigerator.[17] Disc diffusion method disc of 5 Mm was made on nutrient agar medium and each disc was dipped at different concentration (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0) efficiency of prepared Ag-ZnNPs. The pure cultures of fungal pathogens were sub-cultured on an appropriate medium. After incubation at 37°C for 48 h the zones of fungal inhibition were measured. The assays were performed triplicate.

3. RESULTS AND DISCUSSION

3.1 UV-Visible spectral analysis

UV–Vis spectrometer is spectral techniques are widely used to confirm the formation and structural characterization of nanoparticles in colloidal solution. The UV–Vis spec-troscopy was used for the confirmation of silver doped zinc ions reduction by aqueous extracts to form Ag-ZnNPs the colour of the composition has been changed colorless to pale brown colour. The maximum absorbance peak is observed at 453 nm for *S. Muticum* Fig. 1.

3.2 Fourier transform infrared spectrophotometry analysis

FT-IR spectrum of the biosynthesized silver nanoparticles using *S. muticum*. Fig. 2 shows the absorption peaks at 3408, 3400, 1624, 1361, 1151, 1122, 1087, 666, 613, and 599cm-1. The peak at 3408 and 3400cm-1 reveals the presence of N-H stretching vibration, indicating the primary and secondary amines, 1624cm-1 reveals the presence of N-H bend stretching vibration, indicating the presence of primary amines groups, 1361cm-1 reveals the presence

of N-O symmetric stretching vibration, indicating the presence of nitro compounds, 1151 and 1122cm-1 reveals the presence of C-H wag stretching vibration, indicating the presence of alkyl halides This is further confirmed with a reduction in PH of solution during the reaction. The sulfated polysaccharides peaks pointed out the involvement of sulfate groups in the biosynthesis of silver nanoparticles, 1087cm-1 reveals indicating the presence of C-N group that is characteristic of proteins shifted from after the synthesis of Ag-ZnNPs, 666, 613 and 599cm-1 reveals the C-Br stretching vibration of alkyl halides either may be poly phenols associated with synthesized silver doped zinc nanoparticles which is segregated by *S. muticum* extract.

3.3 X-ray diffraction analysis

The sample of Ag-ZnNPs could be also characterized by X-Ray Diffraction analysis of dry powder. The diffraction intensities were recorded from 10°-90° at 2θ angles. Four different and important characteristic peaks were observed at the 2θ of 22.8°, 37.2º, 38.6º, 40.2º, 41.9º, 43.5º, 44.7º, 63.3º, 63.6º, 64.8º, 69.1º, 75.7º, 77.7º, and 81.9° that correspond to (100), (002), (101), (111), (200), (102), (110), (103), (311), (112), (201), (004), (202) and (222) planes, respectively. All the peaks in XRD pattern can be readily indexed to a face centered cubic structure of silver doped zinc as per available literature (JCPDS, File No. 4-0783). The XRD pattern of these peaks indicates the Ag-ZnNPs is crystalline in nature and some of the unassigned peaks were observed it may be due to the fewer bio-molecules of stabilizing agents are enzymes or proteins in the *S. muticum* extract [16] Fig. 3.

3.4 Dynamic Light Scattering Analysis

Particle size and zeta potential values were measured using Nanopartica SZ-100. The particle size distribution spectra for the Ag doped zinc nanoparticles were recorded as diameter (nm) verses frequency (%/nm) spectra with diameter (nm) on x-axis and frequency (%/nm) on y-axis. The zeta potential spectra for the Ag doped zinc nanoparticles were recorded zeta potential verses intensity spectra with zeta potential (mV) on x-axis and intensity (a.u) on y-axis. Particle size of 102.0nm with zeta potential of 36.3mV was recorded for the Ag doped zinc nanoparticles synthesized from *S. Muticum aqueous* extract (Fig. 4a & 4b). The zeta potential indicates the degree of repulsion between adjacent and similarly charged particles in dispersion.

3.5 Scanning electron microscopy and EDX analysis

The surface morphology, size and shape of *S. muticum* silver doped zinc nanoparticles were characterized from the SEM micrograph, it is evident that AgNPs were spherical in shape and were polydispersed Fig. 5. The measured average size of AgNPs was 55 nm and the nanoparticles ranges from 40.4 nm to 57.6 nm occasional agglomeration of the AgNPs has been observed. The electrostatic interactions and hydrogen bond between the bioorganic capping molecules bond are responsible for the synthesis of silver nanoparticles using *S. muticum* extract.[16]

3.6 *Antimicrobial potential assay of formed Ag-ZnNPs tested bacterial and fungal species*

The antimicrobial activity of the synthesized Ag-ZnNPs was carried out using the method proposed by Kirby-Bauer.[18] Ag-ZnNPs obtained from *Sargassum muticum* have very strong inhibitory action against Gram-positive-negative bacteria and fungi Fig. 6 & 7. These isolates were collected from Acharya N G Ranga Agricultural University, Tirupathi. Ten concentrations of Ag-ZnNPs were prepared and were applied against an array of bacterial species *viz, S. aureus* (Gram positive), *E.coli, Bacillus* and *P. aeruginosa* (Gram negative). The higher concentration (1.0) of Ag-ZnNPs showed significant antimicrobial effect compared with other concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9). But when compared to *E. coli* and *S. aureus* (Gram positive) *P. aeruginosa* (Gram negative) and *Bacillus* (Gram positive) shown effective zone of inhibition in all concentrations. While coming to fungal species when compared to *Aspergillus niger* and *Aspergillus flavus, Fusarium* and *Rhizopus* shown very good inhibition activity at higher concentrations (0.6, 0.7, 0.8, 0.9 and 1.0). The inhibitory action of the microbes may be attributed to the loss of replication ability of DNA upon treatment with the silver and zinc ion, besides the fact that expression of ribosomal sub-unit proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated because it is due to the release of ROS (Reactive oxygen species). When ppm concentration decreases the diameter of zone of inhibition was also decreased. The results indicated that antimicrobial effect was done by dose dependent. The mechanism of inhibition of Ag-ZnNPs on microorganisms is not well known. AgZnNPs binds with cytoplasmic membrane and killed the bacterial and fungal cells. This is because the electrostatic interaction between positively charged Ag-ZnNPs and negatively charged cell membrane of microorganisms.[19] The observed results of the antimicrobial assay of Ag-ZnNPs are given in Table.1.

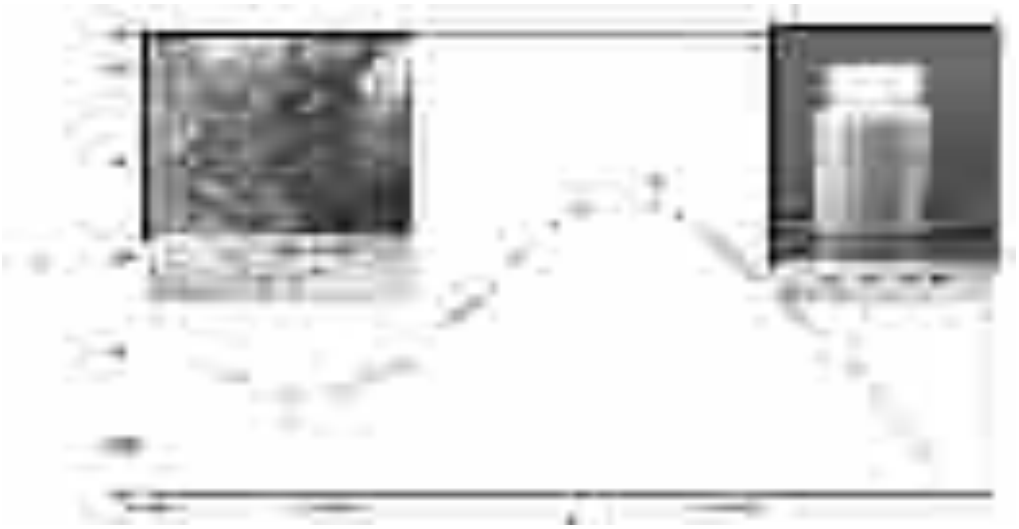


Fig. 1. UV/Visible absorption spectrum of synthesized silver doped zinc nanoparticles from *Sargassum muticum*.

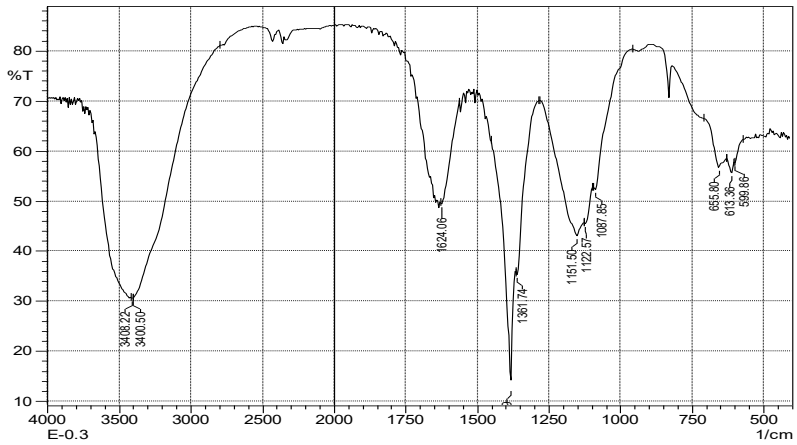


Fig. 2: FT-IR spectrum of synthesized silver doped zinc nanoparticles from *S. muticum*.

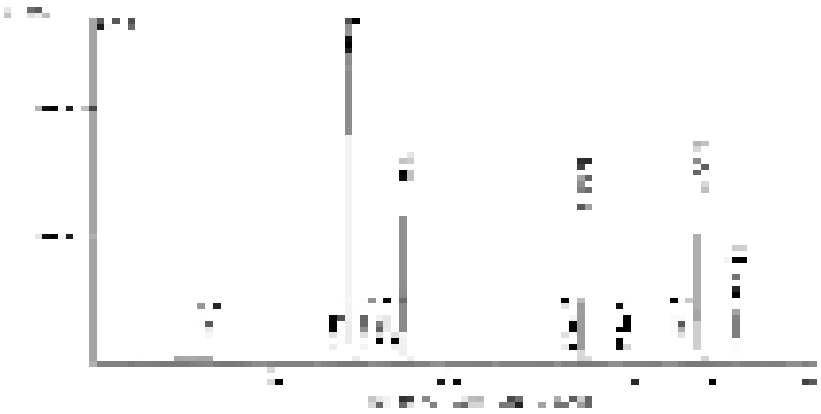


Fig. 3: XRD analysis of synthesized silver doped zinc nanoparticles from *S. muticum*.

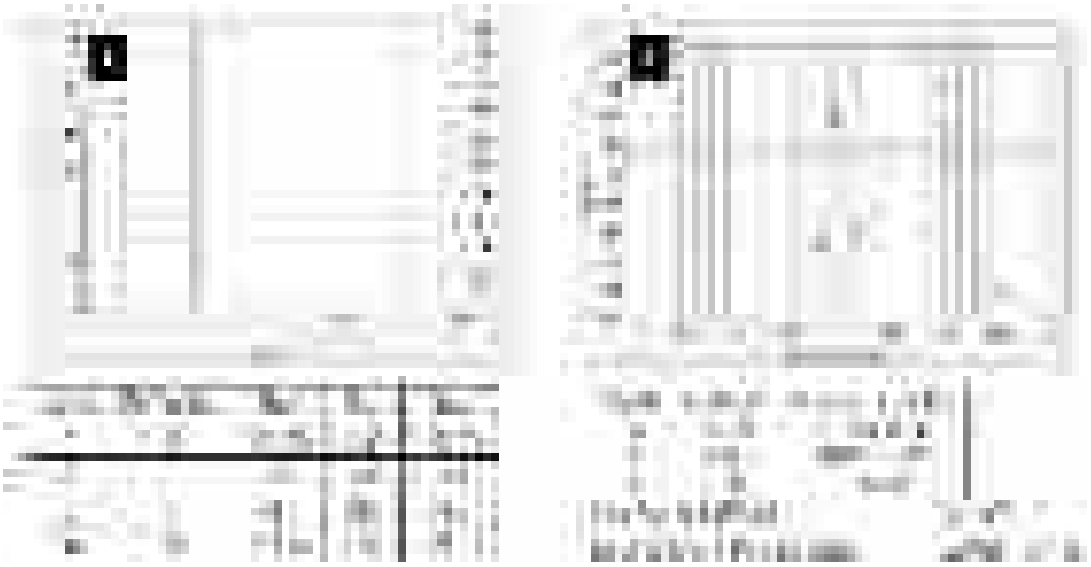


Fig. 4: DLS analysis of synthesized silver doped zinc nanoparticles from *S. Muticum* a) Particle size b) Zeta potential.

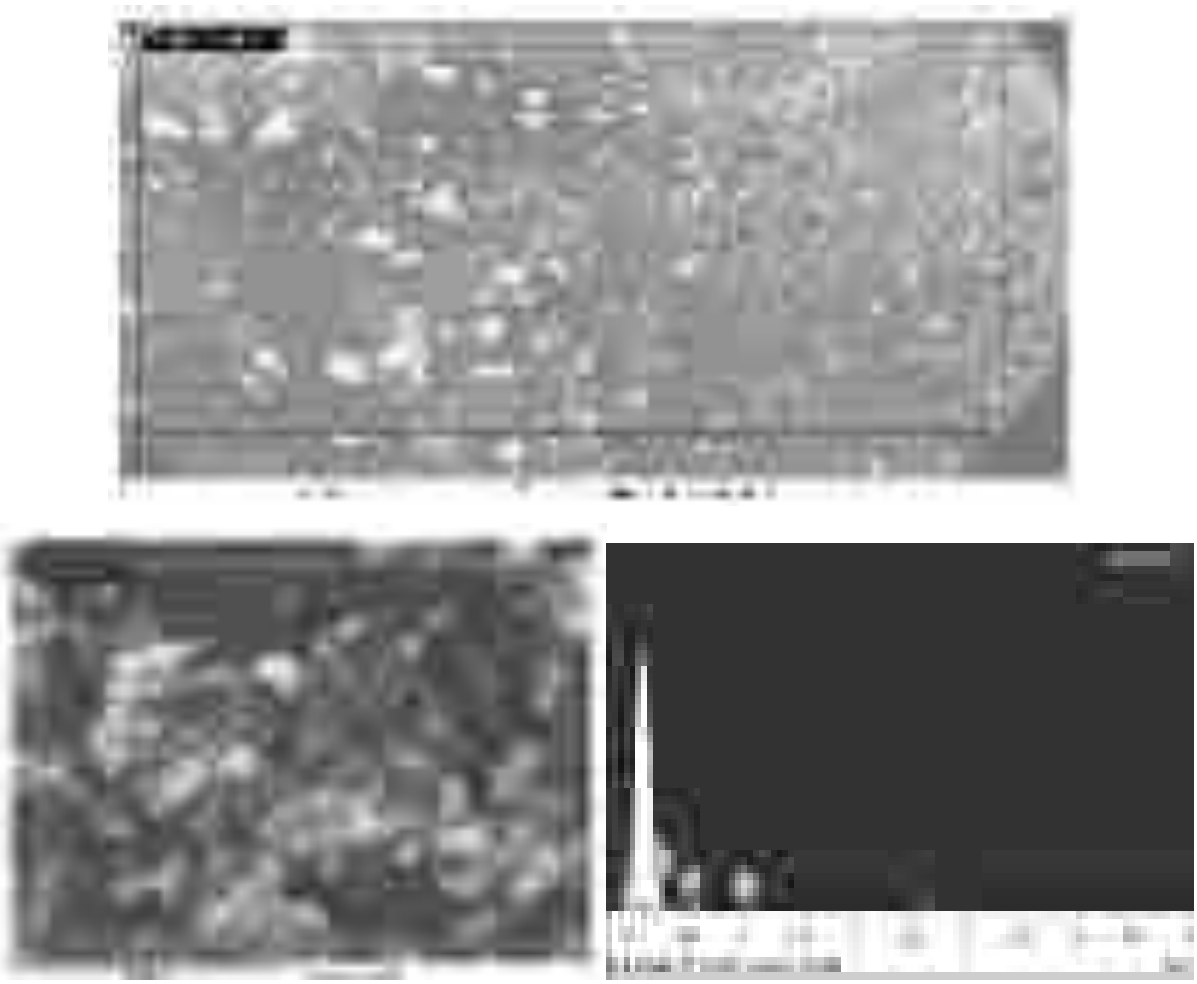
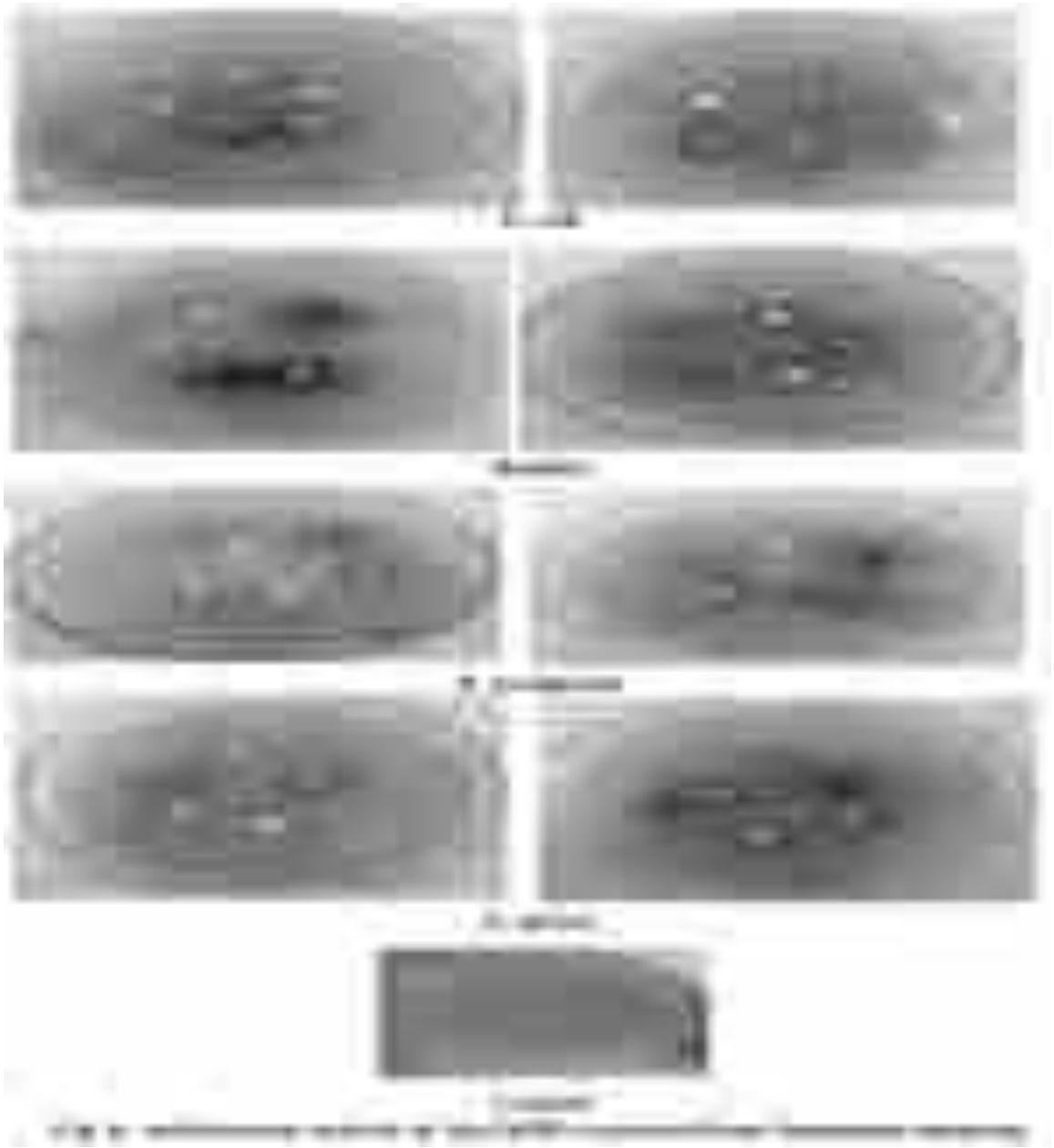
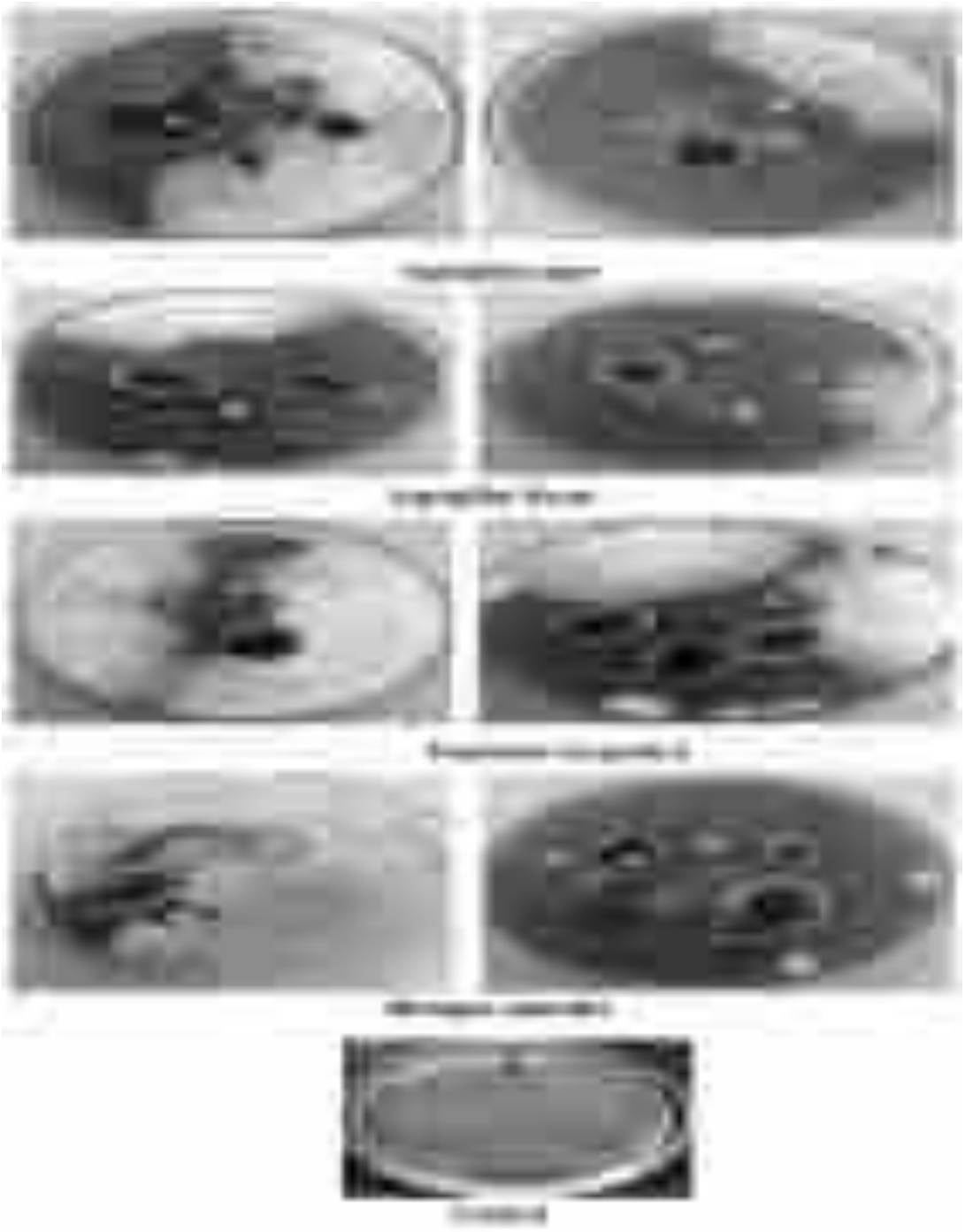
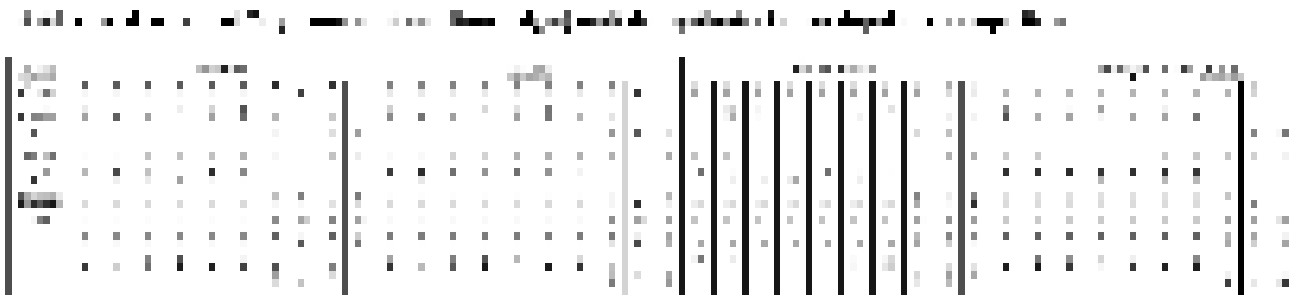
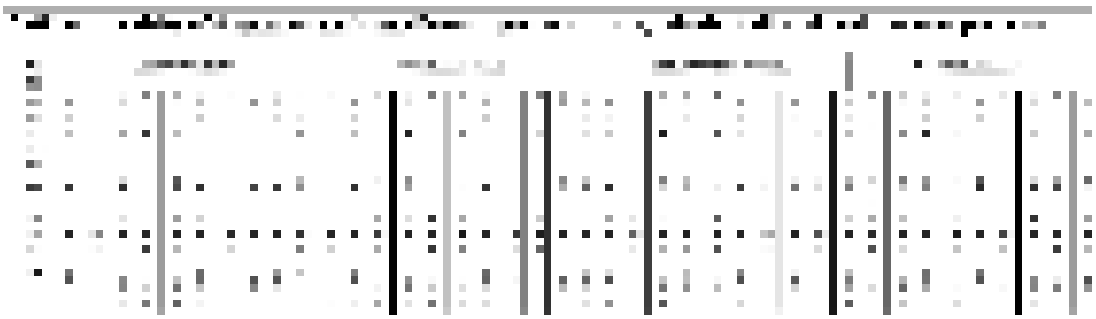


Fig. 5: Showing SEM and EDX of synthesized silver doped zinc nanoparticles from *S. muticum*.









4. CONCLUSION

In the present study, we have used economically important brown seaweed *Sargassum muticum* which is a rich marine source available in abundance. The biomolecules present in the algae must be responsible for the reduction of Silver and zinc ions to Ag doped zinc nanoparticles. Biosynthesis of AgNPs using algal resources is a simple, environmentally friendly, pollutant-free and low-cost approach. This green method of synthesizing AgNPs could also be extended to Medical and industrial applications. This method is inexpensive and highly recommended for large-scale production of Silver doped zinc nanoparticles

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A SYSTEMATIC REVIEW ON THE IMPACT OF ALCOHOL DEPENDENCY: IMPLICATIONS FOR FUTURE RESEARCH AND PRACTICE

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ABSTRACT: *Alcohol abuse has significant individual, familial and social costs. Long term and/or chronic alcohol use has been associated with liver cirrhosis, liver disease, lip, oral cavity and pharynx cancers and heart disease (WHO, 2004). Additionally, intoxication increases risk for road traffic accidents, poisoning and intentional and unintentional injury. Globally alcohol causes 3.2% of all deaths or 1.8 million deaths annually and accounts for 4.0% of disease burden (WHO 2007). This review examines what is known about the impact of alcohol on the health and lives of individuals and families in India. The present study was conducted to examine the prevalence and impacts of alcohol dependency. The study reviewed different studies that are conducted in re relation to alcohol prevalence and impacts. For this purpose, the researcher employed a qualitative approach mainly conventional content analysis. The researcher also used for basic themes that are thought to take the lion share in analyzing the review.Based on the present review, the conclusion made was alcohol greatly impacts in individuals specifically in affecting their health in general and injuries and violence in particular. Based on the conclusion, the recommendation forwarded were raising awareness through education regarding the problem and capacity building for action. Preventive efforts not only create awareness but can also help to de-stigmatize the problem and individuals affected by alcohol misuse,*

Key Words: : *alcohol, dependency, health*

Introduction

Alcohol consumption is a culturally defined activity, impacted by the economics and polity of a society at a given point in time. In the year 2000-2001 the recorded alcohol per capita consumption in India was a low 0.82 liters of pure alcohol as compared to the per capita consumption in US (8.51 liters), Canada (8.26 liters) and UK (10.39 liters) for the same period (WHO, 2004). Post 1995, the unrecorded alcohol per capita consumption in India is an
estimated 1.7 liters (WHO, 2004). While these figures give the impression that India is largely a dry culture, considerable variations exist in the prevalence of alcohol use and misuse within the country. Class, caste, religion and gender are significant factors that define the patterns and nature of alcohol consumption across India. Further, increasingly, globalization and economic liberalization are affecting changes in the social fabric and organization of the Indian society and is likely to have an impact on drinking patterns and cultures in the country as well. This article reviews literature on alcohol use in India to identify impact of alcohol use in India.

Objective

The major objective of the resent paper is to review relevant literature regarding the impact of alcohol dependency, specifically the current review aimed at examining the prevalence alcohol use and providing implication for further research and practice.

Review of literature

Types and prevalence of alcohol use in India

India is a very diverse country with considerable variation in climate, vegetation, natural resources, cultures and traditions. This diversity is also reflected in the types of alcoholic beverages consumed and the cultural meaning associated with alcohol use

Types of Alcoholic Beverages

The most common forms of alcoholic beverages are arrack (made from paddy or wheat), toddy (palm wine), country liquor, illicit liquor, Indian Made Foreign Liquor (IMFL)4, beer and imported liquor (Bennett, Campillo, Chandrashekar, Gureje, 1998; Navchoo&Buth, 1990; Mohan, Chopra, Ray, Sethi, 2001). Alcohol

content in traditional alcoholic beverages such as arrack, toddy, country liquor ranges from 20 to 40 % (WHO, 2004). Alcohol content in illicit liquor is much higher (up to 56%). Illicit liquor production is a serious problem in India. Raw materials used in production of illicit liquor are similar to those used for country liquor, however, illicit liquor is often adulterated using adulterants such as industrial methylated spirit (WHO 2004). Illicit liquor is cheaper than licensed country liquor and therefore popular among the rural and urban poor. In many parts of India, illicit production of liquor and its sale is a cottage industry with each village having one or two units operating illegally (WHO, 2004).

Home production and self- consumption of some alcoholic beverages is also common in certain regions and ethnic communities in India. For instance, in Ladakh, a mountainous region set in the northernmost State in India, certain alcohol (chhang, phaph, gurgur cha) and narcotic (berzeatsink, staspakchek, zimpating) preparations are part of the local diet (Navchoo&Buth, 1990). Arunachal Pradesh, a Northeastern state in India boasts a rice wine called Apong (WHO 2004). Zu and Rohi are locally brewed alcoholic beverages found in the state of Nagaland (WHO 2004). In the Sundarban region in West Bengal, Handia (rice beer) is a traditional drink regarded as food as well as intoxicants by the local adivasis (tribal/indigenous) communities (Chowdhury, Ramakrishna, Chakraborty, Weiss, 2006).

Prevalence of alcohol use in India

Business news reports claim that the Indian market for IMFL is growing at the rate of 8%- 10% a year (Thottam& Hannon, 2009). Sales of IMFL is however likely to account for only a section of the population consumption, namely the middle and the upper middle class consumption. It does not account for traditional or country made liquors consumed primarily by the lower middle class and the urban and rural poor. Further, illicit liquor production, sale and consumption remain unaccounted for.

The dearth of systematic national level epidemiological survey makes it difficult to estimate the prevalence and patterns of alcohol consumption and/or misuse at a country level.

The 2003 National Survey for Alcohol and Drug Abuse found that of the 40697 male respondents (across 25 states, covering rural and urban populations) aged 12-60 years, 74.1% reported life-time abstinence and 21.4% reported being current users (used in last 30 days) of alcohol. Of the total-users, 17% were classified as dependant users (based on the International Classification of Diseases 10) (WHO, 2004). The prevalence rate reported in this study is higher than that in the following secondary two national studies as well as in other regional or community specific epidemiological studies that have been conducted so far. Neufeld, Peters, Rani, Bonu&Brooner (2005) analyzed data from the nationally representative survey (National Sample Survey) of 471,143 people across the country. They reported that the national prevalence of alcohol use was 4.5%. Men were found to be 9.7 times more likely to report regular use of alcohol than women. Further, members of Scheduled Castes and Scheduled Tribes (historically marginalized communities in India) were significantly more likely to report regular use of alcohol as well as tobacco smoking and chewing.

Similar results were reported by Subramaniam, Nandy, Irving, Gordon & Smith (2005) who analyzed the Indian National Family Health Survey for the period 1998-1999. They too reported that members of the schedule castes, schedule tribes and other backward classes were more likely to consume alcohol than members of other caste groups. Further they found that men with no education were more likely to report alcohol use than those with post-graduate education. These studies hint at class, caste and gender variations in alcohol consumption but reveal little in terms of alcohol related problems.

With the exception of studies mentioned above, almost all other epidemiological studies on alcohol use in India have been very region or community specific and their genralizability to the entire country is questionable (Bennett, Campillo, Chandrashekar, Gureje, 1998).

Nonetheless, these studies provide insights into factors determining nature and patterns of alcohol use in India.

Factors determining nature and patterns of Alcohol Use in India

Class, Caste and Ethnicity.

Chowdhury, Ramakrishna, Chakraborty& Weiss (2006) conducted an ethnographic study to identify alcohol consumption patterns and norms in 2 ethnically and economically diverse Development Blocks5 in West Bengal, India. One of the development blocks, Sagar, is inhabited mainly of Hindu migrant workers from another state while Gosaba is home to the adivasis i.e. local tribal (indigenous forest people) communities. Sagar is relatively better developed and has a ferry connection to the mainland. Comparatively, Gosaba is close to a tiger reserve with only country boats to connect them with the mainland.

The researchers found that popular alcoholic beverages in both these blocks were country liquor and toddy (palm wine). The consumption of IMFL was restricted to tourists and 'high status male'. Differences were observed in the culture and pattern of drinking among the tribal community and the migrant community.

Handia (rice beer) was a household brew among the tribal and mainly made for private consumption as part of the diet. Handia was not considered a hard drink and did not have negative connotations associated with alcohol drinking. Like other tribal communities, alcohol consumption by women was not taboo. Handia was also popular among lower caste men and laborers in Sagar. Among the members of low caste and tribal communities country liquor could be used to barter services (boat/ferry ride) or goods (soil beds, betel leaves). Additionally, consumption of alcohol at religious, funeral, marriage ceremonies are reportedly common. Unlike Gosaba, in Sagar consumption of alcohol was not allowed at home or within the immediate community. Alcohol related problems were identified in both these sites and punitive community actions were targeted towards those perceived as alcoholic or engagingin disruptive public behavior (brawls, eve teasing etc.).

Mohan, Chopra, Ray, Sethi (1997) who surveyed 12,157 men and women aged 15 + years from three districts (Mandsaur, Barabanki&Thoubal) in Madhya Pradesh a state in Central India also found significant differences in drinking patterns and attitudes among specific castes and ethnic groups. They reported that men from certain land and cattle owning castes groups (Rajputs, Yadavs and Meghvar) had a culture of alcohol consumption and a reputation for entertaining guests. Drinking was reportedly generally done at home by members of these caste groups. This cultural acceptance of alcohol was limited to the men and complete abstinence from alcohol was expected of women among these Hindu castes groups. In contrast the tribal groups in Thoubal district viewed alcohol as a natural product, a gift of god to be utilized for dietary as well as medicinal purposes by men and women.

Religion.

Religion plays an important spiritual and regulatory role in individual and community life in India. The Census of India identifies Hinduism, Islam, Christianity, Sikhism, Buddhism and Jainism as the five major religions in India. In the 2001 Census, 6,639,626 Indians identified themselves as following religions and persuasions other than the five mentioned above. Of the five major religions three, namely, Islam, Buddhism and Jainism explicitly prohibit alcohol consumption. Across studies, Indian Muslims report the highest abstinence rates (Chowdhury, Ramakrishna, Chakraborty& Weiss, 2006; Gupta, Saxena, Pednekar, &Maulik, 2003; Mohan, Chopra, Ray, Sethi 1997; Subramaniam, Nandy, Irving, Gordon, Smith, 2005). Despite the stronghold of religion in the lives of people in India, its role in alcohol consumption had not been adequately explored.

Gender.

With the exception of tribal societies, abstinence from alcohol consumption by women is a cultural norm in India. General population studies have consistently found a low consumption rate among Indian women - ranging from 2-5%. Cultural and religious norms, limited accessibility and gendered nature of drinking spaces are likely to explain the low rates of alcohol consumption among women in India (Benegal, Nayak, Murthy, Chandra &Gururaj, 2005). Not much is known about women who do drink.

One study examined patterns and context of alcohol consumption among women in urban and rural Karnataka. Benegal, Nayak, Murthy, Chandra &Gururaj (2005) interviewed 1517 males and 1464 women across eight urban and rural centers in the southern state of Karnataka. Of the women interviewed, 84% reported being life time abstainers and 5.8% reported having at least one drink in the last 12 months. Of the 5.8% women, urban working class women and rural women drinkers comprised 2% while affluent urban women comprised the remaining 4%.

This study highlights the differences in drinking patterns amongst women reporting alcohol consumption. Of the 5.8% women, 46.5% women drinkers reported heavy drinking (6 or more drinks per typical occasion). Further it was reported that a larger proportion of rural women than urban women reported drinking weekly or more often as well as drinking more 5+ drinks per occasion. These researchers also found that poor women in rural and urban communities mainly consumed Arrack (country liquor) or moonshine (illicit liquor) and were more likely to drink at home or at off-license retail outlets. These women also reported that they consumed alcohol for tension reduction and stress relief rather than for pleasure. In comparison, alcohol consumption was significantly lower among women from upper and middle socio-economic groups. These women were younger; more educated and reported drinking less per typical drinking occasion.

Impact of alcohol use

Health, Injury and Violence.

Cancela, Ramdas, Fayette, Thomas, Muwonge, Chapuis, Tharaet. al. (2009) interviewed 32,347 participants to evaluate the role of alcohol drinking and patterns of consumption in oral cancer incidence and mortality in 13 panchayats6 in Trivandrum district in Kerela. They found that incidence of oral cancer increased by

49% among current drinkers and 90% among past drinkers than among never drinkers. Current and past drinkers in this study were also more likely to be tobacco smokers and betel- quid chewers than never drinkers. Further, it was reported that the risk of dying from oral cavity cancer was significantly increased among alcoholics in this study. Other studies in Indian have found alcohol consumption to be a risk factor in for cardiovascular diseases (Kusuma, Babu& Naidu 2009) and oral submucousbibrosis (Hashibe, Sankaranarayanan, Thomas, Kuruvilla& Matthews 2002).

Alcohol use has also significantly associated with injury. India has one of the most stringent Blood Alcohol Content (BAC) count allowed for drivers yet in a study by the National Institute of Mental Health and Neurosciences, India it was found that in the city of Bangalore alone, 18-25% of the road injuries are attributable to driving under the influence of alcohol (NIMHANS, 2007).

Benegal, Gururaj, Murthy, Taly, Kiran, Chandrashekar., R &Chandrashekar, H. (2007) sampled 658 injury cases reported to the Emergency Department (ED) of the largest and most reputed general hospital in Bangalore. The injuries represented more than half (54.5%) of all cases seen at the ED during the study period. A high proportion of injuries were found to be alcohol related. It was found that 23.7% of all subjects presenting for treatment of injuries had consumed alcohol prior to the injury occurrence. Of these, 17.9% had BAC readings of .03 and over, which is the legal limit for driving in India. 77.5% patients who reported alcohol use prior to the current injury were also significantly more likely to have had repeated admissions to the ED in the past. Further, subjects who had drunk prior to injury were significantly more likely to drink five or more drinks per sitting, more than 3-4 times a week than subjects without alcohol use prior to injury.

An important gender difference related to indirect alcohol related injury was observed in this study. Of those reported injuries indirectly related to alcohol (use by others) 57% were female and 59% male. Injuries indirectly related to alcohol among women included injuries due to burns, hanging, poisoning and assault. The researchers point out that in the Indian context, a large proportion of burn injuries are not accidental burns but assault and homicidal attempts on women by male relatives. In a family where a woman is already being harassed for dowry, birth of a girl child or lack of male children, alcohol abuse by the husband is likely to intensify physical, emotional and financial abuse (Benegal, Gururaj, Murthy, Taly, Kiran, Chandrashekar., R &Chandrashekar, H. 2007)

A number of studies on domestic violence suggest that while alcohol abuse by the spouse may not be the primary cause of domestic violence, it increases women's vulnerability to violence perpetrated by her spouse or partner. Varma, Chandra, Thomas & Carey (2007) interviewed 203 women attending an antenatal clinic in a public hospital in Bangalore to assess the prevalence of intimate partner violence and sexual coercion and its mental health consequences among pregnant women. 30 of 203 women in this study reported experiencing physical and psychological violence. Further, of these 30 women 15 reported ongoing violence during pregnancy. Prevalence of alcohol use was found to be much higher among spouses of abused women (82%) compared to spouses of non-abused women (18%). This study found that harmful use of alcohol use was a significant predictor of the presence as well as severity of violence.

Parker, Fernandes& Weiss (2003) conducted focus group discussions with women, men, youth and community leaders in a slum in Mumbai to identify the needs of the community for a community based mental health program. Alcoholism emerged as a major problem in the community. Participants reported that alcohol was distilled locally, was readily available and imbibed by 60% to 70% of the male population in the community. Domestic violence was identified as a rampant problem and closely associated with alcohol abuse by men. In addition to physical abuse women reported that their husbands regularly harassed them for money to repay credit taken for alcohol or for further alcohol consumption.

Similar findings were reported by Stanley (2008) who interviewed 75 wives of men enrolled in a de-addiction center in the city of Tiruchirappalli in South India. Like the previous studies, women in this study reported regular psychological, physical and financial abuse. 43.3% women reported that alcohol was consumed by their spouse at all times of the day. 30 % reported that their husbands had borrowed money and 13.3% reported that property was sold to meet the drinking expenditure. Of the sample 96.7% reported being verbally abused and 90% reported being physically abused. 50% of these women also reported physical abuse of children.

These studies indicate that alcohol abuse and woman and child abuse co-occur but do not explore the role of alcohol in domestic violence perpetuation or the nature of injuries caused by violence involving alcohol abuse. While it is unclear whether alcohol use triggers or intensifies violence, the studies suggest that women with alcoholic husbands are at increased risk for injury, victimization and impoverishment.

Recommendations for Social Work Practice

A multifaceted problem such as alcohol abuse requires a multi-pronged and multi-system approach to intervention (Benegal, 2005). As with most social problems, prevention, detection and treatment are key

areas for intervention in addressing alcohol abuse. Social work professionals can make significant contributions by initiating and testing best practice options in each of these areas.

Preventive initiatives usually involve raising awareness through education regarding the problem and capacity building for action. Preventive efforts not only create awareness but can also help to de-stigmatize the problem and individuals affected by alcohol misuse. Schools, colleges, religious organizations, community based and development organizations (such as youth and women's organization) are key community institutions that need to be enrolled in the efforts to address alcohol problems within a given community. Social workers can be instrumental in community mobilization for social change. As change agents social workers can identify, motivate, train and assist stakeholders to draw on community assets for initiating, planning and implementing community specific preventive and treatment strategies.

Educational and awareness raising initiatives must be complimented by adequate detection and treatment efforts. Professionals likely to come in contact with those affected by alcohol abuse including health care providers, development workers, women's organizations, police and traffic police require capacity building to ensure detection and appropriate referrals are made. A review of websites of organizations7 offering alcohol treatment and de-addiction services indicate that some have adopted the Alcohol Use Disorders Identification Test (AUDIT) for screening and assessment. However the extent of its usage in assessment and screening across treatment facilities in India is unknown. Training of health care professionals and social service providers in the use of standardized assessment measures is essential for practice as well as research. Social work professionals are an integral part of many systems including schools, hospitals and law enforcement and can be instrumental in facilitating networking and collaborations between various stakeholders in the community. Additionally social workers can significantly contribute to planning, co-coordinating and implementation of training and capacity building of professionals.

Literature on treatment approaches currently in use in India is sparse. A review of websites of organizations reflect a variety of approaches including detoxification units, yoga, psychotherapy, counseling, brief therapies, residential and non-residential programs, communit based rehabilitations camps and programs involving one or more family members. However the efficacy of these treatment approaches for the particular client population remains to be demonstrated. The current situation warrants the urgent need for social workers to conduct practice evaluation research in order to identify best practices in addressing alcohol related problems.

Implications for Research and Policy

Presently it appears that while the majority of the Indian populace is abstinent, among
those who do drink there are high rates of problematic and harmful drinking (Benegal, Nayak, Murthy, Chandra &Gururaj, 2005; D' Costa, Nazareth, Naik. Vaidya, Levy, Patel, & King, 2007; Rahman, 2003). Systematic and streamlined research on countrywide prevalence, context and impact of alcohol abuse is needed in order to formulate effective policies and implement appropriate interventions. Further, a thorough investigation of the nature, type and effectiveness of alcohol interventions currently in use is essential.

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A COMPARITIVE STUDY OF MICROBIAL ANALYSIS OF DRINKING WATER IN PINNAKOTA AND RAMPALLE PANCHAYATHS IN ANANTHAGIRI MANDAL, VISAKHAPATNAM, ANDHRA PRADESH

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Abstract

In the present study an investigation was done on the microbiological and physico-chemical properties of potable water (stream, bore and well) from the Pinnakota and Rampalle panchayaths of Ananthagiri mandal, Visakhapatnam, Andhra Pradesh. The water samples are subjects to physico-chemical analysis by standard methods. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification. The final identification was done according to the Bergey's Manual. The physicochemical characters of all the five drinking water samples were within the recommended permissible level of WHO. The total plate count was above the WHO guidelines values (<10CFU's/ml) in the three water samples studied the highest count was during August. The MPN in all the three water samples was highest during the rainy season i.e., August and was due to the contribution of all the pathogenic bacteria. During the study period all the three different water samples (i.e. stream, bore and well) from the two panchayaths showed the presence of the eight pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus, Group D Streptococcus, Vibrio cholerae* and *V. parahaemolytics.* Among the pathogenic bacteria *Escherichia coli* was observed to be highest percentage followed by *Staphylococcus aureus* and the least percentage was *Klebsiella pneumoniae*. In conclusion, the presence of these pathogens in the water indicates that none of the water used for drinking purposes in these panchayaths.

Keywords: Drinking water, Pathogenic bacteria, Pinnakota panchayath and Rampalle panchayath

Introduction

Access to safe drinking water is one of the basic human rights and is enormously crucial to health. For a nation to maintain optimal health and development there has to be a continual supply of safe drinking water to its population. In developing countries, about 1.8 million deaths per year are attributed to unsafe water, sanitation and hygiene, mainly through infectious diarrhea (WHO, 2002). Diarrheal disease remains a major killer in children. It is estimated that 17% of all child deaths under the age of 5 years in developing

countries result from diarrheal diseases (WHO, 2003). Hence, lack of safe drinking water supply, basic sanitation and hygienic practices is associated with high morbidity and mortality from feco-orally transmitted diseases. About 1.1 billion people have no access to safe drinking water and diarrheal disease is highly endemic in these societies. This underlines the need for safe drinking water. However, the effectiveness of interventions aimed at improving the quality of drinking water alone may not solve the problem because people can become infected with organisms that cause diarrhea through multiple pathways. Even in developed countries with improved water supplies, diarrhea is often endemic (Wright, 2004; Colford et al., 2006; Roy et al., 2006; Clasen et al., 2007; Johnson et al., 2016). For example, it has been reported that environmental interventions have shown 15 to 17% median reduction in diarrhea from water quality Interventions (Clasen et al., 2007). This indicates that not only water improvements at the source or collection point (protected wells, boreholes, and tap stands) but also improvement at household level and other sources are equally important to minimize the risk of water born diarrhea (Zvidzai et al., 2007).

In India, 88% of the population of 1.2 billion uses drinking-water from such sources, as compared to 72% in 1990. The majority of the infections that is associated with the lack of accessibility to potable water supply and poor environmental sanitation especially in developing countries. The following are micro-organisms associated with water; *Pseudomonas aeroginosa, Salmonella, Mycobacteria, Escherichia coli Proteus, Shigellasonnei, Klebsiella, Cyanobacteria* (Chris, 2004). Hence, the bacteriological quality of drinking water is important and periodical monitoring is essential for potable water. Thus this study was conducted to assess the bacteriological and physico chemical quality of sources of water used for drinking during April 2011 to March 2012 from Pinnakota and Rampalle panchayaths, Anathagiri mandal, Visakhapatnam, Andhra Pradesh, India.

Material and methods:

Ananthagiri ($18^0$17’14”N, 83 ^{0}6’43”E) is about 60km away from Visakhapatnam and lies on the top of the Eastern Ghats. The area of the Ananthagiri mandal is roughly 50sq km and the entire area is inhabited by aboriginal tribes. Of the 25 panchayaths in Anantagiri mandal, Pinnakota panchayath and Rampalle panchayath was selected for the present study. Both the panchyaths contain three different water sources i.e. well, bore and a stream. The water quality of the three water samples (stream, bore and well) from the Pinnakota and Rampalle panchayaths of Ananthagiri mandal, Visakhapatnam, Andhra Pradesh were studied for physicochemical and bacteriological parameters. In the present study, water samples from three sources i.e., a well, a bore and stream of the two panchayths were collected once in a month for a period of 12 month from April 2011 to March 2012, in white plastic bottles, which were previously rinsed with distilled water and sterilized with 70% alcohol. At the collection point, the containers were rinsed thrice with the sample water before being used to collect the samples. The collected samples were placed in a thermocol box. The

microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification (Sherman Cappuccino, 2009). The final identification of resulted isolates was done by the biochemical tests in accordance to the Bergey's Manual (Holt et al., 1984).

RESULTS

Water samples collected from Pinnakota panchayath and Rampalle panchayath for a period of one year i.e., during April 2011 to March 2012 were analyzed for physical, chemical and bacteriological characteristics. The physical characteristic measured is P^H. Among the chemical characteristics Total dissolved solids (TDS) and fluoride contents were measured. For total number of viable bacteria total plate count (CFU/ml), for faecal and total coliforms most propable number (MPN/100ml) and for isolation and identification of bacterial staining, biochemical and growth on selective media were performed.

The mean P^H value of stream water in the both panchayaths was in the range 7. The mean P^H value of bore water in both Pinnakota and Rampalle panchayaths was 7.09 & 7.06 respectively. The mean P^H value of well water in both panchayaths was 7.005. The P^H value in the three water samples is in the safe limit as recommended by WHO.

The amount of total dissolved solids of the stream water in both Pinnakota and Rampalle panchayaths was on the average 107.84mg/l &107.64mg/l respectively and Fluoride content on the average in the both panchayaths was 0.1mg/l. The amount of total dissolved solids of the bore water in both Pinnakota and Rampalle panchayaths was on the average 289.67mg/l & 273.25mg/l respectively and Fluoride content on the average was 0.12mg/l & 0.104mg/l respectively. The amount of total dissolved solids of the well water in both Pinnakota and Rampalle panchayaths was on the average 191.13ma/l & 175.08mg/l respectively and Fluoride content on the average was 0.11mg/l & 0.109mg/l. Both the values in the three samples were in the permissible limits as recommended by WHO.

In stream water the total plate count in both Pinnakota and Rampalle panchayaths fell in the range of 36-64 CFU's/ml & 39-76 CFU's/ml respectively (fig 1). The Pinnakota panchayath water sample showed the maximum number of CFU's (64CFU's/ml) in August and minimum number was noted in June (36 CFU's/ml). The Rampalle panchayath water sample showed the maximum number of CFU's (76CFU's/ml) in August and minimum number was noted in March and June (39 CFU's/ml). In bore water the total plate count in both Pinnakota and Rampalle panchayaths fell in the range of 39-76 CFU's/ml & 58-139 CFU's/ml respectively (fig 3). The Pinnakota panchayath water sample showed the maximum number of CFU's (76CFU's/ml) in August and minimum number was noted in June (39 CFU's/ml). The Rampalle panchayath water sample showed the maximum number of CFU's (139CFU's/ml) in August and minimum number was noted in March (58 CFU's/ml). In well water the total plate count in both Pinnakota and Rampalle panchayaths fell in the range of 58-139 CFU's/ml & 97-236 CFU's/ml respectively (fig 5). The

Pinnakota panchayath water sample showed the maximum number of CFU's (139CFU's/ml) in August and minimum number was noted in March (58 CFU's/ml). The Rampalle panchayth water sample showed the maximum number of CFU's (236CFU's/ml) in August and minimum number was noted in May (97 CFU's/ml). Total plate count for bacteria performed for all water samples showed that the bacteria in all the samples were above the WHO guideline values(<10CFU's/ml). The total plate count in all the three water samples was highest during the rainy season i.e., August and was due to the contribution of all the pathogenic bacteria.

In stream water the MPN index in both Pinnakota and Rampalle panchayaths ranged from 3-15/100ml & 4-28/100ml respectively (fig 2). In Pinnakota panchayath the maximum MPN index was recorded in (15/100ml) August and October and the minimum MPN index was recorded in (3/100ml) April and May. In Rampalle panchayath the maximum MPN index was recorded in (28/100ml) August and the minimum MPN index was recorded in (4/100ml) March. In bore water the MPN index in both Pinnakota and Rampalle panchayaths ranged from 4-21/100ml & 9-64/100ml respectively (fig 4). In Pinnakota panchayath the maximum MPN index was recorded in (21/100ml) September and the minimum MPN index was recorded in (4/100ml) April and March In Rampalle panchayath the maximum MPN index was recorded in (64/100ml) August and the minimum MPN index was recorded in (8/100ml) March. In well water the MPN index in both Pinnakota and Rampalle panchayaths ranged from 28-9/100ml & 20-210/100ml respectively (fig 6). In Pinnakota panchayath the maximum MPN index was recorded in (28/100ml) August and the minimum MPN index was recorded in (9/100ml) January and March. In Rampalle panchayath the maximum MPN index was recorded in (210/100ml) August and the minimum MPN index was recorded in (20/100ml) June. The MPN in all the three water samples was highest during the rainy season i.e., August and was due to the contribution of all the pathogenic bacteria.

During the study period all the three different water samples (i.e. stream, bore and well) from the two panchayaths showed the presence of the eight pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus, Group D Streptococcus, Vibrio cholerae* and *V. parahaemolytics* (Sandhya et al., 2013). (Table 1 &2)

Among the pathogenic bacteria the most dominant species in stream water (figure 7) in Pinnakota panchayath the most dominant species of bacterium was *E.coli* (24.26%) followed by *Staphylococcus aureus* with 19.04%, *Group D Streptococcus* with 12.24%, *Vibrio cholerae* and *V. parahaemolytics* with 10.20%, *Shigella dysenteriae* with 9.75%, *Salmonella typhi* with 9.52% and *Klebsiella pneumoniae* with 4.76% contribution. In Rampalle panchayath was *Escherichia coli* (28.3%) followed by *Staphylococcus aureus* with 15.16%, *Vibrio cholerae* with 12.5%, *Salmonella typhi* with 11.2%, *V. parahaemolytics* with

9.45%, *Shigella dysenteriae* with 8.79%, *Klebsiella pneumoniae* with 7.47% and *Group D Streptococcus* with 7.03%, contribution

In bore water (figure 8) in Pinnakota panchayath the most dominant species of bacterium was *E.coli* (22.25%) followed by *Staphylococcus aureus* with 19.25%, *Group D Streptococcus* with 14.76%, *Salmonella typhi* with 10.28%, *V. parahaemolytics* with 9.71%, *Vibrio cholera* with 9.53%, *Shigella dysenteriae* with 8.59%, and *Klebsiella pneumoniae* with 5.6% contribution. In Rampalle panchayath the most dominant species of bacterium was *E.coli* (27.9%) followed by *Staphylococcus aureus* with 19.9%, *Salmonella typhi* with 10.6%, *Vibrio cholerae* and *V. parahaemolytics* with 9.84% each, *Shigella dysenteriae* with 8.2%, *Group D Streptococcus* with 8.03% and *Klebsiella pneumoniae* with 5.44%, contribution.

Analysis of well water (figure 9) in Pinnakota panchayath the most dominant species of bacterium was *E.coli* (24.712%) followed by *Staphylococcus aureus* with 17.69%, *Group D Streptococcus* with 13.82%, *Salmonella typhi* with 10.15%, *Vibrio cholera* with 10.05%, *Shigella dysenteriae* with 9.73%, *V. parahaemolytics* with 8.48% and *Klebsiella pneumoniae* with 5.34% contribution.In Rampalle panchayath revealed that the predominant bacterium was *E.coli* (26.16%) followed by *Staphylococcus aureus* with 19.57%, *Salmonella typhi* with 12.40%, *Shigella dysenteriae* with 10.65%, *Vibrio cholerae* with 9.30, *V. parahaemolytics* with 8.13%, *Group D Streptococcus* with 7.17% and *Klebsiella pneumoniae* with 6.58%, contribution.

During the study period all the three different water samples (i.e. stream, bore and well) from the two panchayaths showed the highest percentage of *Escherichia coli* followed by *Staphylococcus aureus* and the least percentage was showed by *Klebsiella pneumoniae*.

Table 1: Morphological and Cultural characteristics of Organisms

Morphological & Cultural characters	Organism	Disease caused by the organism
Gram negative rod, forms circular, low convex mucoid, opaque colonies with entire marginal growth on nutrient agar. Green metallic sheen colonies were observed on EMB agar.	*Escherichia coli*	Causal agent of gastroenteritis, urinary tract infections, and neonatal meningitis.
Gram positive coccus, non spore forming and non- motile bacteria. It forms circular, low convex with entire margin, smooth, medium opaque colony on nutrient agar. It forms yellow coloured colonies on mannitol salt agar.	*Staphylococcus aureus*	*S.aureus* incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It causes a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.
Gram positive coccus. It forms thin, even growth on nutrient agar. Black (or) Brown coloured colonies were observed on bile esilin agar.	*Group D Streptococcus*	*Group D Streptococcus* causes urinary tract infections, meningitis, neonatal sepsis, spontaneous bacterial peritonitis, septic arthritis, and vertebral osteomyelitis diseases.
Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and yellow coloured colonies on TCBS agar.	*Vibrio cholerae*	*Vibrio cholerae* is responsible for the occurrence of cholera.
Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and green coloured colonies on TCBS agar.	*Vibrio parahaemolytics*	*V. parahaemolytics* is responsible for gastrointestinal illness in humans.
Gram negative rod. It forms slimy, white somewhat translucent, raised growth on nutrient agar and dark pink coloured colonies on mac - conkey agar.	*Klebsiella pneumoniae*	*Klebsiella pneumonia* is responsible for pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrohea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia.
Gram negative rod. It forms thin even grayish growth on nutrient agar and dark green colonies on SS agar.	*Salmonella typhi*	*Salmonella typhi* causes typhoid.
Gram negative rod. It forms grayish growth on nutrient agar and colourless colonies on SS agar.	*Shigella dysenteriae*	*Shigella dysenteriae* is the bacillary dysentery causing bacterium.

Table 2: Biochemical Characteristics of isolates:

Test						
Catalase	+	-	+	+	+	+
Oxidase	-	-	-	+	-	-
Motility	-	-	+	+	-	
Indole	-	-	+	+	-	+
Methyl-red	-	+	+	-	+	+
Voge-Proskauer	+	-	-	+	-	-
Citrate Utilization	-	-	-	+	+	-
Urease	+	-	-	-	-	-
Hydrogen sulphide	-	-	-		+	-
Starch hydrolysis	-	-	-	-	-	-
Nitrate Utilization	-	-	+	+	+	+
Gelatin liquefication	-	-	-	+	-	-
Lactose fermentation	-	A	AG	AG	-	-
Glucose fermentation	A	A	AG	AG	AG	A
Sucrose fermentation	A	A	A(+)	AG	AG	A+/-
Organism	*Staphylococcus*	*Streptococcus*	*E. coil*	*Vibrio*	*Salmonella*	*Shigella*

A = Acid production only
AG = Acid and gas production
+/- = Variable reaction
\+ = Positive
– = Negative
(+) = Late Positive

Fig 1: Total plate count of stream waters

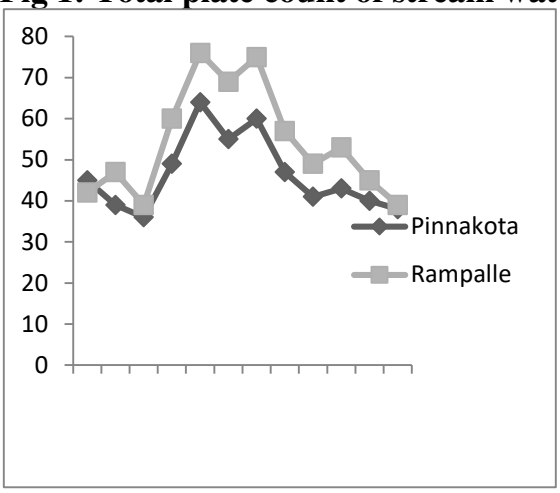


Fig 2: Most probable number of stream water

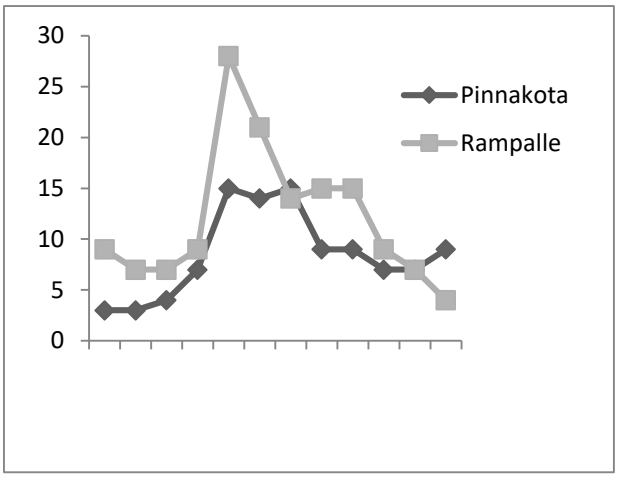


Fig 3: Total plate count of bore waters

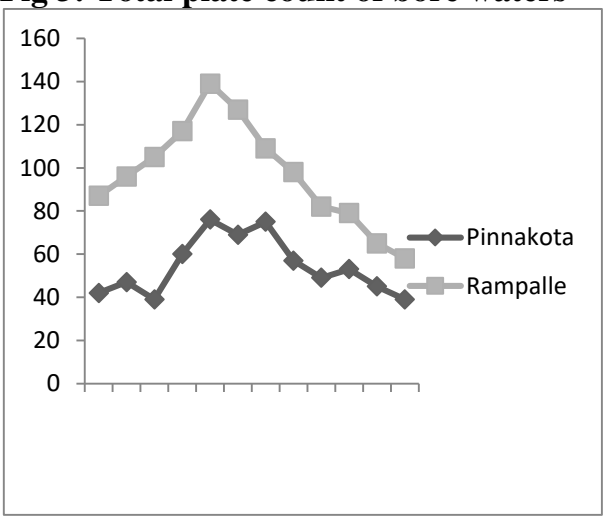


Fig 4: Most probable number of bore water

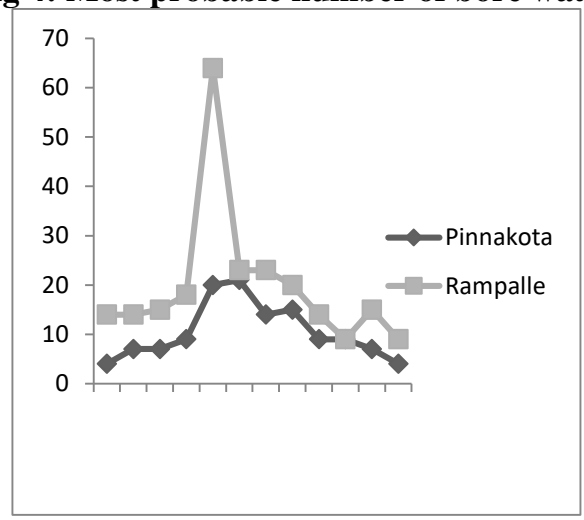


Fig 5: Total plate count of well waters

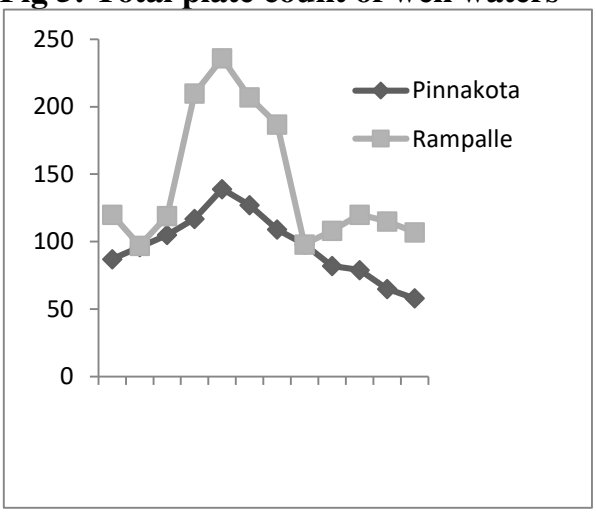


Fig 6: Most probable number of well water

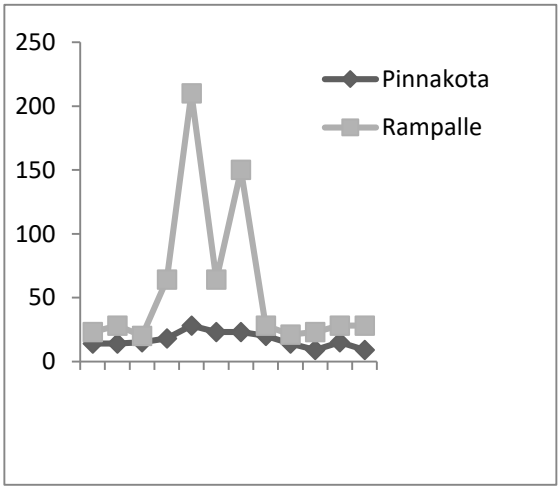


Fig 7: % pathogenic Bacteria in stream

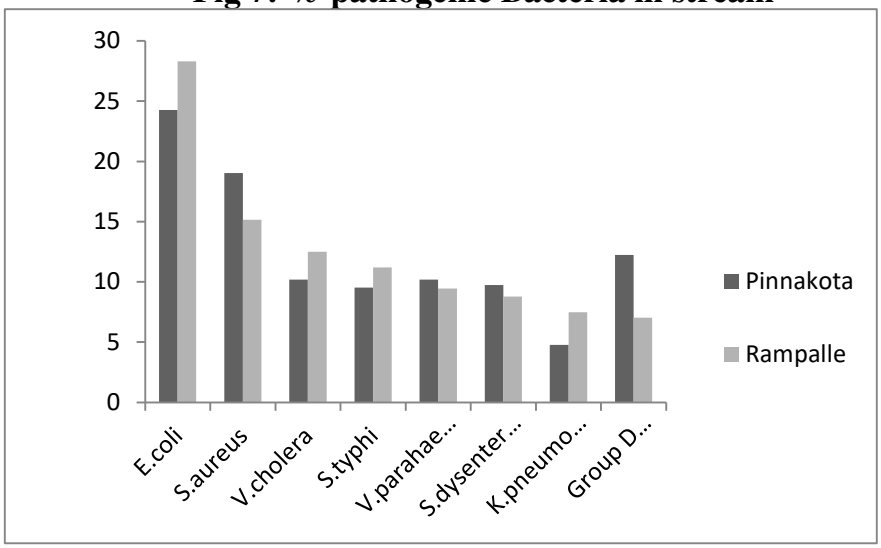


Fig 8: % Pathogenic bacteria in bore

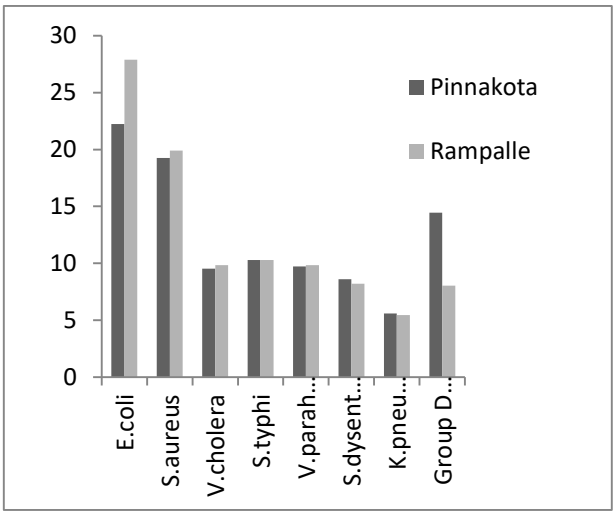
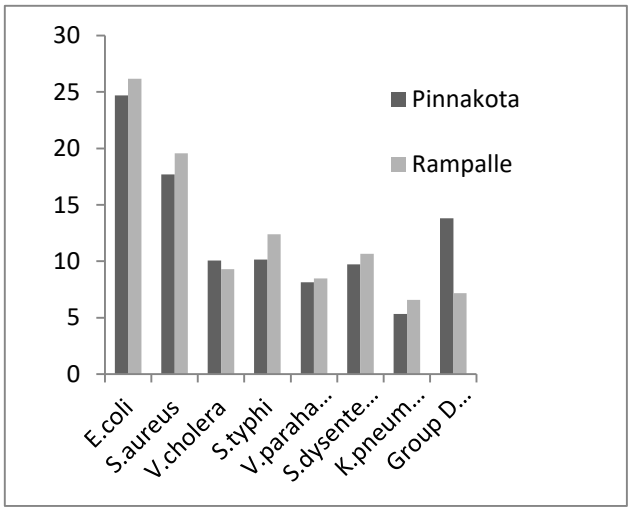


Fig 9:% Pathogenic bacteria in well



Discussion

India is rich in water resources, being endowed with a network of rivers and blessed with snow cover in the Himalayan range that can meet a variety of water requirements of the country (Bhardwaj, 2005). The rivers of India play an important role in the lives of the Indian people. Major factors affecting microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements. The present panchayaths are with mountains as predominant topological features, harbors several natural stream water (SW). This stream water is the primary source of potable water for the population in the both Pinnakota and Rampalle panchayaths. Indicator organisms are commonly used to assess the microbiological quality of surface waters and faecal coliforms (FC) are the most commonly used bacterial indicator of faecal pollution. They are found in water that is contaminated with faecal wastes of human and animal origin. Total coliforms (TC) comprise bacterial species of faecal origin as well as other bacterial groups (e.g. bacteria commonly occurring in soil). The coliforms are indicative of the general hygienic quality of the water and potential risk of infectious diseases from water. High FC and TC counts in water are usually manifested in the form of diarrhoea and sometimes by fever and other secondary complications. Bathing and swimming in streams and river are also common among children and adults in the local community. The probability of ingesting infective dose of disease causing microorganism is very high considering the fact that water borne pathogens generally have low infective dose.

Safe drinking water is one of the most important felt needs in public health in developing countries in the 21st century (Sobsey et al., 2003). The general public today has always been found facing the problems for the availability of safe drinking water as a vast majority routinely suffers from common diseases like diarrhoea etc. and is the leading cause of mortality particularly among the children. The importance of potable drinking water is therefore obvious, emphasizing the need for its utilization (Ahmed et al., 2004). Diseases that spread through the contaminated water principally in areas of poor sanitation are Hepatitis A, Hepatitis E, Typhoid fever, diarrhea and dysentery etc. (Light, 2000).

Physical parameters such as P^H, TDS and fluoride content have a major influence on bacterial population growth. P^H values ranging from 3 – 10.5 could favour both indicator and pathogenic organism's growth (Zamxaka et al., 2004). P^H provides the information about the acidity or alkalinity of water (Katyal and Satake, 1990). It also provides a means of clarifying and for collecting other characteristics or behavior such as corrosive activity (Ghandour et al., 1985). Eye irritation and exacerbation of skin disorders have been associated with P^H values greater than 11. With respect to the water samples the P^H values were in safe limit.

High TDS was commonly objectional or offensive to taste. A higher concentration of TDS usually serves no health threat to human until the values exceed 10,000mg/l (Anonymous, 1997). The TDS values of the all the three water samples were within the permissible limit.

Fluoride testing in water quality analysis should be given importance because fluoride is known to cause a variety of health problems viz dental fluorosis and non skeletal manifestations when the level beyond 1.5ppm. Fluoride has come to stay as number one parameter in causing toxicological and geo-environmental problems in various countries. The fluoride content of all the three water samples was within the permissible limit.

The results of physico–chemical analysis of the present study are in agreement with the study of Germs et al., (2004) who reported that the chemical quality of the Chunies River in South Africa was acceptable for domestic as well as for agriculture. Similary, Nevondo et al., (1999) revealed that the chemical quality of all water samples was acceptable. The chemical analysis of water samples from Egypt carried out by Fadaly et al., (1999) showed that the measured parameters were found within the permissible limits.

Most common and wide spread health risk associated with drinking water is the bacterial contamination caused either directly or indirectly by human or animal excreta. In the present study the heterotrophic plate count was used to estimate the total amount of bacteria in water and indicates the overall microbial status of water. The factors responsible for high microbial counts in the water samples may be due to technically ill planned sewerage network, damaged sewer lines, rust water pipe lines and poorly maintained disinfection system. Muhammad Anjum Zia et al., (2005) found that the ground water of Faisalabad city showed high bacterial counts. The pathogenic bacteria thus isolated were mostly belonging to the Enterobacteriaceae. The presence of Enterobacteriaceae members in the tested water samples indicates the faecal pollution. High level of contamination of ground water with faecal coliforms was found in urban areas of Karachi (Zubair and Rippy 2000). Khan et al., 2000 was also found that more than 50% water samples of Peswar, Nowshera and Charsada were polluted with faecal coliforms. These faecal coliforms were also reported from Umian lake water (Rajurkar et al., 2003) and also in different water samples at Sivakasi (Radha Krishnan et al., 2007). Water sources used for drinking or cleaning purpose should not contain any organism of faecal origin (Sabongari 1982, Fonseca et al., 2000).

Human and animal waste is the primary source of different bacteria in water. The source bacterial contamination include run off feedlots, pastures, dog runs and other land areas where animal wastes are deposited. Bacteria from these sources can enter in taps that are either open at the land surface, or don't have water tight casing or caps, or don't have seal in the annular space (the space between the wall of the drilled tap and the outside of the tap casing). Insects, rodents and animals entering the tap are other sources of contamination. Another way through which bacteria can enter the water supply is through inundation or infiltration by flood waters or by surface runoff. Flood water commonly contains high level of bacteria.

This study reveals that the increased in the microbial loads at the consumer points (i.e. bore and well) was due to the observed activities. At some points, the direct washing of human clothing and washing of other household utensils around the sampling point. The presence of animals and the intense agricultural related activities going on around the consumer point could lead to contamination. The direct washing of legs, hands, clothes and utensils in the stream could also lead to contamination (Banwo, 2006).

Enteric pathogens cannot normally multiply in water hence water is not its mode of transmission to humans (WHO, 1996). However, the presence of enterobacteriae would be enough infective doses in people whose local or general natural defense mechanisms are impaired to significantly low. The people likely to be at risk would be the very old or the very young as well as patients undergoing immunosuppressive therapy. Other immunocompromised individuals suffering from AIDS would also be at risk. Also, water polluted by bacteria when permitted to contaminate food would lead to the multiplication of the pathogens to very large doses.

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RESEARCH ARTICLE

EVALUATION OF SODIUM CHLORIDE STRESS TOLERANCE IN FINGER MILLET (ELEUSINE CORACANA L.) CULTIVARS BY OBSERVING MORPHOLOGICAL CHARACTERS.

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Abstract

Saline soils are unproductive. Plant growth and yield are highly effected by salinity. This may overcome by cultivating salt tolerant varieties. The aim of this study is to figure out, salt tolerant and salt sensitive cultivars of finger millet. In this study 12 cultivars of finger millet (*Eleusine coracana* L.) seeds were grown at different NaCl concentrations. Germination percentage, root length, shoot length and dry weight of the seedlings were assessed using different concentrations of NaCl. Based on the growth parameters, VR-1076 was confirmed as salt tolerant cultivar and VR-988 was confirmed as salt susceptible cultivar.

Introduction:-

Finger millet (Eleusine coracana L.) is an annual herbaceous plant, widely grown as a cereal crop in arid and semiarid areas in Africa and Asia. Abiotic pressures like salt stress limit the crop growth and yield; also limit the land available for farming. It is thus needed to understand, how plants respond to adverse conditions. By studying the effects of environmental stresses, tolerance in plants may be understood (Joseph *et al*., 2010).

High concentration of soluble salts in the soil moisture of root zone is referred to as salinity in agricultural soils. These concentrations of soluble salts through their high osmotic pressures reduce plant growth by restricting the uptake of water by the roots. As the absorption of nutritional ions is restricted, plant growth is affected (Tester and Davenport, 2003).

Salinity creates two major threats to plant growth: osmotic and ionic stress (Flowers and Colmer, 2008). Salinity stress alters different physiological and metabolic processes of plants. The responses of these changes are often accompanied by a variety of symptoms such as the decrease in leaf area, increase in leaf thickness and succulence, abscission of leaves, necrosis of root and shoot and decrease of internode lengths (Gucci and Tattini, 1997; Kozlowski, 1997; Parida and Das, 2005). More recently, climate change has shown a trend that leads to differences in rainfall patterns, temperature extremes and soil composition changes, including salinization (Versules *et al*., 2006). The main aim of this work is to find out NaCl tolerant and NaCl sensitive cultivars of finger millet at the seedling stage.

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Materials and methods:-

For the collected, twelve cultivars of finger millet salt tolerance activity test was conducted with NaCl. In this assay germination of the seeds, shoot length, root length and dry weight were calculated. For salt tolerance activity 10 different NaCl salt concentrations i.e. 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 ppm were used. Initially 20 seeds from each cultivar were taken and sowed in different pots with mentioned concentration of NaCl. After that enough water was added to all the samples. Along with these different salt concentrations one control pot was seeded with 20 seeds for all cultivars. After 48 hours, germination percentage was calculated and tabulated. After incubation, germination percentage, root length, shoot length and dry weight were measured and recorded. According to the result resistant and susceptible cultivars were characterised.

Result Analysis and Discussion:-

In the present study, the effect of NaCl stress on morphological characteristics such as percentage of germination, root length, shoot length and dry weight of seedlings in 12 different cultivars of finger millet were investigated. There was a large range of variation in germination percentage under NaCl stress conditions. From the table-1 it is inferred that as the NaCl concentration increased, the germination percentage declined. The highest NaCl concentration used in this experiment was 5000 ppm, only one cultivar i.e. VR-1076 showed germination (figure 1), six cultivars VR-1101, VR-900, VR-847, VR-708, VR-1099 and PR-202 showed germination up to 3500 ppm, four cultivars VR-762,VR-1117,VR-936,GPU-67 showed germination up to 3000 ppm, cultivar VR-998 showed germination up to 2500 ppm. Similar results were reported in mungbean (Mahajan and Tuteja, 2005; Mahadavi and Sanavy, 2007), rice (Lee *et al*., 1998), durum wheat (Tekalign Mamo *et al*., 1996). The effect is more pronounced at higher salinity levels.

Figure 1:-Figure showing that germination of susceptible cultivar with different salt concentrations in first lane and germination of tolerant cultivar with different salt concentrations in second lane.

Table 1:-Effect of NaCl stress on seed germination in different cultivars of finger millet

Cultivars	Salt (NaCl) concentrations used (PPM)										
	control	500	1000	1500	2000	2500	3000	3500	4000	4500	5000
VR-762	85 %	85 %	70 %	55 %	50 %	35 %	15 %	0	0	0	0
VR-1101	80 %	75 %	75 %	50 %	35 %	25 %	15 %	10 %	0	0	0
VR-900	100 %	75 %	70 %	50 %	35 %	25 %	25 %	15 %	0	0	0
VR-988	85 %	60%	45%	35%	25%	5%	0	0	0	0	0
VR-1076	85 %	85 %	75 %	65 %	50 %	50 %	45 %	40 %	30 %	30 %	25 %
VR-847	75 %	75 %	70 %	70 %	55 %	50 %	40 %	20 %	0	0	0
VR-1117	80 %	75 %	75 %	55 %	40 %	20 %	20 %	0	0	0	0
VR-936	75 %	75 %	55 %	50 %	45 %	45 %	25 %	0	0	0	0
VR-708	80 %	80 %	75 %	70 %	50 %	50 %	30 %	20 %	0	0	0
VR-1099	80 %	80 %	70 %	50 %	40 %	25 %	20 %	10 %	0	0	0
GPU-67	80 %	75 %	55 %	40 %	35 %	25 %	15 %	0	0	0	0
PR-202	75 %	75 %	65 %	55 %	50 %	40 %	40 %	25 %	0	0	0

Table2:-Effect of NaCl stress on root length in different cultivars of finger millet

Cultivars	Root length (Cm)										
	control	500	1000	1500	2000	2500	3000	3500	4000	4500	5000
VR-762	2.51	2.39	1.72	1.07	0.27	0.22	0.22	0	0	0	0
VR-1101	2.48	2.41	1.96	1.32	0.48	0.42	0.34	0.25	0	0	0
VR-900	3.28	2.83	2.05	1.07	0.78	0.56	0.48	0.37	0	0	0
VR-988	2.38	1.89	1.45	1.02	0.69	0.38	0	0	0	0	0
VR-1076	2.45	2.14	1.64	1.07	0.5	0.43	0.4	0.35	0.31	0.26	0.22
VR-847	2.1	1.57	1.05	0.85	0.58	0.41	0.4	0.33	0	0	0
VR-1117	2.7	2.03	1.39	1.01	0.75	0.65	0.43	0	0	0	0
VR-936	1.85	1.06	0.85	0.47	0.39	0.33	0.31	0	0	0	0
VR-708	2.41	2.02	1.65	0.92	0.5	0.42	0.40	0.32	0	0	0
VR-1099	2.41	2.01	1.66	1.1	0.73	0.48	0.39	0.33	0	0	0
GPU-67	2.45	2.12	1.5	1.04	0.64	0.42	0.39	0	0	0	0
PR-202	1.98	1.3	1.02	0.96	0.76	0.51	0.32	0.30	0	0	0

The seedling root lengths of various cultivars were recorded in table-2. The highest root length was found in VR-900 (3.28 cm), followed by VR-1117 (2.7cm), VR-762 (2.51 cm), VR-1101 (2.48 cm), VR-1076, GPU-67 (2.45 cm), VR-708, VR-1099 (2.41 cm), VR-988 (2.38 cm), VR-847 (2.1 cm), PR-202 (1.98 cm), VR-936 (1.85 cm). Significant decrease in root length with increase in NaCl concentration was observed.

Table3:-Effect of NaCl stress on shoot length in different cultivars of finger millet

Cultivars	shoot length (Cm)										
	Control	500	1000	1500	2000	2500	3000	3500	4000	4500	5000
VR-762	3.55	3.48	2.81	2.16	1.36	0.98	0.47	0	0	0	0
VR-1101	3.52	3.5	3.05	2.41	1.57	1.16	0.55	0.4	0	0	0
VR-900	4.32	3.92	3.14	2.16	1.06	0.82	0.51	0.33	0	0	0
VR-988	3.42	2.13	1.27	0.83	0.39	0.33	0	0	0	0	0
VR-1076	3.49	3.23	2.73	2.16	1.59	1.32	1.14	0.99	0.48	0.4	0.31
VR-847	3.14	2.66	2.14	1.39	1.17	0.85	0.48	0.33	0	0	0
VR-1117	3.74	3.12	2.48	2.10	1.84	1.13	0.77	0	0	0	0
VR-936	2.89	2.15	1.48	1.19	0.95	0.48	0.25	0	0	0	0
VR-708	3.45	3.11	2.74	2.01	1.59	1.10	0.72	0.3	0	0	0
VR-1099	3.45	3.10	2.75	2.19	1.82	1.14	0.59	0.33	0	0	0
GPU-67	3.49	3.21	2.59	2.13	1.73	1.31	0.84	0	0	0	0
PR-202	3.02	2.39	2.11	1.74	1.13	0.95	0.34	0.22	0	0	0

The highest shoot length was observed in cultivar VR-900 (4.32 cm), followed by cultivars VR-1117 (3.74 cm),VR-762 (3.55 cm), VR-1101 (3.52 cm), VR-1076 (3.49 cm), GPU-67 (3.49 cm), VR-708 (3.45 cm), VR-1099 (3.45 cm), VR-988 (3.42 cm), VR-847 (3.14 cm), PR-202(3.02 cm) and least growth was observed in cultivar VR-936 (2.89 cm) under unstressed condition. Increasing salinity levels decreased the shoot lengths. Except one cultivar i.e. VR-1076, remaining cultivars failed to germinate at highest concentration (5000ppm) of NaCl. The shoot length observed at 5000 ppm in cultivar VR-1076 is 0.31 cm, it is less than 10% of control. As NaCl concentration is increased the shoot length was found to be decreased in all cultivars.

Table3:-Effect of NaCl on dry weight in different cultivars of finger millet

Cultivars	Dry weight (mg)										
	Control	500	1000	1500	2000	2500	3000	3500	4000	4500	5000
VR-762	45	43	41	34	30	23	18	0	0	0	0
VR-1101	48	45	45	40	35	53	25	20	0	0	0
VR-900	41	41	37	33	30	28	25	22	0	0	0
VR-988	48	41	31	28	22	20	0	0	0	0	0
VR-1076	53	50	50	45	43	38	35	32	27	23	20
VR-847	53	52	50	45	42	32	27	20	0	0	0

VR-1117	45	45	43	42	36	33	21	0	0	0	0
VR-936	51	51	45	41	35	26	22	0	0	0	0
VR-708	42	40	40	36	30	25	25	20	0	0	0
VR-1099	47	45	43	40	37	30	28	28	0	0	0
GPU-67	42	40	40	34	28	28	25	0	0	0	0
PR-202	40	38	33	29	26	26	22	20	0	0	0

From the table-4 it was observed that with increasing NaCl levels all the cultivars showed decreased quantities of dry weights. Highest dry weight was recorded in VR-1076 and VR-847 (53 mg) followed by VR-936 (51 mg), VR-1101, VR-988 (48 mg), VR-1099 (47 mg), VR-762, VR-1117 (45 mg), VR-708, GPU-67 (42 mg), VR-900 (41 mg), PR-202 (40 mg).

Similar results were obtained by Kaliappan et al., (1967), Panigarh et al., (1978), Onkware (1993) and Anantaraju (2001) while screening finger millet genotypes for salt tolerance at seedling stage.

Salt stress inhibited the seed germination, root length and shoot length. The results help in studying the effect of NaCl stress on plants (Jamil and Rha, 2004).Unbalanced nutrient uptake by seedlings is due to toxic effects $Na^+$and Cl^- ions present in the salt might be the reason for reduction in seedling growth. Decreased water uptake by roots might affect the growth of roots and shoot (Werner and Finkelstein, 1995). The findings of the study are in accord with the findings of Demir and Arif (2003) who reported that the root growth was more adversely affected compared to shoot growth by salinity. Hussain and Rehman (1997) also reported that the roots of seedlings were more sensitive than shoots.

The results obtained in this study deal with the salt tolerance of different cultivars at seedling stage. The tolerance observed in the cultivar VR-1076, at NaCl concentration 5000 ppm and cultivars VR-1101, VR-900, VR-847, VR-708, VR-1099 and PR-202 at NaCl concentration 3500 ppm may or may not be conferred at the adult stage. Nevertheless, tolerance observed at the seedling stage is of great importance because it has been emphasised by many workers that the screening of cultivars for salt tolerance at vegetative stage of plants has considerable value in determining the ultimate tolerance of the species (Akbar and Yabuno, 1974; Asraf and McNeilly, 1987).

Conclusion:-

Based on the germination percentage, root length, shoot length and dry weight, it was observed that cultivars VR-1076, VR-1101, VR-900, VR-847, VR-708, VR-1099, PR-202 were found to be salt tolerant cultivars and cultivar VR-988 was very much sensitive to NaCl. The salt tolerance levels observed in different cultivars of finger millet studied in laboratory may have a considerable value, the cultivars which were observed to grow at salinity levels 3500 ppm-5000 ppm VR-1076, VR-1101, VR-900, VR-847, VR-708, VR-1099, PR-202 need to be tested in field experiments for the conformation.

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Research Article

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Phyto-biological Cu-Zn bimetallic nanoparticles synthesis, characterisation, its applications for photodegradation of malachite green dye and antimicrobial activity

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Abstract: An effortless method was reported for synthesis of Cu-Zn bimetallic nanoparticles by using leaf extract of *Areva lanata* as a reducing and capping agent. Characterization of the prepared nanoparticles was done by UV-Visible spectroscopy, FT-IR, SEM, EDX and TEM. These synthesized Cu-Zn bimetallic nanoparticles were used as photocatalyst for 82.6% degradation of Malachite Green dye by sunlight irradiation under optimum conditions and also used to study the antimicrobial activity against bacteria *Staphylococus aureus, Streptococcus pyogenes, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia* by well diffusion method.

Key words: Bimetallic nanoparticles (BMNPs), *Areva lanata*, Malachite green (MG), Photodegradation, Antimicrobial activity.

INTRODUCTION

Nanotechnology is the wing of science which deals with very small structures of 0.1 to 100 nm in size[1]. In recent year's nanotechnologies and nanomaterial attract tremendous attention because of its huge applications[2]. Synthesis of nanoparticles can be achieved by bottom-up process which comprises

building up of the atoms or molecules. Top-down process includes making tinier and tinier structure by etching from the bulk material[3].Nanoparticles have physical properties like specific area, shape, aggregation and size, state of size distribution, surface morphology and structure containing crystallinity, solubility and defect structure. Nanoparticles have Chemical properties the elemental distribution of nanomaterials and its surface chemistry like photocatalytic properties and zeta potential [4,5]. Physical and chemical properties exhibited by nanoparticles are unique when compared to the bulk material.ls[6]. Nanoparticles used in various fields like medicine, manufacturing material, environmental, electronic and energy[7].

Various metallic nanoparticles such as silver, zinc, magnesium, gold, alginate, silver and copper can be produced by chemical, physical and biological methods[8]. Due to some toxic chemicals absorbed on the surface of metals more harmful effects have been associated with chemical synthesis methods. Biological methods are eco-friendly and best alternative towards chemical and physical methods for the synthesis of nanometals. Nanoparticles synthesized by using plants or plant extracts[9, 10], microorganisms[11,12], fungus[13] and enzymes[14] are developed as ecofriendly and important branch of nanotechnology. Bimetallic nanoparticles consists of two different metals have more concern when compared to monometallic nanpoparticles from both technological and scientific point of view[15, 16]. Now a day's biosynthesized bimetallic nanoparticles are used in imaging, labeling, luminescence tagging, drug delivery and biomedical field because of their rapport in *invivo* are screening[17]. Biosynthesized bimetallic nanoparticles also have great catalytic activities[18].

Environmental pollution is one of the major and most urgent problems of the modem world. Textile industry releases coloured water during fabrics dyeing process and industries such as dyestuff, distilleries, tanneries and paper mills are also releases highly coloured waste water into environment which causes pollution[19].Malachite Green (MG) is a cationic green crystalline dye belongs to triphenylmehane group and water soluble[20]. MG is largely used wood, leather, jute, silk and cotton industries for dyeing. When MG enters into water bodies and food chain because of its carcinogenic and mutagenic nature MG can cause toxic effects on living organisms[21]. For elimination of dyes photocatalytic process is more advantageous and can degrade many organic chemicals when compared to other degradation processes of dyes.

Traditional methods like herbal extracts used to the synthesis nanometal particles have shown extensive consent in medicine. These synthesized nanometal particles have great bactericidal activity than bulk metals because of its adsorption at bacterial surface[22].Metals like copper, silver, gold, nickel etc., nanometal are hypothesized to be able to participate in sub-cellular reactions as their size is comparable to biological molecules[23].Bimetallic nanoparticles composed of two different metals have drawn a greater interest than the monometallic nanopaticles due to the properties differ from pure elemental particles include unique size dependent, optical, electronic, thermal, catalytic and biological effects[24].

Herein, an effortless method for synthesis of Cu-Zn bimetallic nanoparticles (BMNPs) by using leaf extract of *Areva lanata* as a reducing and capping agent was reported and by using these BMNPs photodegradation of MG dye by solar light irradiation was also studied.

EXPERIMENTAL

1. Materials: Chemical reagents used (copper sulphate and zinc sulphate) in this study were of analytical grade. Deionised water was used to clean glassware, prepare chemical solutions and throughout experimental procedure. Fresh leaves of *Areva lanata* were collected from agricultural fields located at Neelavathi village in Vizianagaram district, Andhra Pradesh, India.

2. Preparation of plant leaf extract: 100g of fresh leaves were weighed and thoroughly cleaned with running tap water to eliminate debris on surface of leaves followed by deionised water to remove other contaminants from leaves and dried up under shade for 9 days. These leaves were sliced into tiny pieces and made powder by using home blender. The obtained powder placed in refrigerator at 4^{o}c which was taken in air tight container. Now 200 mL deionised water was taken in 500 mL beaker to this 10g stored powder weighed and added. The contents in the beaker boiled for 30 minutes with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled leaf broth was filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4^{o}c. This was taken as leaf extract throughout the experiment.

3. Synthesis of Cu-Zn bimetallic nanoparticles: Equimolar (25 mM) concentrations of copper sulphate and zinc sulphate solutions were prepared separately in 100 ml volumetric flask by dissolving 0.6242 gms, 0.4368 gms weight of $CuSO_4$ and $ZnSO_4$ respectively. Synthesis of Cu-Zn BMNPs was done by taking 50mL of copper sulphate solution in beaker to this 90ml of leaf extract was added drop wise through burette. 50mL of zinc sulphate solution was added to contents in the beaker by drop wise. During addition process beaker was placed on a magnetic stirrer for continues agitation. Now this mixture was heated up to 50^{o}c for 40 minutes. These synthesized BMNPs were accomplished by doing centrifugation at 6000 rpm for 15 minutes. The obtained BMNPs were washed by using deionised water 2 times to remove unwanted constituents. The resultant BMNPs particles used for characterization.

4. Photodegradation experiment: Photocatalytic activity of Cu-Zn BMNPs was examined for degradation of malachite green dye. Initially, 50 ppm of malachite green stock solution prepared. Then Reaction mixtures were prepared by adding certain amount of Cu-Zn BMNPs to 100 mL of malachite green distinct concentrations. The pH of the reaction mixtures were altered by adding H_2SO_4 or NaOH solutions when required. Now this mixture was agitated for 30 minutes in dark condition to attain adsorption-desorption between malachite green and Cu-Zn BMNPs. Sun light used as irradiating source to reaction mixture for study degradation during 11am to 2 pm. At regular 10 minutes time intervals aliquot of the reaction mixture is collected, centrifuged to remove the photocatalyst particles and analyzed for absorbance using UV-Visible Spectrophotometer (200 nm to 800 nm). The effect of concentration of BMNPs on catalyst loaded and also based on concentration of dye was observed. Malachite green shows the highest absorption[25] at 617nm. To determine the percentage of degradation of MG solution following equation {1} was used.

$$\% \text{ degradation} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100 \quad \ldots \quad \{1\}$$

Where, A_0 is the initial absorbance of the MG solution at 0 minutes and A_t is the absorbance of the degraded solution after time t minutes[26].

5.Characterization: Formation of Cu-Zn BMNPs was confirmed by UV-Visible absorption spectra are measured using UV-2450 SHIMADZU double beam spectrophotometer, FTIR using Bruker, SEM, EDX studies are done by using Hitachi S-3700N machine and the morphology of BMNPs was done by TEM analysis Jeol/JEM 2100 machine

RESULTS AND DISCUSSION

1. UV-Visible spectral analysis: UV-Visible absorption spectra of Cu-Zn BMNPs is revealed in Figure.1.The characteristic surface plasmon resonance (SPR) band observed in Cu-Zn BMNPs which is not observed for mono Cu and Zn nanometals[27-29].

Figure.1: UV-Visible absorption spectra of Cu-Zn bimetallic nanoparticles

2. FTIR spectral analysis: FTIR describes chemical bonds in molecules by generating an infrared absorption spectrum. FTIR measurements are used to identify different functional groups present in biomolecules responsible for the bioreduction of Cu^{+2}, Zn^{+2}precursors and capping/stabilization of copper zinc BMNPs. The intense, broad bands were observed and compared with standard values to analyze the functional groups in *Areva lanata* leaf extract and biosynthesized Cu-Zn BMNPs. FTIR spectrum of *Areva lanata* leaf extract and synthesized Cu-Zn BMNPs by using *Areva lanata* leaf extract were shown in **Figure.2 (a) and Figure.2 (b).** The comparison of the FTIR spectra of both Cu-Zn BMNPs and leaf extract of *Areva lanata* clearly indicates the existence of the plant extract phytomolecules on the surface[30] the Cu-Zn BMNPs.

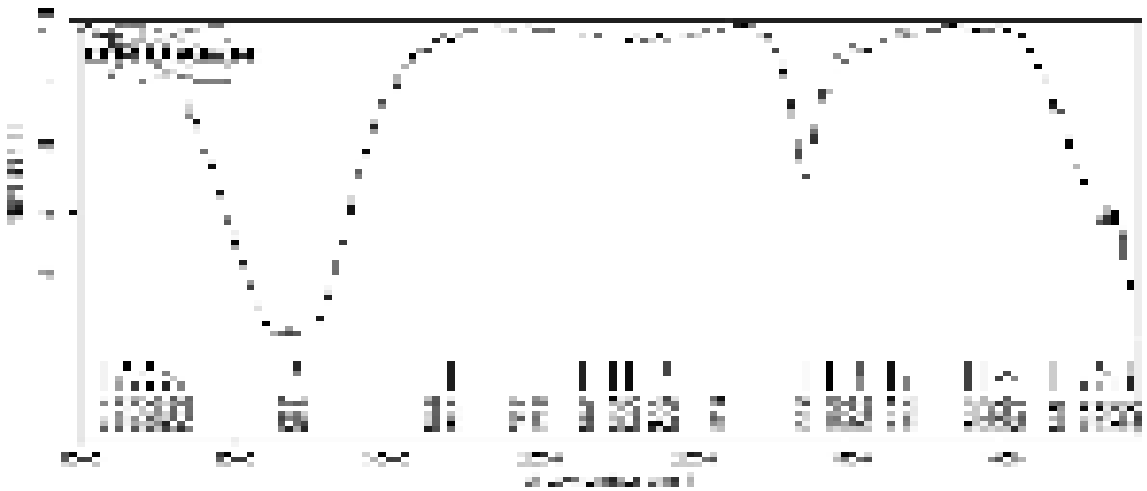

Figure.2 (a): FTIR spectrum of Areva lanata leaf extract

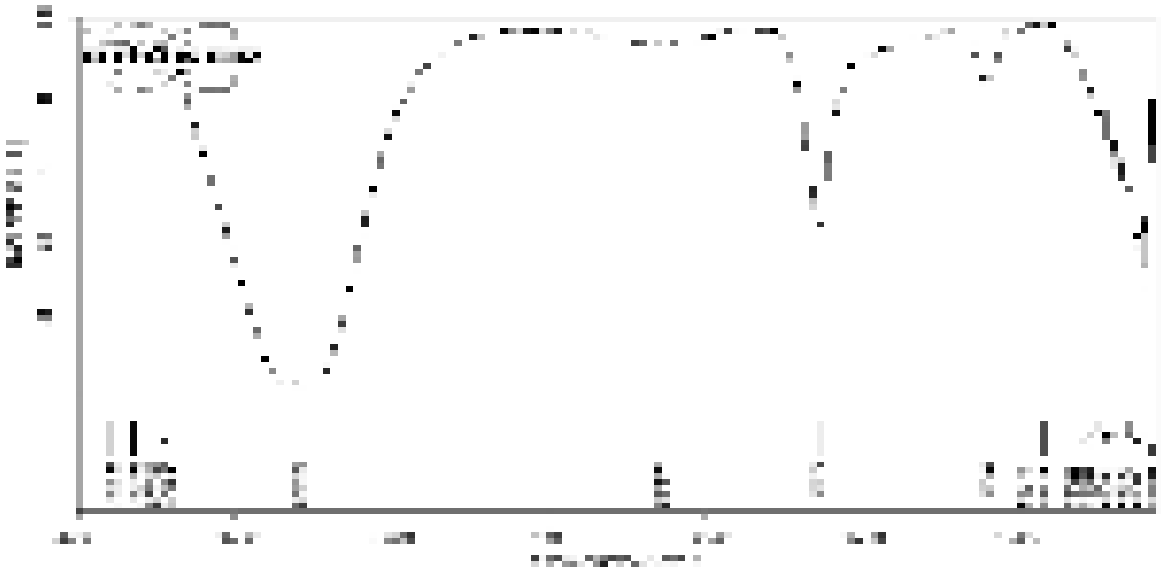

Figure.2 (b): FTIR spectrum of Cu-Zn bimetallic nanoparticles

The spectrum of *Areva lanata* leaf extract shows major peak positions at 3329, 3290, 2860, 1640, 1556, 1019, 825 and 690-570cm^{-1}.A broad and intense band at 3329 and 3290cm^{-1} are due to O-H vibrations of phenols, alcohol and carboxylic acid group or N-H stretching vibration of amines present in the extract the other peaks at 2860, 1640, 1556, 1019, 825 and 690-570cm^{-1}corresponds to vibrations of C-H of aldehyde, C=O stretching, N-H bending of amines, C-O stretching, C-Cl groups respectively. The spectrum of Cu-Zn BMNPs shows major peak positions at 3296, 2141, 1637, 1098 and 746-582cm^{-1}. The change in band between 690-570 cm^{-1} to 746-582 cm^{-1} shows presences of Cu-Zn nanoparticles which are not observed in individual nanoparticles[31,32].

3. SEM and EDX analysis: Figure.3 shows scanning electron microscopic images of Cu-Zn BMNPs with various magnifications. From this, it can be clearly noticed that bimetallic nanoparticles prepared are in size of <100 nm in diameter From energy dispersive X-ray can analyze all the elements present in prepared BMNPs by *Areva lanata* leaf extract. **Figure.4** and **Table.1** shows EDX spectrum and elemental composition indicates the presences of Cu, Zn and O which confirms the formation of Cu-Zn bimetallic nanoparticles. The standard electrode potential of Zn^{2+} is less than of Cu^{2+}. Therefore Cu remains in the reduced form Cu^0 while the Zn metal encapsulates copper in its Zinc oxide from. This is also supported by the EDX study.

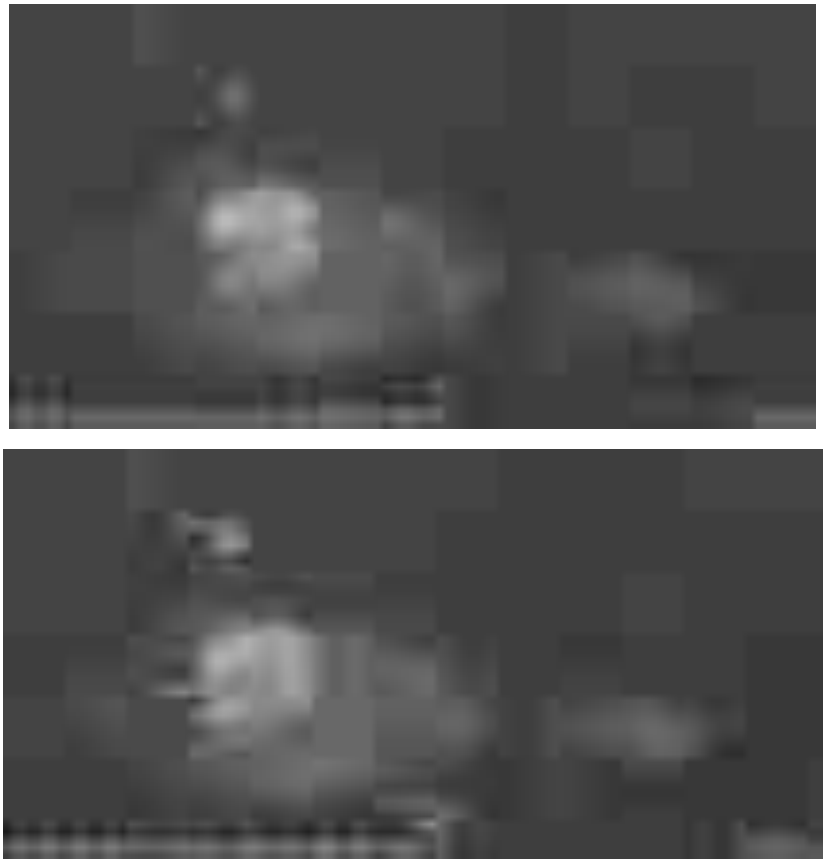

Figure 3: SEM images of Cu-Zn bimetallic nanoparticles

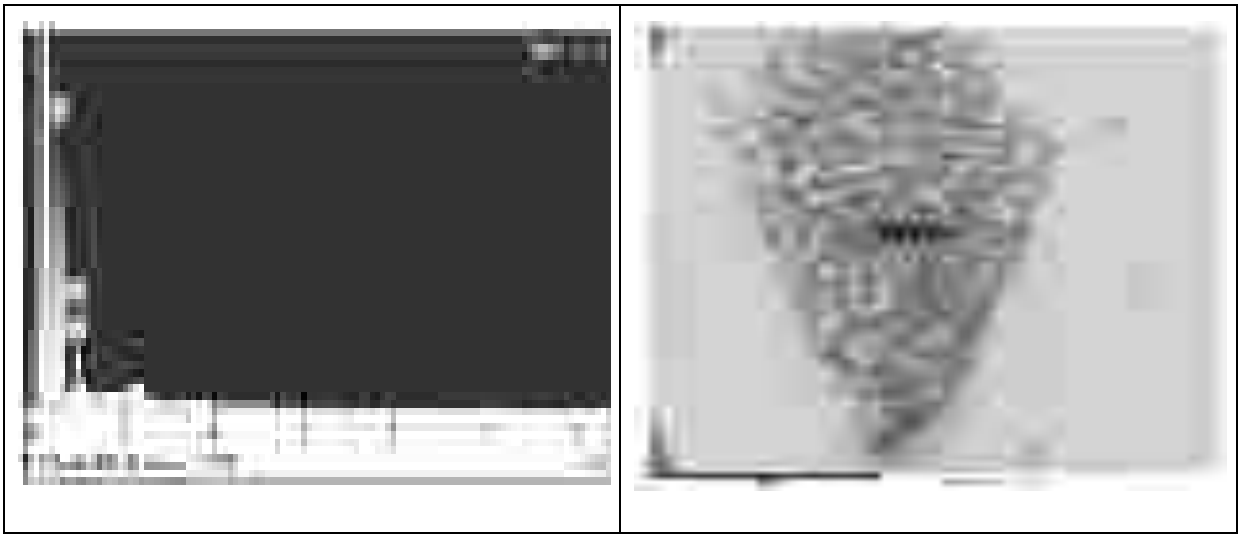

Figure.4: Cu-Zn bimetallic nanoparticles EDX spectra

Table 1: Elemental analysis of Cu-Zn bimetallic nanoparticles

Element	Weight%	Atomic%
O K	74.95	92.28
Cu L	19.92	6.17
Zn L	5.13	1.55
Total	100	

4. TEM analysis: Figure.5 shows the transmission electron microscopy (TEM) images and selected area electron diffraction (SAED) pattern for synthesized Cu-Zn BMNPs from *Areva lanata* leaf extract. From these images, it was observed that Cu-Zn BMNPs were formed with spherical morphology and crystalline structure below 100 nm in size. This is in good agreement with SEM images.

Figure 5: TEM and SAED images of Cu-Zn bimetallic nanoparticles

5. Photodegradation of MG: To study the photocatalytic activity of the Cu-Zn BMNPs on malachite green visible region in UV-visible spectrophotometer was used. Absorption spectrum of 10 ppm malachite green was shown in **Figure 6(a).** Highest absorption peak at 617 nm was observed and taken as to monitor the photodegradation of malachite green dye.

Effect of contact time: Photodegradation capacity of the Cu-Zn BMNPs on malachite green dye was studied by batch mode experiments. The efficiency of BMNPs on degradation of MG is increased by increasing contact time. The effect of contact time is carried out by taking 10 ppm of 100 ml MG and 10 mg of BMNPs which is shown in **Figure 6(b).** An optimum contact time of 90 minutes is taken for MG dye degradation onto BMNPs. Initially, degradation of dye by using BMNPs is begun to be fast and then become constant with the increase of contact time[33]. This is due to strong attractive forces between the dye and the BMNPs and also all BMNPs sites were vacant at during initial period but after some time it steadily approached about consistent value when equilibrium was reached because of decrease in the number of vacant sites being available for further dye removal[34,35].

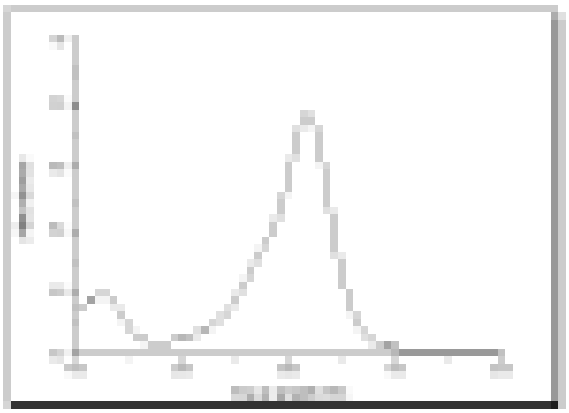

Figure.6 (a): Absorption spectrum of 10 ppm Malachite Green dye

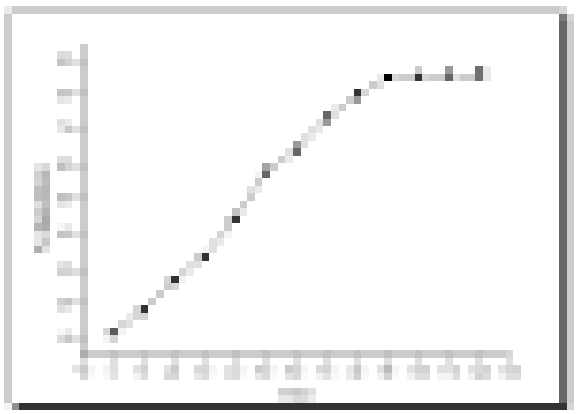

Figure.6 (b): The effect of contact time on % of degradation

Effect of concentration of MG: Initial concentration of MG effects photodegradation rate. For this, concentration of BMNPs was kept constant at 10 mg and time of irradiation 90 minutes. Different initial concentrations 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm of MG dye solutions were prepared. The rate of degradation can be shown graphically in **Figure 6(c).** The maximum degradation observed at 5 ppm then decreases by increase in the concentration of MG. As the MG dye initial concentration increases the % of degradation decreases[36,37]. This is due to large dye molecules were adsorbed on surface of BMNPs at initial concentration of dye. As the increase in dye concentration, intense coloured solution was formed which decreases the path length of photons entering into solution there by only fewer photons attain BMNPs surface. Therefore, the % of photodegradation reduced when concentration of dye is high there by the path length was further decreased[38,39].

Effect of pH:pH of dye solution can influence the adsorption of dye on photocatalyst. Originally, Malachite green dye solution is having pH value at 5. The initial concentration of malachite green solution 10 ppm and the concentrations of the photocatalyst 10 mg were kept constant with time of irradiation 90 minutes. Different solutions of various pH values of 4 to 9 were prepared. Degradation efficiencies were compared after 90 minutes which was shown in **Figure 6(d).**

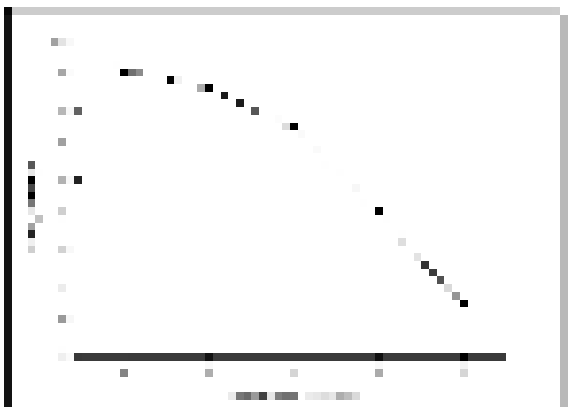

Figure.6 (c): The effect of concentration of dye on % of degradation

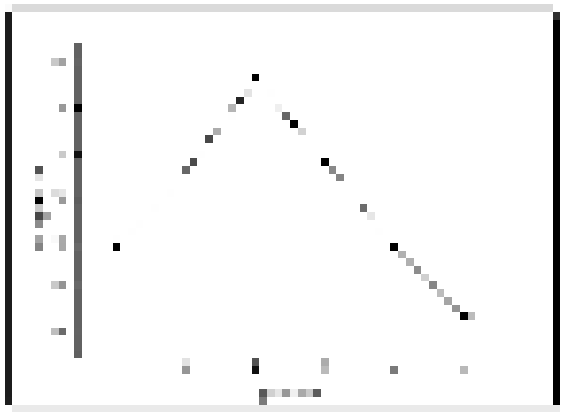

Figure.6 (d): The effect of pH on % of degradation

It is observed that by increasing pH of the MG dye solution, the degradation of MG dye on the photocatalyst enhances up to pH 6 and then onwards decreases. Upto pH 6 i.e. under acidic conditions, the catalyst has stabilized semiconductor structure which help in the photo induced degradation of the dye. But after pH 6 i.e. in neutral and even in basic conditions, the surface structure of the semiconductor undergoes changes so that it does not support the degradation process. This may be because of the surface either gets reduced to zinc oxides.

Effect of dose of photo catalyst: In photodegradation process, one among parameters of decolorizing of dye solution is photocatalyst dosage[40]. To avoid wastage and provide the entire absorption of photons optimization of catalyst dosage is important[41]. For this dosage amount was varied from 10 mg to 80 mg taken in 100 ml of 10 ppm MG dye at pH 6 with contact time 90 minutes. The degradation of MG was shown in **figure 6(e).**

This concluded that, by increasing dosage of catalyst from 10 mg to 30 mg in 100 ml, the degradation of MG dye enhances because the increase in amount of catalyst would increase the reactive sites that produce more reactive species[42]. Further increase of catalyst dosage 40 mg to 80 mg degradation of MG dye decreases because the suspension has more turbidity, which reduces the solar light penetration[43,44]. 10 mg of catalyst was taken as optimum dosage for degradation.

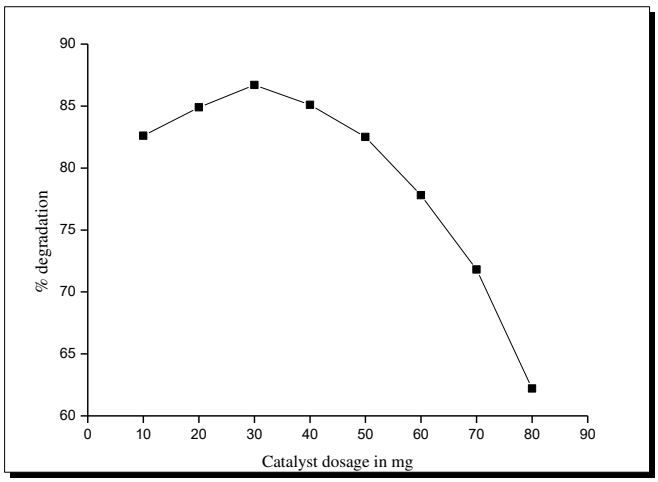

Figure.6 (e): The effect of dosage of catalyst on % of degradation

6. Antimicrobial activity of Cu-Zn BMNPs: The Cu-Zn BMNPs are used in antibacterial screening *in vitro* for six organisms *Staphylococus aureus, Streptococcus pyogenes, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia* by well diffusion method and the obtained results are given in **Table 2** and illustrated in **Figure 7.** The Cu-Zn BMNPs inhibit the growth of different bacteria. The Cu-Zn nanoparticles showed maximum inhibition on *Klebsiella pneumonia* (17mm), followed by *Escherichia coli* (15mm), *Staphylococcus aureus* (14mm), *Micrococcus luteus* (12mm), *Pseudomonas aeruginosa* (6mm) and minimum antibacterial activity on *Streptoconccus pyogenes* (2mm). Cu-Zn BMNPs show high antibacterial activity agaist *Klebsiella pneumonia*. Cu-Zn BMNPs show better activity on *Klebsiella pneumonia* than standard Rifampicin drug.

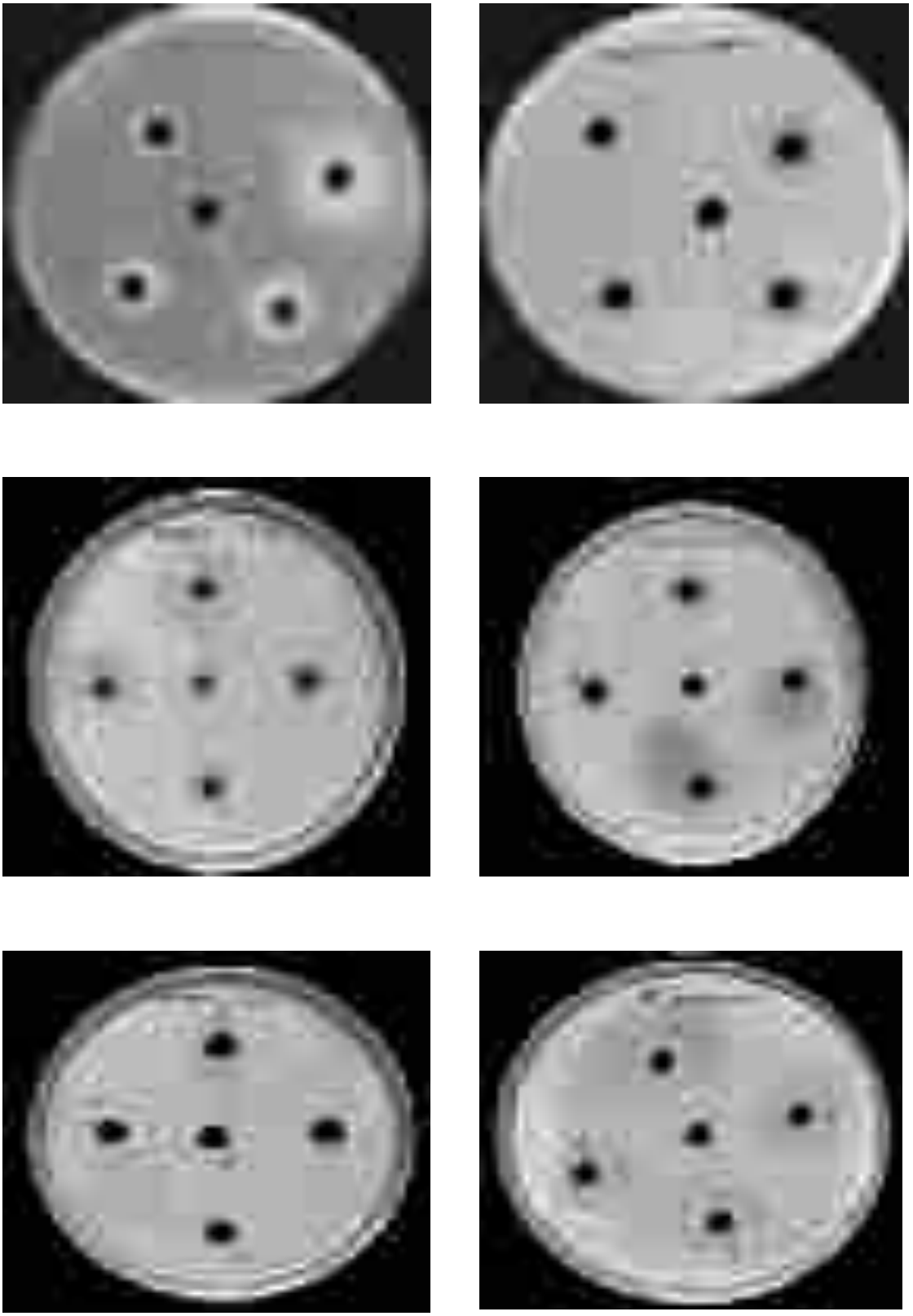

Figure 7: Antibacterial images of Cu-Zn BMNPs against *Staphylococcus aureus, Streptococcus pyogenes, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Table 2: Antimicrobial activity of Cu-Zn BMNPs

Bacteria	**360 mg/mL**	**180 mg/mL**	**90 mg/mL**	**45 mg/mL**	**Activity**
Staphylococcus aureus	14 mm	11 mm	8 mm	4 mm	Moderate
Streptococcus pyogenes	2 mm	1 mm	Nil	Nil	Low
Micrococcus luteus	12 mm	9 mm	3 mm	1 mm	Moderate
Escherichia coli	15 mm	10 mm	5 mm	3 mm	Moderate
Pseudomonas aeruginosa	6 mm	3 mm	Nil	Nil	Low
Klebsiella pneumonia	17 mm	12 mm	10 mm	5 mm	Moderate

The solvent control of methanol and DMSO had no effect on bacterial growth of antibacterial drug Rifampicin have varied activity against bacteria tested. Rifampicin has shown highest activity, ZOI (zone of inhibition), on *Micrococcus luteus, Staphylococcus aureus* on concentration of 5 mg/ml. The Zone of inhibition values of standard drug Rifampicin shown in **Table 3**.

Table 3: ZOI values of standard drug Rifampicin

Bacteria	**ZOI values**	**Activity**
Staphylococcus aureus	22 mm	High
Streptococcus pyogenes	Nil	No
Micrococcus luteus	42 mm	High
Escherichia coli	21 mm	High
Pseudomonas aeruginosa	Nil	No
Klebsiella pneumonia	10 mm	Moderate

CONCLUSION

An environmentally friendly method is projected to synthesize bimetallic Cu-Zn nanoparticles from *Areva lanata* leaf extract. From SEM and TEM analysis results, the formed Cu-Zn BMNPs are in spherical morphology and crystalline structure with size below 20 nm. From EDX study the BMNPs appears to be in the form of Cu-ZnO due to the presence of excess of oxygen and also the synthesis reation being an open system. The photocatalytic activity of these nanoparticles is examined under sunlight for degradation of MG dye which is environmental pollutant. The % photodegradation of MG

dye changes with parameters such as contact time, concentration of MG dye, pH, photocatalyst dosage. From this research study on bimetallic Cu-Zn BMNPs synthesized from *Areva lanata* leaf extract, the optimum conditions found in the degradation of MG dye is pH 6, weight of catalyst 10 mg, dye concentration of 10 ppm and contact time of 90 minutes. The degradation is obtained as 82.6% under these optimum conditions. . Synthesized Cu-Zn BMNPs showed antimicrobial activity against six bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*). Cu-Zn gave better results against *Klebsiella pneumonia* among six bacteria than standard drug Rifampicin.

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"Development Of Silver Nanoparticle Loaded Green Activated Carbon As An Efficient Composite Material For Removal Of Pathogenic Bacteria In Drinking Water Samples Of Mudasarlova Reservoir In Visakhapatnam."

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ABSTRACT

The present work highlights the high efficiency of silver nanoparticles (Ag-NPs) coated onto activated carbon (AC) granules in antimicrobial activities for water purification. Silver nanoparticles (Ag-NPs) were prepared by chemical reduction method. The formation of silver nanoparticles was monitored using UV-Vis spectroscopy. Green Activated carbon (AC) granules were synthesized from agricultural waste, the rice husk. The green activated carbon granules were coated with silver nanoparticles by impregnation of AC in super saturation solutions with different concentrations of Ag-NPs. The resulted Ag-NPs/AC were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The antimicrobial susceptibility of the synthesized Ag-NPs/AC was investigated using inhibition zone and shake flask techniques against E. coli. The inhibition zone diameter increased with increasing NPs concentration. Thus, the minimum inhibition zone was 9 mm at Ag-NPs/AC (50 µg/g) whereas the maximum inhibition zone diameter was 20 mm at Ag-NPs/AC (500 µg/g). In the Shake flask test, it was observed that the number viable bacterial cells incubated with the nanoparticles reduced drastically within 1 hour after treatment, while a 100% reduction in *E.coli* count was found after 3 hours incubation. This study reveals that the silver nanoparticle coated activated carbon can achieve effective antibacterial activity and can be efficiently utilized as an efficient composite material for removal of pathogenic bacteria in drinking water samples.

Keywords: Green Activated Carbon. Silver Nanoparticles. Antimicrobial activity. Water purification.

INTRODUCTION

Removal of microorganisms from drinking water to obtain potable water is a critical requirement in various societies across the world, and particularly in India. World Health Organization (WHO) investigation showed that 80% of diseases are due to contaminated drinking water. For that reason, there is an urgent need to re-evaluate conventional disinfection methods and to consider innovative approaches that enhance the reliability and robustness of disinfection. Water quality improvements inspired by these goals are currently focused on reducing diarrheal illnesses and, hence, are focused on biological contamination and related pathogen removal. To meet the increasing need for potable water, the use of nanotechnology has been increasing in recent years. Advancements in nanotechnology are being applied in the water purification industry, to keep harmful bacteria out of drinking water.

While the effectiveness of Activated Carbon (AC) to act as adsorbents for a wide range of contaminants is well documented (Junaid Saleem *et al*., 2019), the activated carbon is not easily and economically available because of its expensive cost. This has led to a search for low-cost and locally available materials, which could be considered the source of adsorbents. Hence, the need for production of activated carbon from locally available agricultural wastes is gaining prominence as they offer the most available and cheapest of all the known raw materials (Kushwaha *et al*., 2013; Rafatullah *et al*., 2013).

However, carbon on its own does not remove or kill microorganisms. Silver nanoparticles (AgNPs) deposited or impregnated materials (metal, polymer, metal oxide, carbon, cellulose) have been widely investigated for their unique physico- chemical properties such as optical, electrical, catalytic and anti-microbial, due to their unusual interfacial effects. Among the available metal nanoparticles, silver and related materials have been utilized in many nano based commercial products for their antimicrobial property. Studies suggested that the antimicrobial performance is enhanced due to an increased surface area/reduced particle size. Hence, an intensive research effort has been made to introduce AgNPs in various materials for the effective antimicrobial properties. Recently, nanocomposites with multifunctional properties receive more attention as they represent a novel approach for the development composite films with antimicrobial activity. Hence, in order to impart antimicrobial properties to activated carbon, it is necessary to establish the maximum possible contact area between Ag with any microorganisms present in the water flowing through the activated carbon (Abdel Hameed *et al*., 2013). We expect this to be achievable, if Ag is coated as nanoparticles on the external surface of the granules of AC.

In the current proposed research, we will address different parameters – isolation of the water borne *E. coli* from the Mudasarlova Reservoir, a drinking water supply system for Visakhapatnam city in Andhra Pradesh and the synthesis of silver nanoparticles coated activated carbon from agricultural wastes. Finally, we will investigate the efficacy of silver coated activated carbon (Ag-AC) as a composite material for the elimination of *E. coli* in drinking water samples.

MATERIALS AND METHODS

Collection of water samples and isolation of water borne *E.coli*

Water samples were collected from the Mudasarlova Reservoir, a drinking water supply system for Visakhapatnam city in Andhra Pradesh. The water samples were serially diluted and inoculated onto the nutrient agar media and incubated at 37ºC for 24 hrs. The obtained bacterial colonies were observed and *E. coli* were isolated based on the macroscopic and microscopic morphological methods as per Bergey manual. *E. coli* was purified by quadrant streaking on nutrient agar and maintained on the same medium as a working culture, while the stock was maintained in 10% glycerol at -20°C.

Synthesis of Green Activated Carbon from Agricultural wastes

Based on the main objectives of this research, as raw materials to produce activated carbon, rice husk was collected from the local market. The rice husk was placed in the furnace for thermal activation at about 500°C for

5 hours. Carbonization was followed by activation with treatment with 25% solution of $CaCl_2$ for 24 hours. The activated carbon was then rinsed thoroughly and placed in an oven at approximately 100°C.

Preparation of silver nanoparticles

Silver nanoparticles were prepared according to the chemical reduction method adapted by Fang, *et al*., (2005). 50 ml of 3 M silver nitrate was prepared, and then heated till boiling and 5 ml of 1% tri-sodium citrate was added drop by drop. The solution was mixed vigorously and heated until the colour changed to pale brown followed by stirring until cooled to room temperature. The aqueous solution was air dried up to 4 days so as to obtain a powdered form of sliver nanoparticles.

Coating of Silver Nanoparticles onto the Activated Carbon Granules:

The activated carbon granules were impregnated in 20 ml of silver nanoparticles solution of different concentrations (0.1, 1.0 and 1.5 mol/L) under vigorous stirring at room temperature overnight to make sure the coating was complete. The activated carbon coated with silver were then cured in a vacuum oven at 110°C for at least 2h to allow full coating of the silver nano-particles onto the activated carbon.

Characterization of Ag-AC

The ultraviolet–visible (UV–Vis) spectra was used for structural characterization of silver nanoparticles. The crystalline structure of Ag-Ac was examined by X-ray diffractometry (XRD) and Scanning electron microscopy (SEM).

Antibacterial test

Silver nanoparticles impregnated activated carbon was tested for their antibacterial effect against waterborne pathogenic *E.coli* under test. If this organism is killed, as a standard, all other borne-disease-causing organisms are assumed killed.

(a) Plate Assay Method (Qualitative test)

The *E.coli* seeded agar plates were prepared and seven mm diameter holes are made in the seeded agar using a sterilized cork borer. Different concentrations (50 µg/g, 100 µg/g, 250 µg/g and 500 µg/g) of Ag-AC particles were added in the holes and incubated at 37^{o}C for 24 hours and the antibacterial effect is measured referring to the inhibition zone diameter. The inhibition zone of Ciproflaxin 20µg is considered as control.

(b) Shake flask test in saline (Quantitative test)

For the shake flask test, 50 ml of sterile saline (0.9% NaCl) was inoculated with 1 ml bacterial suspension. 50 mg of Ag-AC nanoparticles were added to the flask and the contents were stirred on a rotary shaker at 37 ºC. The samples were drawn periodically (0, 1, 3 & 24 hrs) from the flask and tested for the surviving *E.coli* by plate count method on M-Endo agar using standard procedures.

RESULTS AND DISCUSSION

Isolation of water borne *E.coli*

The three basic tests to detect coliform bacteria in water i.e. presumptive, confirmed and completed were performed sequentially for each water sample by means of the most probable number tests (MPN). Measured aliquots of the water to be tested were added to a lactose broth (LB). These tests detect the presence of coliforms, Gram negative, non-spore forming bacilli that ferment lactose broth with the production of acid and gas that is detectable following a 24 h incubation period at 37°C. After the presumptive analysis, bacterial strains that showed close resemblance with E. coli were selected and analyzed for colony and cell morphology. Strains that differ in morphology were selected and streaked on their respective medium such as Eosin Methylene Blue Agar (EMB) or nutrient agar. Finally, bacterial strain that was sorbitol non-fermenting E. coli were selected for further study.

Characterization of Ag-AC

Silver nanoparticles coated green activated carbon granules were prepared as mentioned in the above section. The structural characterisation of silver nanoparticles was monitored using UV-Vis spectroscopy. A single broad peak centered at 430 nm in Figure 1 was observed that corresponds to the formation of silver nanoparticles (Lee *et al*., 2007). The crystalline structure of Ag-AC was examined by X-ray diffraction (XRD) Philips Model PW 3710. Scanning electron microscopy (SEM) observations (Figure 2) were carried out on a JEOL JSM5900LV equipped with an OXFORD EDX probe. For SEM analysis, the loaded activated carbon sample was coated with a thin film of Au. The XRD pattern of impregnated Ag over active carbon showed amorphous carbon phase and silver in metallic phase (Kumar *et al*., 2004). All prominent peaks in Figure 3 at respective 2θ values known for zerovalent fcc silver representing the 111, 200 and 220 crystal planes due to Bragg's reflections are present (Khanna *et al*., 2007).

Figure 1: UV-Vis Spectra of Silver Nanoparticles (AgNPs)

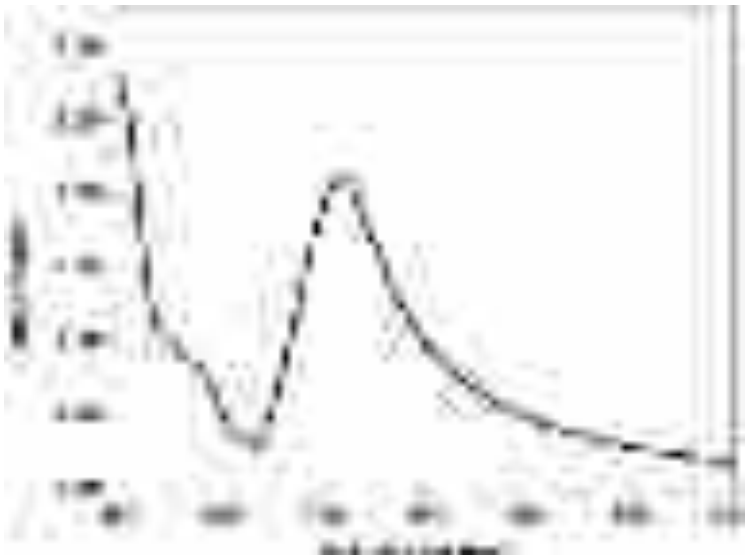


Figure 2: SEM of Ag-AC

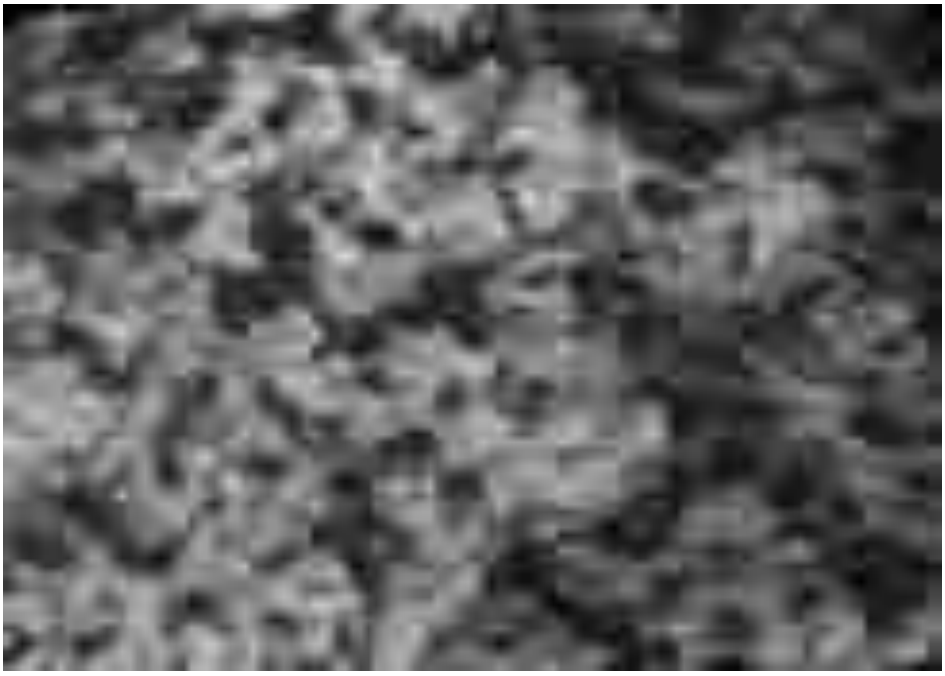
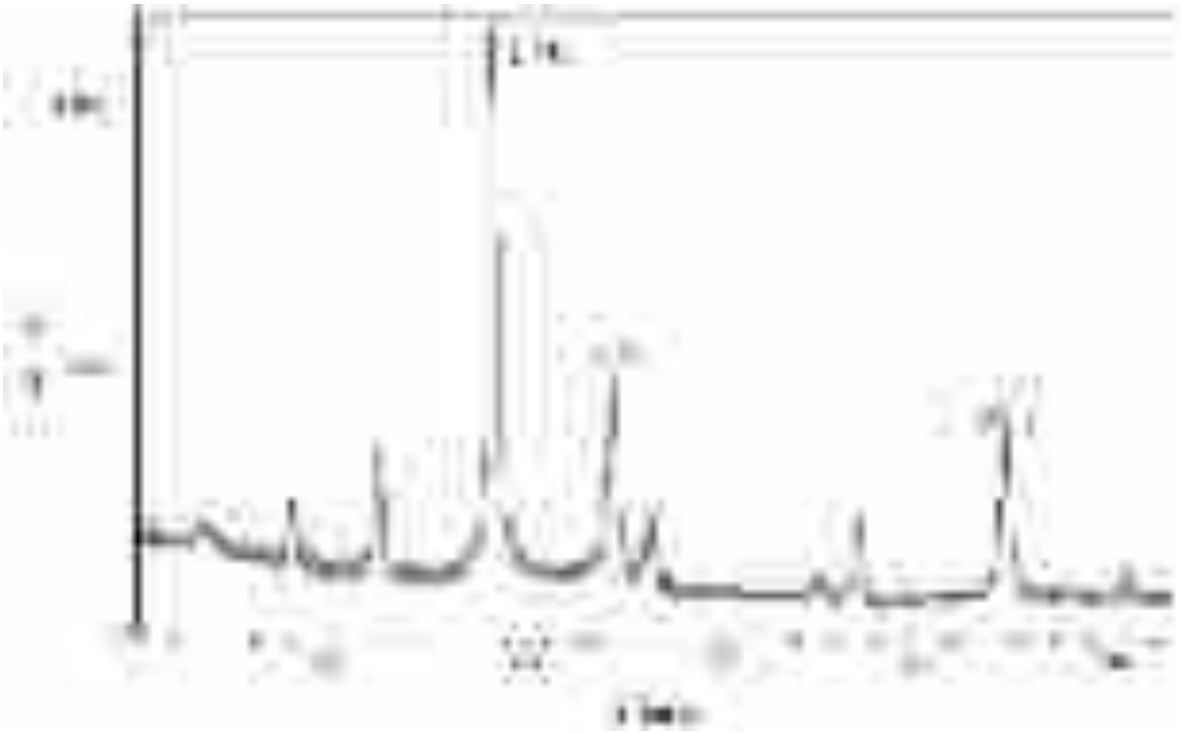


Figure 3: X-ray diffraction (XRD) of Ag-AC



Antibacterial test

(a) Plate Assay Method (Qualitative test)

The antimicrobial susceptibility of synthesized Ag-NPs/AC was investigated using inhibition zone. The diameter of inhibition zone (in mm) around the different concentrations of Ag-NPs granules with E.coli are shown in Table (1) which shows the inhibitory effect of silver impregnated activated carbon. It is revealed that inhibition of E.coli was obtained with all concentrations (Figure 4). The inhibition zone diameter increased with increasing NPs concentration. Thus, the minimum inhibition zone was 9 mm at Ag-NPs/AC (50 µg/g) whereas the maximum inhibition zone diameter was 20 mm at Ag-NPs/AC (500 µg/g).

Table 1: Inhibition zone (mm) test results for E.coli with different concentrations of Ac/Ag NPs granules.

Ag-NPs/AC 50 µg/g	Ag-NPs/AC 100 µg/g	Ag-NPs/AC 250 µg/g	Ag-NPs/AC 500 µg/g	Ciproflaxin 20µg
9.00	11.00	18.00	20.00	29.00

Figure 4: : Inhibition zone for E.coli with different concentrations of Ac/Ag NPs granules

(b) Shake flask test in saline (Quantitative test)

In the Shake flask test (Table 2) to determine the antibacterial effect of the Ag-AC particles against E.coli, it was observed that the number viable bacterial cells incubated with the nanoparticles reduced drastically within 1 hour after treatment, while a 100% reduction in E.coli count was found after 3 hours incubation. Thus the activated carbon coated silver nanoparticles showed efficient antibacterial activity against E. coli that was similar to that found by Rastogi *et al*., 2011.

Table 2: Total viable count as affected by the exposure time to different nanoparticles, using shake flask test.

Contact time (hr)	CFU/ml x 10^4
0	100.00
1	14.0
3	0.0
24	0.0

CONCLUSION:

In this study, silver nano-particles were coated onto green activated carbon from rice husk. The prepared Ag-AC NP were characterized by UV-Vis spectrophotometer, XRD and SEM. The active antibacterial property was measured by the clear zone formed by the silver containing activated carbon granules on the plate. The batch method showed that with the increase in contact time and the decrease in the bacterial count and complete inhibition was achieved. This study reveals that the silver nanoparticle coated activated carbon can achieve

effective antibacterial activity and can be efficiently utilized as an efficient composite material for removal of pathogenic bacteria in drinking water samples.

Acknowledgments

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Performance Of Various Financial Institutions In Implementing PMMY – An Analytical Study

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ABSTRACT

Pradhan Mantri Mudra Yojana(PMMY) is a scheme launched by the Honorable Prime Minister Sri Narendra Modi on 8th April 2015 for providing formal credit to small and micro enterprises. Micro Units Development and Refinancing Agency(MUDRA) Ltd., is a refinancing agency. MUDRA provides financial support to Micro and Small units through Commercial Banks, NBFCs, RRBs and various other financial agencies. This paper aims to analyze the performance of various financial Institutions in the implantation of MUDRA scheme by using the data providedby the Annual Report 2017-18 of PMMY.

Micro, Small and Medium Enterprises play a vital role in the Indian economy as they contribute large share in Gross Domestic Product, employment and exports. Despite of the contribution of MSMEs to Indian economy, very few of them have access to formal credit. In 2015 Central Government initiated the Pradhan Mantri Mudra Yojana to bring the micro and small enterprises into the scope of organized credit.

Types of MUDRA loans:

The MUDRA loans can be classified into 3 categories.
1.Shishu: Covering loans up to RS. 50000
2.Kishor: Covering loans above Rs. 50000 and up to Rs. 5 lakhs.
3.Tarun: Covering loans above Rs. 5 lakhs and up to Rs. 10 lakhs.

The loans under Mudra Scheme are guaranteed by Credit Guarantee Fund for Micro Units(CGFMU). This guarantee cover is available for 60 months.

The financial support under Mudra scheme can be divided into two types. They are Micro Credit scheme and Refinance scheme.

The Micro Credit Scheme is implemented mainly through Micro Financial Institutions(MFIs). MFIs provide the credit up to Rs. 1 lakh for various micro and small business units. The credit will be provided to individuals and Self Help Groups.

Refinancing Scheme is operated through Commercial Banks, Regional Rural Banks, Non Banking Financial Companies(NBFCs) and Small Finance Banks.

Types of Financial Institutions and their performance:

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Table 1

Various Financial Institutions &their performance during 2016-17 and 2017-18

Rs. In crores

Agency	Sanctioned amount 2016-17	Sanctioned amount 2017-18	Growth rate (%)
Public Sector Banks	71953.67	92492.68	29
Private sector Banks	39042.60	49545.11	27
Regional Rural Banks	12099.52	15454.51	29
Small Financial Banks	6729.01	19022.89	183
Micro Finance Institutions	45338.22	50143.75	11
NBFCs	5455.53	27018	396
Total	180528.55	253677.10	41

The following observations can be made with the given data.

Public Sector Banks: The public sector Banks are offering MUDRA loans at a lower rate of interest when compared to other agencies. Public Sector Banks are largest lending agencies under MUDRA Scheme. It can be observed a 29 percent growth rate in MUDRA loans during 2017-18. State Bank of India, Canara Bank and Punjab National Bank stood in the top positions among the Public Sector Banks.

Private Sector Banks: Private Sector Banks have also performed very well in the last two financial years. They recorded 27 percent growth in providing MUDRA loans. Among Private Sector Banks, Bandhan Bank stood top by providing Rs. 17657.36 crore loans.

Regional Rural Banks: They registerd 29 percent growth rate during 2017-18 and contributed only six percent of total MUDRA loans. PragatiGramin Bank, Kerala Gramin Bank and Karnataka Grameen Bank and Andhra Bank stood top among the RRBs.

Small Financial Banks: Small Financial Banks are exceptionally performed well in providing MUDRA loans. They registered 183percent growth rate in 2017-18 when compared to the previous year.

Micro Financial Institutions: Micro Financial Institutions achieved 11 percent growth and their market share in MUDRA loans reached to 20 percent in 2017-18. MUDRA is encouraging MFIs by reducing the refinancing rate of interest from 9.45% to 6.85%.

Non Banking Financial Companies: NBFCs are supporting MUDRA Scheme in two ways by providing loans to micro Units and also by refinancing the Micro Financial Institutions. They registered highest growth rate of 396% during 2017-18.

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We can also analyze the performance of various Financial Institutions by comparing the percentage of target reached and their share in total amount of MUDRA loans sanctioned.

Table 2

Percentage of target reached by Financial Institutions and their share in total MUDRA loans

Agency	Target(Rs.Crores) 2017-18	Sanctioned amount (Rs.Crore) 2017-18	Percentage of target reached	Percentage Share of total sanctioned amount
Public Sector Banks	94495	92492	98	36
Private Sector Banks	47150	49545.11	105	20
Regional Rural Banks	18255	15454	85	6
Small Financial Banks	17250	19022	110	7
MicroFinancial Institutions	57800	50143	87	20
NBFCs	9050	27018.16	299	11
Total	244000	253677.10	104	100

Source: Annual Report 2017-18 of PMMY

Analysis of this data shows following conclusions.

1. NBFCs, SFBs achieved highest growth rate during 2017-18 and other agencies have also registered good growth rate.
2. In reaching the targets, NBFCs have shown very good performance. They achieved 299percent of target.
3. SFBs and Private Sector Banks have also achieved more than 100 percent target.
4. The Public Sector Banks occupied the highest share in providing loans. They are providing 36 percent of total MUDRA loans during 2017-18.

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5. Regional Rural Banks have not reached the target and their share in total loans provided is very less.
6. MFIs and Private Sector Banks are occupying second place in the share of total loans by providing 20 percent loans by each agency.
7. Although the share of Non Banking Financial Companies is limited to 11 percent of total MUDRA loans provided, they achieved a very high growth rate of 396 percent.

In both the parameters of target reach and growth rate which are shown in the table 1 and table 2 the NBFCs have outperformed the Commercial Banks. This may be due to the focus of NBFCs on unorganized sector which was neglected by commercial banks. NBFCs are processing loans more quicker than Commercial Banks. Moreover NBFCs are also offering loans at competitive rates. NBFC sector may be expected to expand further in providing MUDRA loans in the coming years.

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2. SBI website

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Isolation and characterisation of halotolerant bacteria from saline habitats

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Abstract: *Salinity in the soil was one of the major problems to the agriculture in coastal areas. Suppressed growth and reduced yields are the effects of salinity on agriculture. Microorganisms that live in saline environments adapted to salinity, such halotolerant microorganisms provide a means of cultivation of crops in salinity influenced areas. Halotolerant bacteria which have the plant growth promoting activity, plant growth promoting bacteria (PGPB) may be used to mitigate the effect of salinity in the field. Aim of the present study is to isolate halotolerant bacteria from saline environments and to characterize them morphologically and biochemically. In the current research six bacterial isolates were studied for their morphological and biochemical characteristics. 16s rRNA gene analysis was carried out for one isolate i.e. MGST-02, which showed growth at 8% of NaCl concentration.*

Key Words: *halotolerance, salinity, plant growth promoting bacteria, morphological and biochemical characters.*

1. INTRODUCTION:

Conditions like extreme temperatures, drought, salinity, flooding, pollution, toxicity are unfavourable to plant growth. Worldwide this abiotic stresses greatly affecting the yields of major crops[1].Extreme temperatures, altering rainfall patterns and soil salinization are the resultants of climate change [2] . Over exploitation of irrigation practices used especially in arid and semi-arid regions leading to soil salinization. Approximately 20% of the cultivated and irrigated lands are negatively salt affected with some estimates being as high as 50%[3]. Due to global increase in salt affected area by 1-2% every year, salinity is becoming one of the leading issues in the coming decades.[4]. It is estimated that world's population will reach 9.5 billion by 2050[5]. There is a need to use unproductive saline and barren lands for cultivation to meet the food demand of the growing population[6].Various practices and techniques enhancing crop growth and productivity under salinity stress conditions must be followed. Though molecular breeding programmes and transgenic approaches are useful but have certain limitations in terms of different ethical issues and time requirement[7]. Therefore application of an alternative approach needs to be considered. One such strategy could be the use of bacterial strains tolerant to higher salinity levels with plant growth promoting activity, plant growth promoting bacteria (PGPB), either free living in the soil, rhizosphere, rhizoplane or phyllosphere[8]. Until the discovery of extremophilic microorganisms, for long time it was considered that the extreme environments were free of life[9]. Extremophilic microorganisms can survive in extreme environments such as unusual levels of salt, P^H, pressure and temperature. Those which adapted thrive in hypersaline habitats are considered as halophiles[10].

Previous reports suggest that bacteria belong to genera such as, *Microbacterium, Pantoea, Achromobacter, Rhizobium, Pseudomonas, Bacillus, Paenibacillus, Enterobacter, Burkholderia, Methylobacterium, Azospirillum, and Variovarax* etc. are helpful in tolerance to abiotic stresses[11,12]. To alleviate abiotic stresses these microorganisms are helpful in agricultural lands[13,14,15]. Studies on several microorganisms of the soil reported that these microbes support the plant growth during stress conditions by producing gibberllins, Indole Acetic Acid (IAA) and some other compounds which enhance the root growth and also nutrient content thus improving the plant health under stress[16, 17]. The main aim of this study is to isolate halotolerant bacteria from saline habitats. Selected halotlerant bacterial isolates were evaluated for their morphological, biochemical characters. One isolate i.e. MGST-02 was identified up to species level through 16s rRNA gene analysis.

2. MATERIALS AND METHODS:

Collection of soil sample

Soil sample was collected from provinces coastal area of Visakhapatnam, Andhra Pradesh, India. Soil sample was collected from the depth of 10-12 inch in sterile polythene bag and samples were kept at room temperature until used.

Isolation and Screening of Bacteria:

Soil suspension was prepared with 5g of soil in 20ml of sterile double distilled water and vortexed and serially diluted. 100 µl of 3rd and 4th dilutions were spread on 3% salt amended nutrient agar plates (Himedia) and incubated for 48h at 37°C for isolation of different bacteria.

Salt tolerance assay

All isolated bacteria were subjected to salt tolerance activity, nutrient broths were added with different concentrations of NaCl. The NaCl concentration ranges from 2.5% to 10%. After 24 hours of incubation all bacterial cultures were spectrophotometrically analysed at 660nm.

Biochemical Characterization:

Biochemical analysis of isolates were carried out according to Bergey's Manual of Determinative Bacteriology and classified primarily through morphological, physiological and biochemical observation.

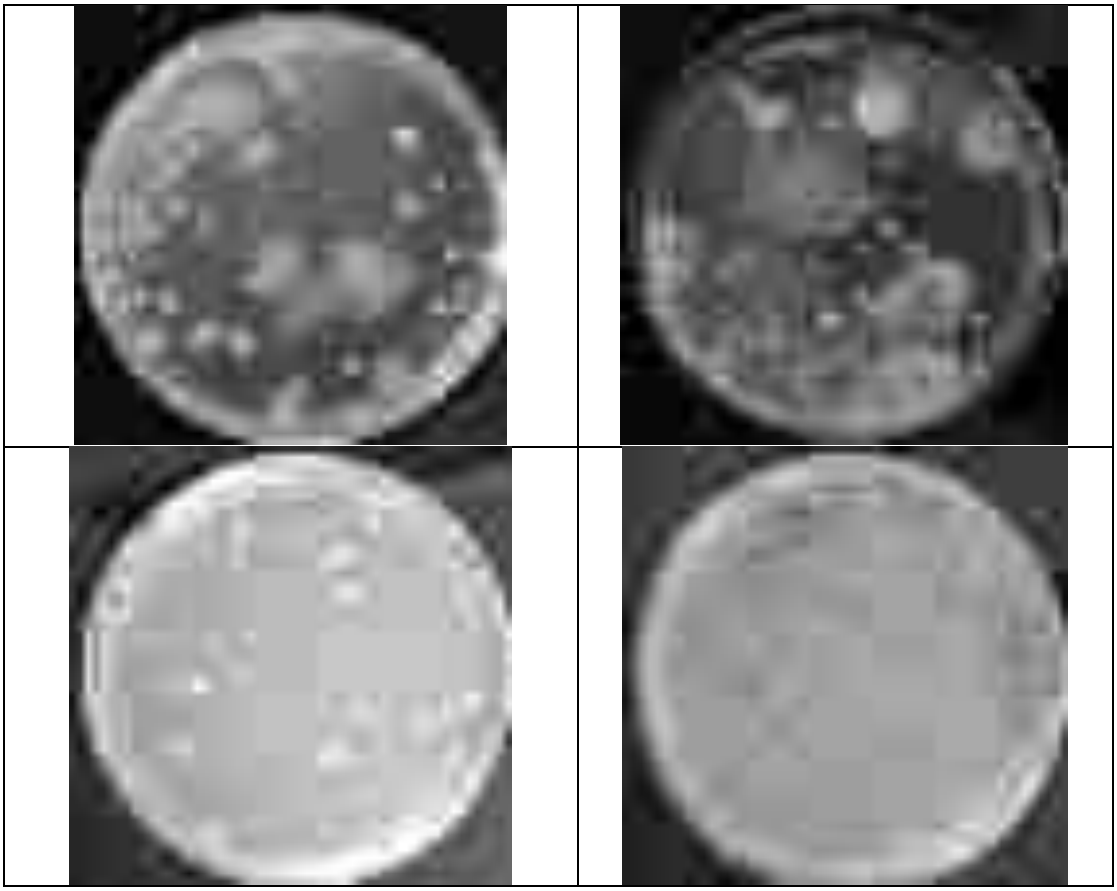
16S rRNA gene sequence analysis

DNA isolation was carried by the SDS extraction method described by Xia et al., (1995)[18] with minor modifications. Two universal primers 27F (5'AGAGTTTGATCMTGGCTCAG 3') and 907R (5'CCGTCAATTCMTTTRAGTTT3') were used to amplify 16S rRNA genes. PCR reaction mixture of 25 µl total volume, containing 1/10 volume 10× *Taq* buffer, 2 mm $MgCl_2$, 1 unit *Taq* DNA polymerase, 0.2 mM dNTP, 20 pmol forward primer, 20 pmol reverse primer and 100 ng DNA. DNA amplification was carried out in a Biorad Mini thermocycler with the following procedure: an initial denaturing step at 94°C for 5 min; 40 cycles for 1 min at 94°C (denature), 1 min at 48°C (annealing), 2 min at 72°C (extension) and a final elongation step at 72°C for 5 min. PCR products were separated by electrophoresis on 1.5 % agarose gel containing 0.5 µg/ml ethidium bromide, and photographed. The standard DNA samples (100 bp DNA ladder marker) were used as molecular size marker. The purified PCR products was subjected to Sanger's di-deoxy sequencing, in both forward and reverse direction ns, using Big Dye terminator v3.1 cycle sequencing kit on ABI Prism3700 DNA Analyzer (Applied Biosystems Inc., USA) as per manufacturer's instructions.

3. RESULT:

From the different soil samples collected from the coastal area six bacterial isolates were isolated based on the morphological characters of colonies. All six isolates can tolerate NaCl concentration up to 3%. Later these isolates were proceeded to calculate the maximum NaCl tolerance and biochemical and molecular characterization.

Figure: 1 Salt tolerant bacterial isolation plates showing the diversified colonies.



Morphological characterization

A total of six isolates obtained as a result of spreading technique followed by streaking. The isolates were coded as MGST-01, MGST-02, MGST-03, MGST-04,MGST- 05 and MGST-06. The colonies of isolates MGST-01, MGST-02, MGST-04 and MGST-06 were circular and the colonies of isolates MGST-03 and MGST-05 were irregular. All the colonies except MGST-04 which was yellow were white in colour. Colonies of isolates MGST-02 and MGST-06 were flat; colonies of isolates MGST-01, MGST-03, MGST-4 and MGST-05 were raised. Isolates MGST-02, MGST-03, MGST-04,MGST- 05 and MGST-06 were spherical in shape (cocci), MGST-01 was rod shaped (bacillus).Isolates MGST-01 was motile, remaining five isolates MGST-02,MGST-03,MGST-04 ,MGST- 05 and MGST-06 were non motile.

Table 1: showing morphological characteristics of the bacterial isolates

Biochemical tests	Bacterial isolates					
	MGST-01	MGST-02	MGST-03	MGST-04	MGST-05	MGST-06
Colony Colour	White	White	White	yellow	White	White
Colony shape	Circular	Circular	Irregular	Circular	Irregular	Circular
Elevation	Raised	Flat	Raised	Raised	Raised	Flat
Shape	bacilli	cocci	Cocci	Cocci	Cocci	Micrococci
Motility	+	-	-	-	-	-

Biochemical characterization

It was inferred that three bacterial isolates MGST-01, MGST-02 and MGST-03 were gram +ve, other isolates MGST-04, MGST- 05 and MGST-06 were gram –ve. Citrate utilization test indicates the capacity of bacteria to utilize citrate as a source of carbon and energy. CO_2 and nitrogen were released which later formed carbonate and hydroxide respectively. Blue colour indicated +ve for this test, isolates MGST-01, MGST-02 MGST-04 and MGST-06 were +ve and isolates MGST-03 and MGST-05 were –ve. Starch hydrolysis test was based on colour reaction of non-hydrolysed starch with iodine, it provides deep blue colour but its breakdown products progressively become violet, brownish red and finally colourless. Isolates MGST-01, MGST-02, MGST-03 and MGST-04 were +ve for starch hydrolysis test, isolates MGST-05 and MGST-06 were –ve for the test. Isolates MGST-04 and MGST-05 were +ve for Methyl Red test, four isolates MGST-01, MGST-02 MGST-03 and MGST-06 were –ve for MR test. Voges Proskauer test (VP test) determines the capability of some microorganisms to produce non-acidic or neutral end products, such as acetyl methyl carbinol, from the organic acids that results from glucose metabolism. Pink colour in the medium indicates +ve result. Isolates MGST-02 MGST-05 and MGST-06 were +ve as pink colour was found in the medium, isolates MGST-01, MGST-03 and MGST-04 were –ve for VP test. When bacteria grown in a medium containing nitrate as the only source of nitrogen, if the bacteria possess nitrate reductase then they convert nitrate into nitrite. This can be observed by adding sulphonic acid which forms diazonium salts, which in turn respond with α-naphylamine, results in formation of red azo dye. Three isolates MGST-02, MGST-05 and MGST-06 were +ve for nitrate reductae test. Isolates MGST-01, MGST-03 and MGST-04 were –ve for nitrate reductase test. Formation of bubbles on addition of $H_2O_2$to bacteria indicates presence of catalase. Isolates MGST-01, MGST-02 and MGST-06 were +ve for catalase test and isolates MGST-03, MGST-04 and MGST-05 were –ve for catalase test.

Table 2: Biochemical characters of bacterial isolates

Biochemical tests	Bacterial isolates					
	MGST-01	MGST-02	MGST-03	MGST-04	MGST-05	MGST-06
GramStaining	+ve	+ve	+ve	-ve	-Ve	-ve
Citrate	+	+	-	+	-	+
Starch Hydrolysis	+	+	+	+	-	-
MR	-	-	-	+	+	-
VP	-	+	-	-	+	+
Nitrate Reductase	-	+	-	-	+	+
Catalase	+	+	-	-	-	+

Positive (+), Negative (-)

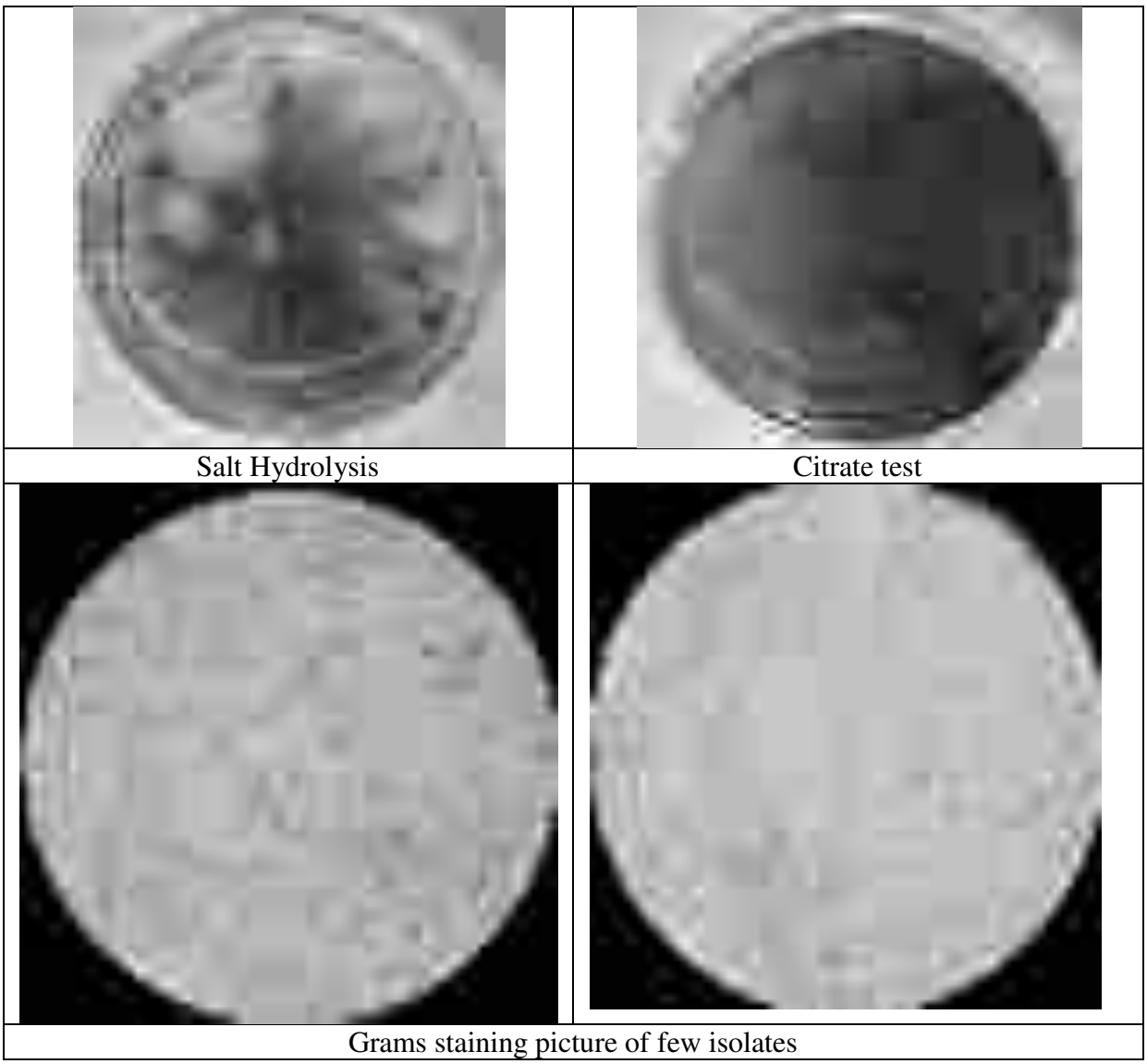


Figure 2: Biochemical characters of bacterial isolates

Salt tolerance assay

The six bacteria studies in this experiment exposed to gradient concentration of NaCl from 2.5 to 10 %. Each bacterium had their own tolerance level. It is evident that highest OD value was shown by MGST-02 followed by MGST-05, MGST-02 and MGST-03, MGST-01 and MGST-04 under unstressed condition. OD values gradually decreased with increased NaCl concentrations. It is clear that bacterial growth was shown to be decreased as the concentration of NaCl increased in the medium. Highest growth was recorded for the isolate MGST-02 the isolate showed growth up to 8% of NaCl concentration. No growth was observed in the medium exceeding 8% of NaCl concentration. Least growth was observed in isolates MGST-01 and MGST-04 for these isolates growth was observed up to 3.5% of NaCl concentration in the medium.

Table 3: Bacterial growth kinetics under salt stress.

Bacterial Isolate	OD at 660 nm													
	Control	NaCl concentration												
		2.5%	3%	3.5%	4%	4.5%	5%	5.5%	6%	6.5%	7%	7.5%	8%	8.5%	9%
MGST-01	1.5	1.1	0.58	0.41	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MGST-02	2.20	2.11	1.97	1.48	1.33	1.19	0.67	0.44	0.38	0.33	0.29	0.23	0.12	0.0	0.0
MGST-03	1.5	1.32	1.26	0.64	0.36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MGST-04	1.29	1.12	0.56	0.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MGST-05	1.93	1.88	1.54	1.27	1.09	0.96	0.74	0.31	0.18	0.0	0.0	0.0	0.0	0.0	0.0
MGST-06	1.80	1.17	0.62	0.48	0.31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

16s rRNA gene sequencing analysis

In order to screen useful microorganisms inhabiting the rhizospheric soil near coastal areas of Visakhapatnam, six strains were isolated. One isolate i.e. MGST-02 which showed growth at 8% NaCl concentration was characterised phylogenetically by sequencing PCR-amplified 16S rRNA gene. The sequence identity was searched by the BLAST analysis using NCBI database. Phylogenetic analysis using 16S rRNA revealed that the isolate MGST-02 was 99.05% similarity with *Acinetobacter calcoaceticus*. The phylogenetic relationship between the isolated 16S rRNA gene sequence compared to those of representative species were illustrated in the figure 4.

MGST-02

TAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCTTAACACATGCAAGTCGAGCGGGGTGAT
GGTGCTTGCACTATCACTTAGCGGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGAC
AACATTTCGAAAGGAATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTGC
GCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCTGTA
GCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
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TTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTCTAGTTAATACCTAGAGATAGTGGACGTTACTCGCA
GAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGATTT
ACTGGGCGTAAAGCGCGCGTAGGCGGCTAATTAAGTCAAATGTGAAATCCCCGAGCTTAACTTGGGAAT
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TAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAACACTGACGCTGAGGTGCGAAA
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GCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTA
AAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGA
AGAACCTTACCTGGCCTTGACATAGTAAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTTACAT
ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
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AAAGGGTTGCTACCTAGCGATAGGATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATTGGAGTCTGCA
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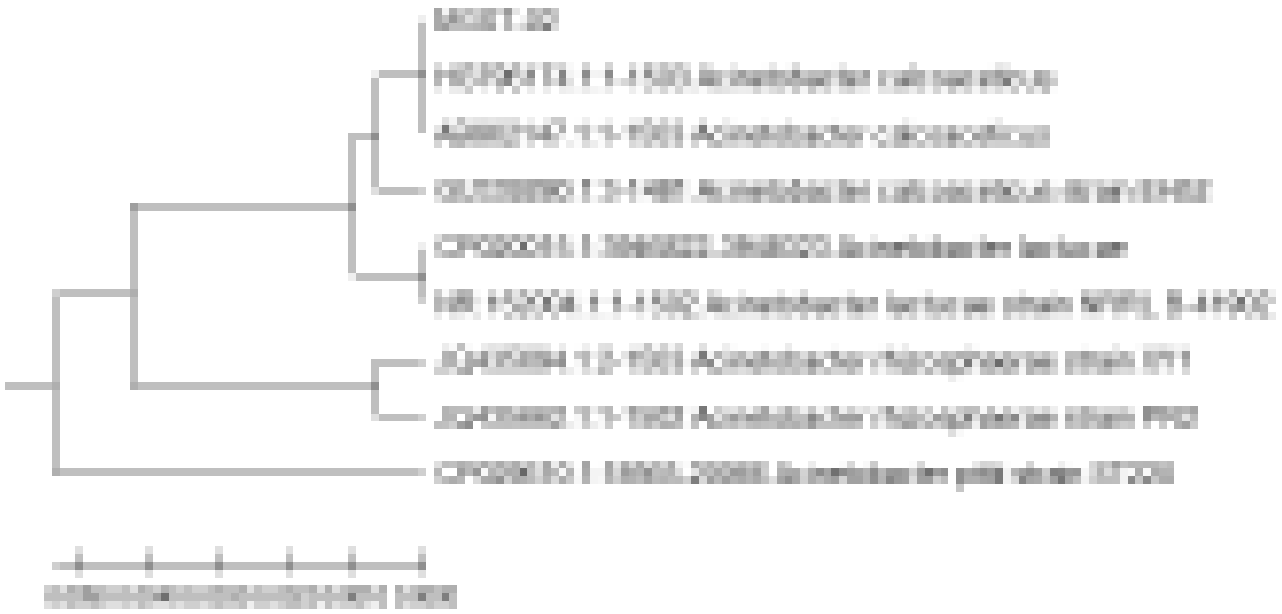

Figure: 4 UPGMA phylogenetic tree

4. Discussion:

The organisms isolated during this study were unique having moderate salt tolerance capacity. However animal pathogens viz. *E.coli, Micrococcus, Staphlococcus, Vibrio* etc. were also known to be naturally halotolerant microbes[19,20,21]. Extremophiles have been isolated from environments in which they are not expected to grow actively[22]. Our current research has shown that bacteria isolated from sample collected from coastal area of Visakhapatnam, Andhra Pradesh were halotolerant bacteria with different characteristics. During the past decades, the studies on ecology, physiology and taxonomy halophilic and halotolerant organisms revealed an impressive diversity[23]. In this study all the six isolates were grown on medium supplemented with different NaCl concentrations (i.e. from 2.5-10%). No growth was observed on medium containing above 8% NaCl concentration. This indicates that the isolates were halotolerant organisms which are in agreement with those reported by Bowers et al., (2009)[24] who reported that halotlerant bacteria can grow over a wide variety of salt concentrations. Verma, (1993)[21] was also reported growth of *Staphylococcus* in nutrient medium containing 10% NaCl which is also a normal salt concentration for halotolerant organisms. It was reported that *Micrococcus luteus* a halotolerant bacterium could tolerate up to 25% NaCl. Halotolerant bacteria grow best at the temperatures range 28-37^{0}C on medium supplemented with 3-4% NaCl.

5. Conclusion:

Six organisms were successfully isolated from coastal area of Visakhapatnam, that could grow in salinity range from 2.5-4% NaCl. From the study it is evident that the bacterial isolate MGST-02 was salt tolerant it can tolerate up to 8% of NaCl concentration. The results revealed that, the isolated MGST-02 is *Acinetobacter calcoaceticus* and is moderately salt tolerant. The ability to thrive in salty environments suggests their possible use for bioremediation purpose. The bacteria to be tested for plant growth promoting activity. Halotolerant plant growth promoting bacteria enhance crop growth and productivity. In future, these can be utilized as biofertilizer to ameliorate salt stress and increase crop production in an economically sustainable manner.

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STRESS AMONG PARENTS OF CHIDREN HAVING INTELLECTUAL DISABILITY: A CASE FOR VISAKHAPATNAM SCHOOL STUDENTS.

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ABSTRACT

Raising a child with intellectual disability considerably affects the family's daily life and the life course of the child's parents. This is not merely a single crisis or stressful event that requires a one-time mobilization of the family, but rather a complicated process which continues throughout a lifetime, and has its vicissitudes. The present study was conducted to examine the parental stress having children with intellectual disability. The participants of the study consisted of 60 parents comprises (30 fathers and 30 mothers) of hearing impaired children who were studying in special education schools and centers in Visakhapatnam district. Data were gathered through parental stress index developed by Abidin(1995). The data were analyzed using the disruptive statistics like frequency, percentage, mean and standard deviation were employed to examne the ovel all parental stress. Independent sample t test, one way and two way ANOVA were employed to see whether there is significant mean difference on parental stress as a functions of gender, parental income and parental education. Based on the present study findings, conclusion and recommendations were made.

Key words: ***stress , parents, intellectual disability*** .

Introduction

Developmental Intellectual Disability means actual limitations in general functioning. It is characterized by intellectual functioning significantly lower than the average, which exists along with related limitations, in two or more areas of the following functional adaptation skills: communication, self-care, household management, social skills, community orientation, self-guidance, health and safety, academic skills, utilizing leisure time, and occupational competence (Luckasson et al., 1992).

The complexity of the definition of disability is expressed inits amended version: "Mental retardation is a disability characterized by significant limitations in intellectual functioning and adapting behavior as expressed in the adaptive, perceptive, social and practical skills" (AAMR, 2002: p. 8). The definition includes three mandatory components which must exist in the individual in order for him to be defined as having an intellectual disability: 1) Considerable limitation of intellectual functioning; 2)Limitation of adapting behavior, in all its aspects, related to the intellectual disability; and 3) The intellectual disability occurs before the age of 18. According to the amended definition, classification of people with mental retardation is conducted according to an assessment of the level of support which the person requires rather than according to IQ levels, and this indicates the substantial role of the environment in the development and functioning of the person with intellectual disability.

Raising a child with intellectual disability considerably affects the family's daily life and the life course of the child's parents. This is not merely a single crisis or stressful event that requires a one-time mobilization of the family, but rather a complicated process which continues throughout a lifetime, and has its vicissitudes (Poehlmann, Clements, Abbeduto, & Farsad, 2005). Families are required to cope with ongoing challenges and crises which affect various aspects of daily life: economic, personal, couple relationship, familial and social (Davis &
Gavidia-Pyane, 2009). The constant care requirements continue even after the child becomes an adult.

Diagnosis of intellectual disability in a child provokes a period of disequilibrium followed eventually by an adjustment to life with or without undue stress. There are various stresses which emerge and reemerge over time. Discrepancies between expectations and the performance of the developmentally disabled child continue bringing feelings of grief (Wikler,1981).

A parent shows a series of reactions after knowing that their child is disabled. These include shock, denial, guilt, sorrow, rejection and acceptance. Questions like 'why me?' 'How can it be?' keep arising without answers. Some of them undergo tremendous guilt feelings, experience deep sorrow, have strong under expectations of achievement, may have unrealistic goals, may want to escape form reactions and ultimately turn to accept the child (Berdine, 1985). All this can affect different parents differently. Their physical and mental health may actually suffer or they perceive it to suffer. Wikler (1981) mentions that tremendous amount of stress chronically affects their lives.

Farber (1960) found that initial stress in parents appears to be sex-linked which shifted with time. Mothers of intellectually disabled children and neurotic children undergo more stressful
experience than mothers of chronically ill or normal children. According to Faber (1963), jealousy and resentment may develop in siblings if the disabled child requires most of his parent's attention, leaving short tempers and impatience for the others.

Jani (1967) in a study examined the social problems related to the presence of a intellectually disabled child. Results indicate that parental feelings were marked by anxiety about future. Also, negative effects towards other siblings, psychological stress, decreased interaction with neighbors and relatives, misunderstandings within family and economic loss were significant facts associated with presence of a child with intellectual disability in the family.

Dupont (1967) found in a review of a four year caseload of a small community mental health centre, that the community mental health services often had a policy of not providing services to persons with intellectual disability.

Loeb (1979) has expressed that parents of intellectually disabled children face many special stresses. They have little opportunity to explore their own needs and difficulties.

Fishman et al.(1989) in their study examined the role of parenting stress and parental depression and marital intimacy among parents of disabled children and developmentally normal children. Results showed that mothers and fathers of autistic children significantly showed greater stress and depression as well as marital intimacy than mothers and fathers of children with Down Syndrome.

Heller et al. (1997) found that in comparison with fathers of intellectually disabled children, mothers spent more time providing care, offered more types of support and perceived more caregiving burden. The behavior and health of the children had a greater impact on mothers than on fathers.

Peshawaria et al (1998) stated that there were gender differences in facilitating and inhibiting factors that affect coping in parents of children with intellectual disability in India. Mothers are under more pressure to balance childcare needs and household chores. Physical support was a relief to them.

Seshadri et al (2000) reported a direct relationship between the degree of perceived burden, social emotional burden, disruption of family routine and disturbance in family interactions for women with intellectually disabled children rather than men.

Hedov et al. (2000) studied self perceived health in Swedish parents of children with Down Syndrome (DS). They found mothers of children with DS had significantly lower, less favorable scores on self perceived health than did the fathers of DS children and control group.

Shin and Crittenden (2003) in their study provided explanations for well being of Korean and American mothers of children with intellectual disability. Causes of stress for the American mothers was specific to the individual variables. For Korean mothers, cultural values that carry social influence were strongly associated with their experience of stress.

Laurvick et al. (2006) while working on physical and mental health of mothers caring for a child with Rett Syndrome found that factors positively related with better mental health were the following: the mother working full time or part-time outside the home, the child not having a fracture in last two years, less reporting of facial stereotypes and involuntary facial movements, being in a well adjusted marriage and low stress scores.

Kermanshahi et al. (2008) in their study on perceptions of lives with children with intellectual disability found six major themes: challenging the process of acceptance, painful emotional reactions, the interrelatedness of mother's health and child's well being, struggles to deal with oneself or the child, inadequate support from the family and the community, and the anxiety related to child's uncertain future.

Studies in literature indicate that parents of children with intellectual disability, more specially the mothers, would have high stress and low health scores. On the other hand, Mahoney (1958) documented some positive effects. He found that the disabled child can have an integrative effect by focusing the family's energy in a concerned, loving manner, thereby minimizing some of the other day to day problems. Some parents expressed a new appreciation for life and ordinary things they used to take for granted.

The present study attempted to handle this issue through answering the following sets of questions:

- What is the level of stress among parents having children with intellectual disability?
- Are there significant mean differences in parents' stress level as the function of gender, parental order of birth and number of children?

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Objectives

The major purpose of this study was to examine the impact of stress on parents having children with intellectual disability, specifically the purpose of this study was to:-

•Examine the level of stress among parents having children with hearing imparement.

• Explore whether there exist significant mean difference in parents' stress level as the function of age, parental group, number of children and order of birth.

METHOD

Research Design

Likewise, to achieve the purpose of the study and to answer research questions, this study is principally organized around a cross sectional survey research design. The reason behind selecting this design is, primarily it is "…the most commonly used design in the social science" (Kumar, 1996:81). The second reason is that a cross sectional survey research design is best suited to studies intended at finding out the prevalence of a phenomenon, circumstance to problem of attitude or issue by taking cross section of the population.

Participants

The target population of this study has been students of visakapatnam special school students. The sample consisted of 60 parents comprises (30 fathers and 30 mothers) of hearing impaired children who were studying in special education schools and centers in Visakhapatnam district. The sampling .techniques employed were covinience sampling technique.

Instruments

Parenting Stress Index: to measure parental stress 36 items questionnaire was adapted form Abidin(1990). The questionnaire had five scales ranging from 1=strongly agree 5=strongly disagree.

Data Analysis

After all to answer the to the first research question, disruptive statistics like frequency, percentage, mean and standard deviation were employed. To answer the second leading question independent sample t test and one way and two way ANOVA were employed to see whether there is significant mean difference on parental stress as a functions of gender, parental income and parental education.

RESULTS

Socio-demographic Context of Participants of the Study

Before turning to the foremost analyses of the study, the main socio-demographic characteristics of the respondents are summarized in Table 1 below

Table 1

Characteristics of Demographic Variables (N=120)

Variable		n	Percentage
Gender	Fathers	60	50.0
	Mothers	60	50.0
Age	21-30 Years	48	40.0
	31- 40 Years	55	45.8
	41-50 Years	17	14.2
Order of Birth of Child	First born	68	56.7
	Second born	38	31.7
	Third born	14	11.7
Number of Children	One	31	25.8
	Two	71	59.2
	Three and above	18	15.0

In order to develop a better insight of the sample, a summary of demographic characteristics of the study sample is presented in Table 1 above . A total sample 120 participants were collected data and the majority of participants 55 (45.8%) in the range of 31 to 40 years as compared to 48 (40.0%) in the age group of 41 to 50 years and17 (14.2%) in the 21 to 30years. Father group 60 (50%) and Mother group 60 (50%) participants are in the equal proportion. Of those participants, 68 (56.7%) have first born child, 38 (31.7%) belong to second child, 14 (11.7%) are third born respectively. The number of children of parents includes 71 (59.2%) are two, 31 (25.8%) with one child, and 18 (15.0%) three and above respectively.

As clealy indicated in table two below the parents have higher level of stress which requires them to have clinical interventions. As per Abedin (1995) a stress level score above 90 score would be considered as chronic.

Table 2 Discriptive statistics indicating the level stress level

	N	Minimum	Maximum	Sum	Mean	Std. Deviation
Parental Total Stress	120	46.00	155.00	11705.00	97.5417	21.79156

A one-way between-groups analysis of variance was conducted to explore the impact of participant's age to their overall stress level (Table 3). Participants were divided into three groups according to their age levels (Group 1: 21-30 Years; Group 2: 31- 40 Years; Group 3: 41-50 Years;). There was a statistically significant difference at the $p < .01$ level in parental stress scores for the three age groups: $F(2, 117) = 5.690$, $p = .004$. Post-hoc comparisons using the Tukey HSD test indicated that the mean score for Group 1 ($M = 89.9583$, $SD = 20.23054$) was significantly different from Group 2 ($M = 101.2727$, $SD = 21.15766$) and Group 3 ($M = 22.48856$, $SD = 22.48856$).

Table 3

Results of One-way Analysis of Variance Results for parental stress as a function of Respondents age level.

Parental stress	*SS*	*df*	*MS*	*F*	*p*
Between groups	5009.201	2	2504.601	5.690	.004
Within groups	51500.590	117	440.176		
Total	56509.792	119			

A two-way between-groups analysis of variance was conducted to explore the impact of parents group and order of birth of children on parental stress (Table 4). Participants were divided into three groups according to their children's order of birth (Group 1: First born; Group 2: Second born; Group 3: Third born). The interaction effect between parents group and order of birth of the children was not statistically significant, $F(2, 114) = .612$, $p = .133$. There was no a statistically significant main effect for order of birth, $F(2, 114) = 914.386$, $p>.01$; There was no a statistically significant main effect for parents group, $F(2, 114) = 115.298$, $p>.01$.

Table 4

Two-way ANOVA results and Descriptive Statistics for Overall Parental stress by Parents group and Order of Birth of the Children

Variable	Mean	SD	n
Fathers Group			
First born	101.5000	24.88915	34
Second born	100.6316	20.00892	19
Third born	99.2857	15.47810	7
Mothers Group			
First born	86.7647	19.93027	34
Second born	103.7368	19.94393	19
Third born	103.7143	15.68135	7

Source	SS	*df*	MS	F

Order of Birth	115.298	2	115.298	.259
Parents group	1828.772	1	914.386	2.051
Order of Birth × Parents	2443.764	2	1221.882	2.740
Error	50829.580	114	445.874	

Note: R^2 = .101, adj. R^2 = .061.
p >.01

A two-way between-groups analysis of variance was conducted to explore the impact of parents group and number of children on overall parental stress (Table 5). Participants were divided into three groups according to their number children (Group 1: Single child; Group 2: Two children; Group 3: Three children). The interaction effect between parents group and number of children was not statistically significant, F (2, 114) = . 833 , p = .438. There was no statistically significant main effect for number of children, F (2,114) = .494, p>.01. There was no statistically significant main effect for parents group, F (1, 114) = 1.406, p>.01.

Table 5
Two-way ANOVA results and Descriptive Statistics for Overall parental stress by Parents group and Number of Children

Variable	Mean	SD	n
Fathers Group			
Single child	101.1333	23.00890	15
Two children	101.4444	23.88478	36
Three children	98.7778	14.52393	9
Mothers Group			
Single child	87.8750	18.66503	16
Two children	94.9143	22.60189	35
Three children	102.1111	16.46545	9

Source	SS	*df*	MS	F
Number of Children	664.084	1	664.084	1.406
Parents group	466.159	2	233.079	.494
No. of Children × Parents	786.284	2	393.142	.833
Error	53829.560	114	472.189	

Note: R^2 = .047, adj. R^2 = .006.
p >.01

Discussion

The results in the descriptive analysis revealed that parental stress level is very chronic. The data reported here appear to support the assumption that parents who have children with intelctuality disability develop the higher level of stress. The results of the present study are consistent with previous research studies. Studies conducted by (Davis & Gavidia-Pyane, 2009).depicted that parents having children with intellectual

disability will develop higher stress . Thus, creating clinical and conseling provisions will improve the parents stress.

The results in the one way analyses of variance revealed age of parents affects the overall stress level of parents. These results would seem to suggest that parents whose age is higher will be hilghy affected by stress. The results of the current study are in lined with previous research works. Research conducted by Kermanshahi et al. (2008) depicted that parental age is one of the essential factor on parents having children with intellectual disability.

The findings of two-way between-groups analysis of variance showed that there was no significant parental stress means score difference among different parents parental group, order of birth and number of children. This result is in consistent with Heller et al. (1997)who found out number of children and order of birth have an impact on overall parental stress. Thsis probably due to the fact that participants of the present study might have handled the problem related to number children and order of birth in efficient manner.

Conclusion

Based on the findings of the study the following conclusions were drawn:

- parents seem to have chronic level of stress because of having children with intelectul disability.
- Parents age seem to have an effect on overall parental level of stress.
- Parental stress is common for parents having one or more children

Recommendations

Based the conclusions the following recommendations could be given:

- Teachers should play their role as counselor both for the students with intellectual disability and their parents.
- Seminars and workshops should be conducted to create the awareness in parents about the acceptance in the way that it could be helpful in the positive development of children.
- Schools should have a policy that addresses the needs of parents of intellectual disability

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Plant Mediated Green Synthesis of Novel Bimetallic Nanoparticles: Characterization and Investigation of Photocatalytic Activity for Degradation of Malachite Green Dye

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ABSTRACT

An ecofriendly facile method was reported for green synthesis of Ag-Co bimetallic nanoparticles by using Aerva Lanata leaf extract. The phytochemicals present in the extract are acting as reducing agents, stabilizing and capping agents for the biofabricated nano particles. The characterization studies were done by UV-VIS , FTIR spectroscopies , SEM, XRD, EDX and HRTEM analyses. These nanoparticles were further applied as photocatalysts for degradation of Malachite Green dye by irradiation under sunlight in optimum conditions. It was observed that the maximum photodegradation 88.6% was achieved for 10 ppm of dye solution at pH 8 with a catalyst dose of 20 mg under irradiation for 120 minutes.

KEY WORDS

Bimetallic nanoparticles (BMNPs), *Aerva Lanata*(AL), Malachite Green (MG), photo degradation

1.INTRODUCTION

Nanotechnology is the branch of science which deals with very small structures of size 0.1 to 100 nm [1]. Now a days nanotechnology and nanomaterials entice great attention because of their numerous applications[2]. Synthesis of nanoparticles can be achieved with the aid of bottom-up technique which includes constructing up of the atoms or molecules. Top-down technique includes making tinier and tinier structure via etching from the bulk material[3]. Nanoparticles have distinct physical properties like large surface area, shape, aggregation and size, state of size distribution, surface morphology ,crystallinity, solubility and defect in structure[4]. The photocatalytic activity and stability of nanoparticles can be influenced by their surface chemistry and zeta potential [5]. Physical and chemical properties exhibited by nanoparticles are unique when compared to the bulk materials[6]. Nanoparticles are also used in various fields like medicine, manufacturing materials, environmental, electronics[7] etc .

Various metal nanoparticles such as gold, silver, platinum, palladium, ,copper, iron, nickel and cobalt can be produced by means of chemical,physical and biological methods[8]. Due to some poisonous chemical substances absorbed on the surface of metals more dangerous results have been related with chemical synthetic methods [9]. Biological methods are environmentally safe and cost effective in comparison with chemical and physical strategies for the synthesis of nanometals. Nanoparticles synthesized through the use of vegetation or plant extracts [10], microorganisms[11,12], fungus[13] and enzymes[14] are recognized as ecofriendly and vital branch of nanotechnology. Bimetallic nanoparticles consists of two one of a kind metals have remarkable properties when compared to monometallic nanpoparticles from both technological and scientific point of view[15,16]. Biosynthesized bimetallic nanoparticles are used in several contemporary fields viz. imaging, labeling, luminescence tagging, drug delivery and biomedical field because of their superior properties.[17].Biosynthesized bimetallic nanoparticles additionally have been found to possess extraordinary catalytic activities[18].

Environmental pollution is one of the major and most pressing problems of the modern world. Textile industry releases coloured water during fabric dyeing process into the environment. Industries rigorously dealing with harmful materials such as dyestuff, distilleries, tanneries and paper mills also release highly coloured waste water into ecosystem which reasons pollution[19].

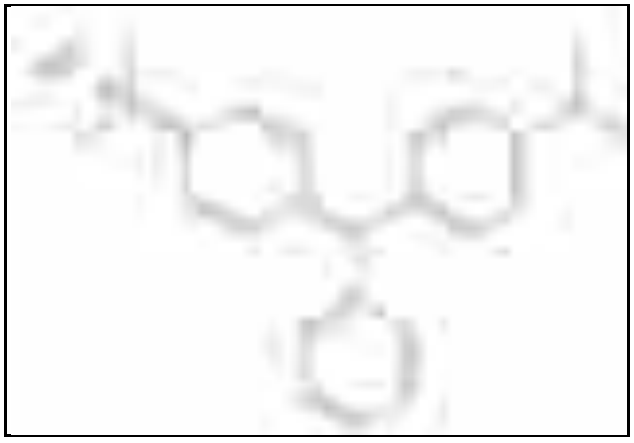


Figure 1 (a) : Structure of Malachite Green dye

Malachite Green (MG) is a water soluble cationic green crystalline dye that belongs to triphenylmethane group [20] (Figure 1(a)). It is largely used in wood, leather, jute, silk and cotton industries for dyeing. When MG enters into water bodies there are obvious possibilities that it may enter food chain and can cause toxic effects on living organisms because of its carcinogenic and mutagenic nature [21]. For elimination of such harmful dyes photocatalytic process has been reported more advantageous and can degrade many organic chemicals when compared to other conventional and routine degradation strategies of dyes.

Herein, an effortless green method for synthesis of Ag-Co bimetallic nanoparticles (BMNPs) by using leaf extract of *Areva lanata* as a reducing and capping agent was reported and by using these BMNPs as catalysts for photodegradation of MG dye under solar light irradiation was also studied at various reaction conditions.

2. EXPERIMENTAL

2.1. Materials: Chemical reagents used (siver nitrate and cobalt nitrate) in this study were of analytical grade. Deionised water was used to clean glassware, prepare chemical solutions and throughout experimental procedure. Fresh leaves of *Aerva lanata* were collected from agricultural fields in S.Kota, Vizianagaram district, Andhra Pradesh state, India.

2.2. Preparation of *Aerva lanata* leaf extract: 100g of fresh leaves were weighed and thoroughly washed with running tap water to remove debris on surface of leaves followed by deionised water to eliminate other contaminants from leaves and dried up under shade for 10 days. These leaves were cut into tiny pieces and made homogenized powder by using home blender. The obtained powder placed in refrigerator at 4^{o}C which was kept in an air tight container. Now 200 mL deionised water was taken in 500 mL beaker to this 10g stored powder was weighed and added. The contents in the beaker heated for 30 minutes at 50^{o}C with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled leaf broth was filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4^{o}C. This was taken as leaf extract throughout the experiment (Figure :1(c)).

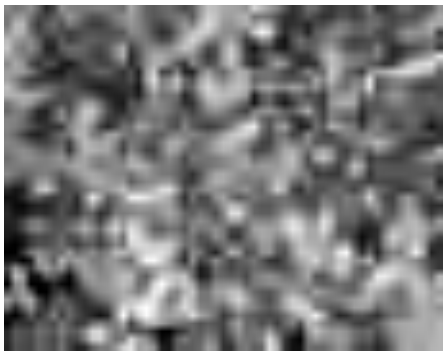


Figure 1(b): Aerva Lanata plant

Figure 1(c):Aerva Lanata leaf extract

2.3. Synthesis of Ag-Co bimetallic nanoparticles:

Equimolar (25 mM) concentrations of silver nitrate and cobalt nitrate aqueous solutions were prepared separately in 100 ml volumetric flask by dissolving 0.4246 gms, 0.7275gms weight of $AgNO_3$ and $Co(NO_3)_2$ in water respectively. Synthesis of Ag-Co BMNPs was done by taking 100mL of $AgNO_3$ solution in a 500 mL beaker, to this 90ml of leaf extract was added drop wise through burette. 100mL of $Co(NO_3)_2$ solution was added to contents in the beaker by drop wise. During addition process beaker was placed on a magnetic stirrer for continuous agitation. Now this mixture was stirred at 50^{o}C for 40 minutes at pH 7 on magnetic stirrer. These synthesized BMNPs were

separated out by doing centrifugation at 5000 rpm for 30 minutes. The obtained BMNPs were washed with using deionised water 2 times to remove unwanted constituents. The resultant BMNPs particles were collected (figure: 1(d)) and used for characterization.

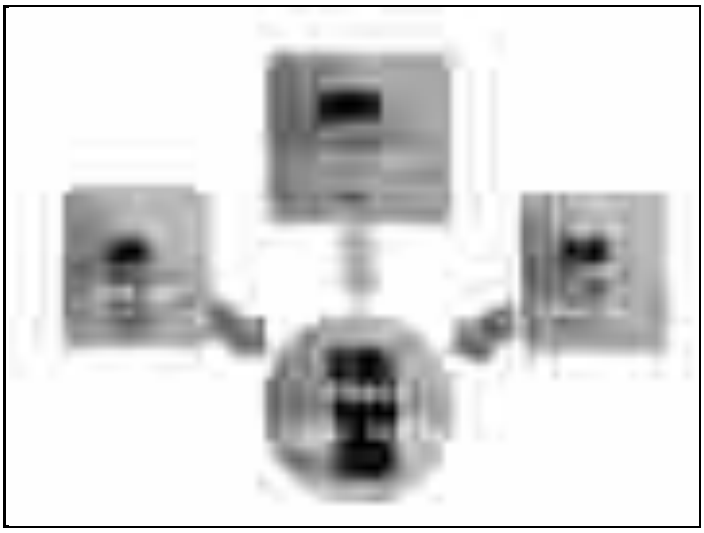


Figure : 1(d) . Synthesis of Ag-Co BMNPs

2.4. Characterization:

Formation of Cu-Zn BMNPs was confirmed by UV-Visible absorption spectra using UV-2450 SHIMADZU double beam spectrophotometer, FTIR using Bruker, SEM, EDX studies are done by using Hitachi S-3700N machine and the morphology of BMNPs was elucidated by HRTEM analysis with FEI Technai machine.

3. RESULTS AND DISCUSSION

3.1.UV-Visible spectral analysis:

UV-Visible spectrum of leaf extract is given in Figure2(a). UV-Visible absorption spectrum of Ag-Co BMNPs is revealed in Figure.2(b). The characteristic surface plasmon resonance (SPR) band at around 441 nm is observed in Ag-Cos BMNPs which confirms the nano size of the synthesized paricles [27] .

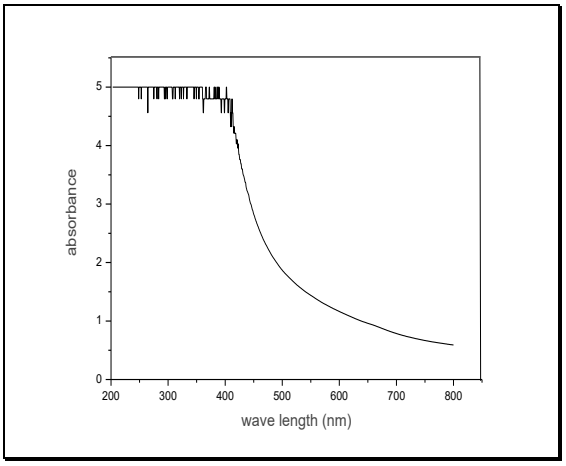


Figure 2 (a): UV-VIS Spectrum of leaf extract

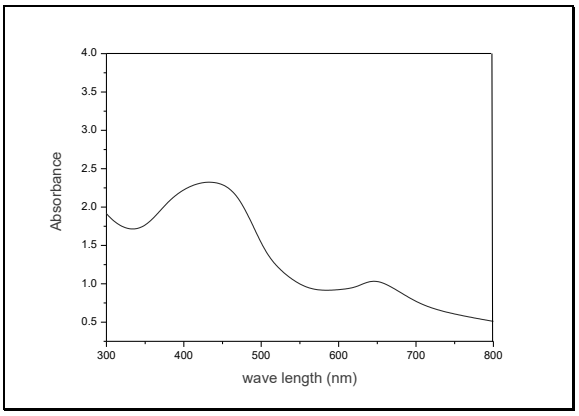


Figure.2(b): UV-Visible absorption spectrum of Ag-Co BMNPs

3.2. FTIR spectral analysis:

FTIR spectral data is used to identify different functional groups present in biomolecules of leaf extract.These groups are responsible for the bioreduction of Ag^{+}, Co^{+2}precursors and also for capping and stabilization of Ag-Co BMNPs. The intense peaks were observed and compared with standard values to analyze the functional groups in *Aerva lanata* leaf extract and greensynthesized Ag-Co BMNPs. FTIR spectra of *Aerva lanata* leaf extract and synthesized Ag-Co BMNPs by using *Aerva lanata* leaf extract were shown in **Figure.2 (a)** and **Figure.2 (b)** respectively**.** The comparison of the FTIR spectra of both Ag-Co BMNPs and leaf extract of *Aerva lanata* clearly indicates the existence of the plant extract phytomolecules such as polyphenols ,terpenes, amides, carbohydrates, amines etc.on the surface[22] of the Ag-Co BMNPs

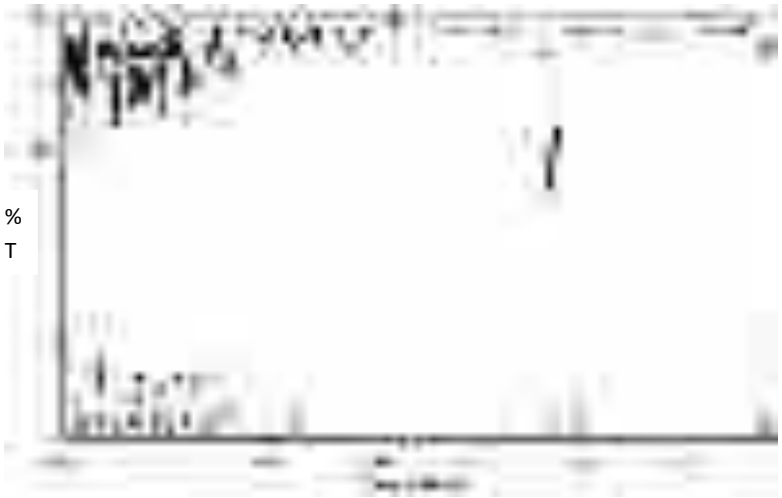


Figure.3 (a): FTIR spectrum of Aerva lanata leaf extract

Figure 3(b). FTIR spectrum of Ag-Co BMNPs in leaf extract

The strong intense peaks between 3200 cm^{-1} to 3950^{-1}may be due to N-H ,O-H stretching of 1^0 amines and polyhydroxy groups present in the extract.The strong absorption at 1643 cm^{-1}indicates the presence of C=O group of amides.This result gives us the evidence about the high protein content of the extract.The small peak at 2892 cm^{-1} is may be due to C-H symmetrical stretching of methylene groups.The peak position at 1519 cm^{-1} may be due to C=C stretch of aromatic ring.The peak at 598 cm^{-1} is denoting the presence of C-Cl group.

The FTIR spectrum of Ag-Co BMNPs exhibits major peak positions at 3215 cm^{-1},3411 cm^{-1} and 3507 cm^{-1}which indicate the N-H stretching vibrations of amines and O-H stretching of hydroxyl groups of alcohols ,phenols.Small peak at 2926 cm^{-1} is due to C-H stretching of alkyl groups.Intense peak at 1640 cm $^{-1}$is due to C=O stretching of amide group. [23]

FTIR analysis clearly confirms that all the aforementioned peaks of the extract are imperceptibly shifted in the FTIR spectrum of Ag-Co BMNPs as the phyto molecules of the extract act as bioreducing agents,capping and stabilizing agents for the synthesized nanoparticles. The existence of these IR bands also in the Ag-Co BMNPs confirmed that the surface of the nanoparticles was covered by plant secondary metabolites such as carbohydrates, glycosides, Saponin, steroids and phytosterols, phenolic compounds, tannins, flavonoids, proteins, aminoacids, diterpenes with functional group phenols, carboxylic acids, amides, ketones, alkyl halides

3.3.**SEM and EDX analysis:**

From energy dispersive X-ray analysis we can analyze all the elements present in prepared BMNPs by *Aerva lanata* leaf extract. **Figure.4** and **Table.1** show EDX spectrum and elemental composition respectively. This indicates the presences of Ag and Co which confirms the formation of Ag-Co bimetallic nanoparticles. This is also supported by the EDX study which gives quantitative data of siver and cobalt compositions in BMNPs **.** Scanning electron microscopic (SEM) images of Ag-Co BMNPs with various magnifications are given in **Figure 5**. From this, it can be clearly noticed that Ag-Co bimetallic nanoparticles prepared are in the size range of 30-100 nm in diameter.

Table: 1. Quantitative results of Ag-Co BMNPs

Element	Weight %	Atomic %
Co L	26.27	39.47
Ag L	73.73	60.53
Totals	100.0	100.0

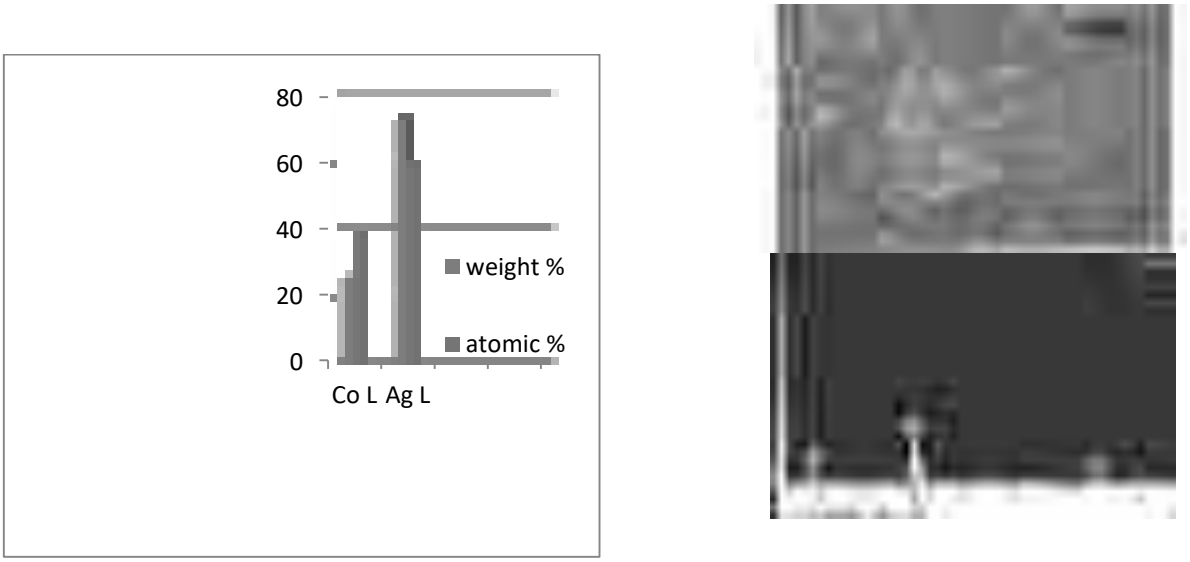
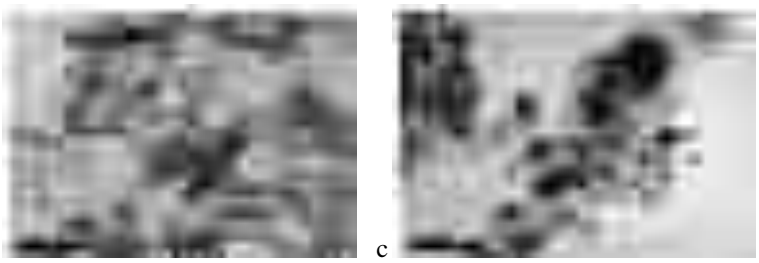


Figure 4 : EDX Analysis of Ag-Co BMNPs

Figure.5 : SEM images of Ag-Co BMNPs

3.4. **HRTEM analysis:** **Figure.5** shows the high resolution transmission electron microscopy (HRTEM) images for synthesized Ag-Co BMNPs from *Areva lanata* leaf extract. From these images, it was observed that Ag-Co BMNPs were formed with spherical morphology and crystalline structure below 100 nm in size. Indeed more explicitly the two metals nanospheres appear to be conjointly placed adjacent to each other giving an overall bilobal structure. This is also in good agreement with SEM images.



c

Figure .6 : HRTEM images of Ag-Co BMNPs

3.5. XRD analysis :

The XRD spectrum of green synthesized Ag-Co BMNPs from leaf extract is shown in figure: 7.

The peaks appeared at 2θ values of 32.123^0 ,37.972^0,44.122^0,64.253^0,77.216^0 corresponding to the Bragg's reflections of (220) (111),(200),(220) and (311) planes respectively of face centered cubic crystal structure[24].

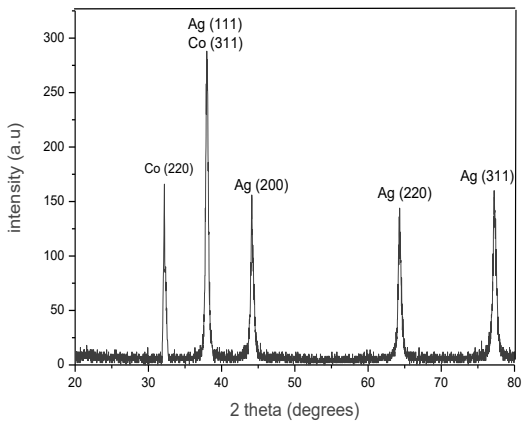


Figure:7 . XRD spectrum of Ag-Co BMNPs

The average size D (in nm) of Ag-Co bimetallic nanoparticles was calculated by using Debye-Scherrer equation (1).

$$D=\frac{K\lambda}{\beta\cos\theta} \quad \text{.....(1)}$$

D = crystalline size of Ag-Co BMNPs

λ = wavelength of x-ray source (0.15406 nm) used in XRD

β = full width at half maximum (FWHM) of the diffraction peak

K = Scherrer constant = 0.9

θ = Bragg's angle

S.no.	2θ (degrees)	θ (radians)	Cos θ	β (radians)	D (nm)
1	32.123^0	0.2803	0.9610	0.00678	21.2804
2	37.972^0	0.3313	0.9456	0.00714	20.5365
3	44.122^0	0.3850	0.9268	0.00664	22.5309
4	64.253^0	0.5607	0.8469	0.00686	23.8658
5	77.216^0	0.6738	0.7814	0.00673	26.3660

The numerically calculated value of the synthesized Ag-Co BMNPs materials corresponds to an average particle size of 22.916 nm.

4. Photodegradation studies on Malachite Green dye using Ag-Co BMNPs :

The photo degradation experiments are carried on Malachite Green dye using green synthesized Ag-Co BMNPs acting as a catalyst. . Initially, 50 ppm of malachite green stock solution was prepared. Then reaction mixtures were prepared by adding certain amount of Ag-Co BMNPs(10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg,70 mg,80 mg) to to 100 mL of malachite green for distinct concentrations(5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm). The pH of the reaction mixtures was altered (for pH 3,4,5,6,7,8,9,10) by adding 0.1 N H_2SO_4 or 0.1 N NaOH solutions when required. Now this mixture was agitated for 20 minutes in dark condition to attain adsorption-desorption equilibrium between malachite green and Ag-Co BMNPs. Sun light was used as irradiating source to reaction mixture for studying degradation during 11.00 am to 3.00 pm. At regular 30 minutes time intervals aliquot part of the reaction mixture is taken, centrifuged to remove the photocatalyst particles and optical absorption properties were analyzed by using UV-Visible Spectrophotometer . The absorbance was observed by varying parameters like changing the time of contact between the catalyst and the dye, pH of the reaction mixture, concentration of the dye solution, dosage of the catalyst. Malachite green shows the highest absorption [25] at 617nm. To determine the percentage of degradation of MG solution following equation (2) was used.

$$\%\ \text{degradation} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100 \quad \text{... (2)}$$

Where, A_0 is the initial absorbance of the MG solution at zero minutes and A_t is the absorbance of the degraded solution after time t minutes[26].

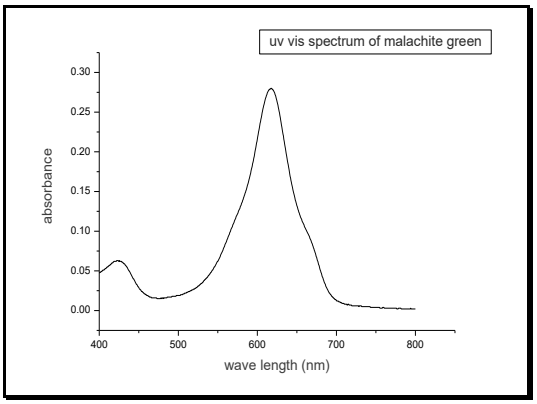


Figure 8(a): UV VIS spectrum of Malachite Green

To study the photocatalytic activity of the Ag-Co BMNPs on malachite green visible region of the light source was selected on UV-visible spectrophotometer was used. Absorption spectrum of 10 ppm malachite green solution was shown in **Figure 8(a).** Highest absorption peak at 617 nm was observed and this maximum absorption peak was considered to monitor the photodegradation reaction of MG dye for all further studies in this paper.

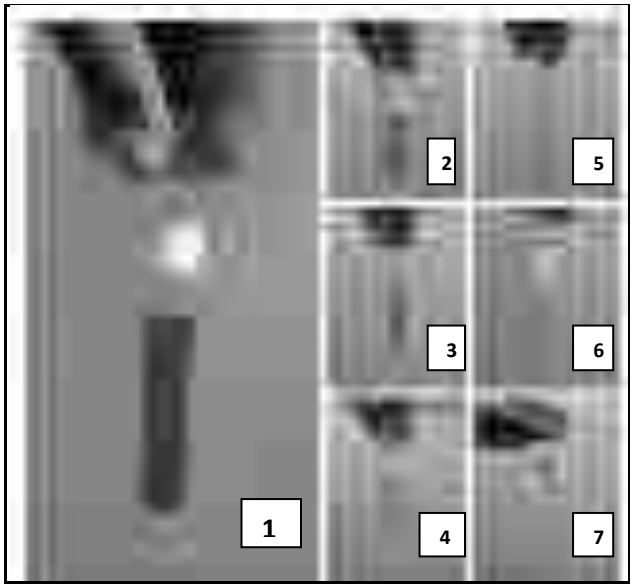
4 (a) . Plausible Mechanism of Photocatalytic degradation

By absorbing suitable wavelength radiations MG dye goes to its first excited singlet state.Then by undergoing intersystem crossing (ISC) to it goes the triplet state. Meanwhile Ag-Co BMNPs also absorbs the radiant energy to excite its electron from valence band to the conduction band. The hole abstracts an electron from H_2O to generate $\cdot OH$ radical and H^+. $\cdot OH$ radical oxidizes the MG dye to its leuco form degrades into colourless product. The electron will be abstracted by oxygen molecule generating superoxide anion radical ($\cdot O_2^-$). The formed anion radical reduce the MG dye to its leuco form, which degrades to products .

4.1. Effect of time of contact :

Photodegradation capacity of the Ag-Co BMNPs on malachite green dye was studied by batch mode experiments. The efficiency of BMNPs on degradation of MG is expected to be increased by increasing contact time. The effect of contact time was carried out by taking 10 ppm of 100 ml MG dye solution and 10 mg of BMNPs (pH at 7) as catalyst load which is shown in **Figure 8(b)** and **Figure 8**

(c). Initially, degradation of dye by using BMNPs was found to be fast and then it almost levels-off as % degradation attains more or less constant value with this increase in contact time[27]. This is due to strong adsorption forces prevailing between the dye and the BMNPs and as the number of the reactive sites on the catalyst were vacant during initial periods of contact time.. But after 100 minutes of contact period, % degradation steadily approached to a more or less constant value when equilibrium was almost reached because there is a steady fall in the number of vacant sites available for further dye adsorption.[28,29]



(1) 0 mins (2) 30 mins (3) 60 mins (4) 90 mins
(5) 120 mins (6) 150 mins (7) 180 mins

Figure 8(b): Colour change in MG dye after addition of Ag-Co BMNPs at various time intervals

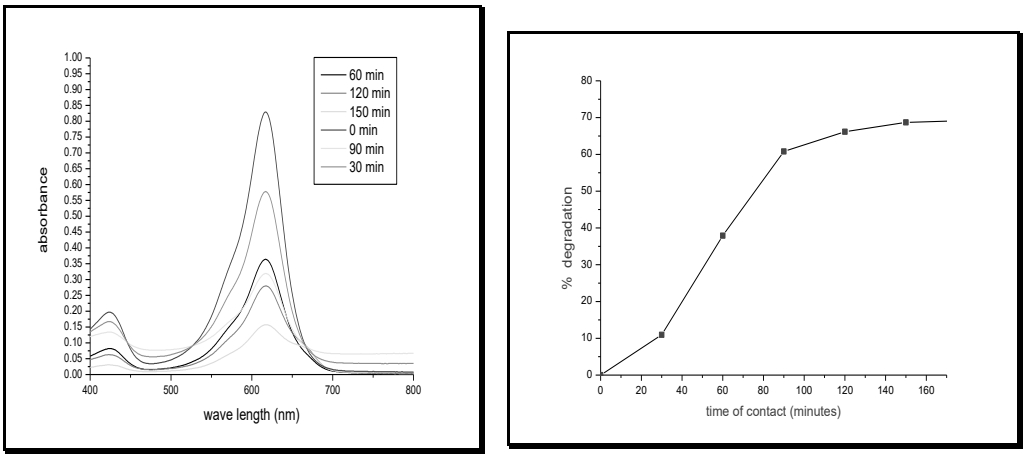


Figure 8(c): Effect of contact time on photo degradation

4.2. Effect of initial concentration of MG dye solution :

Initial concentration of MG dye solution is also expecting to effect the rate of photo degradation. To investigate this fact, dosage of BMNPs nano-catalyst was kept constant at 10 mg , keeping solution pH at 7 and the time of irradiation was maintained as 180 minutes. Whereas the initial concentrations of the MG dye solutions were varied at 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm. The rate of photodegradation can be represented graphically in **Figure 8(d).** It can be observed from the figure that the maximum degradation is found at 5 ppm, then it decreases with further rise in in the concentration of MG dye solution. Thus as the initial concentration of the dye increases beyond 5 ppm, % degradation decreases[30,31]. This observation can be explained by the fact that at the low initial concentration of the dye solution, large number of dye molecules could be adsorbed on the surface of BMNPs . As the concentration of the dye increases , deep coloured solution was formed due to large number of dye molecules which obstruct the photons from entering entering into solution there by only fewer photons can reach the BMNPs surface. Therefore, the % photodegradation is reduced when concentration of dye is high[32,33]. Thus in the present case 5-10 ppm dye solutions can be considered optimum concentration to produce efficient photodegradation upto 85-78% on a 10 mg nanocatalyst under the sunlight.

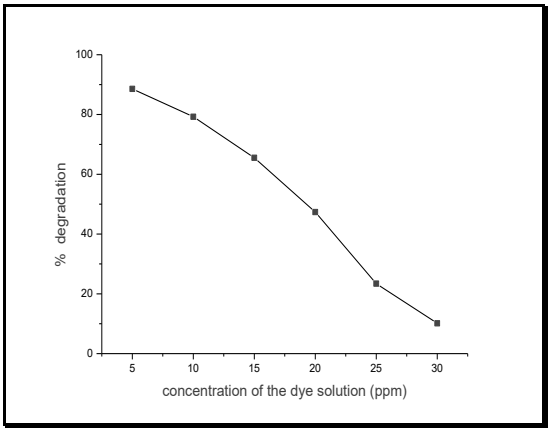


Figure.8(d): Effect of concentration of MG dye solution on photodegradation

4.3. Effect of pH:

pH of dye solution can influence the adsorption of dye on photocatalyst. Originally, Malachite green dye solution has pH value at 5. The initial concentration of malachite green solution 10 ppm and the concentrations of the photocatalyst 10 mg were kept constant with time of irradiation 120 minutes. Different solutions of various pH values of 3 to 10 were prepared. Degradation efficiencies were compared which was shown in **Figure 8(e).**

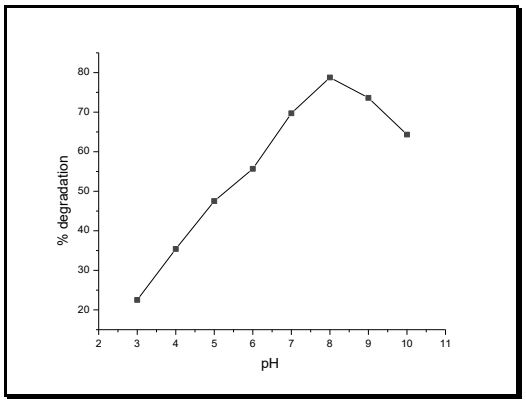


Figure.8(e): Effect of pH on photo degradation

It is observed that by increasing pH of the MG dye solution, the degradation of MG dye on the photocatalyst enhances up to pH 8 and on further increase of pH, the rate of photodegradation was diminished. The initial increase of rate of degradation may due to more availability of the OH^- ions at higher pH values. These ions will form $OH^{\cdot}$ Radicals by combining with positive holes (h^+) of semiconductor and hence they are responsible for photodegradation. But after pH 8, on further increase in pH of the reaction mixture there was a decline in the rate of degradation. This may be due to MG does not confine to its cationic form because of high concentrations of OH^- ions and there will be repulsion between the dye and negatively charged semiconductor surface. As a result the rate of photodegradation decreases.[34]. pH 8 was taken as optimum pH for photodegradation.

4.4. Effect of dosage of photo catalyst:

In photodegradation process, one of the important parameters of decolourizing of dye solution is photocatalyst dosage[34]. To avoid wastage of expensive catalyst and attain the maximum absorption of photons optimization of the catalyst dosage is important[35]. For this, dosage amount was varied from 10 mg to 80 mg taken in 100 ml of 10 ppm MG dye at pH 7 with contact time 90 minutes. The degradation of MG was shown in **figure 8(f).**

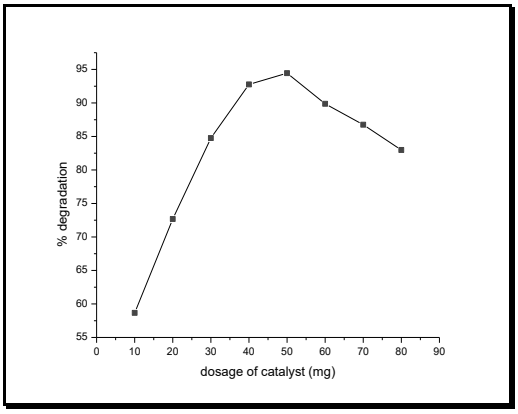


Figure 8(f): Effect of dosage of catalyst on photo degradation

This concluded that, by increasing dosage of catalyst from 10 mg to 80 mg in 100 ml, the degradation of MG dye enhances because the increase in amount of catalyst upto 50 mg would increase the reactive sites that produce more reactive species[36]. On further increase of catalyst dosage %degradation of MG dye decreases because the catalyst particles form more turbid suspensions on higher loadings, which reduce the penetration of the solar light into the reaction mixture[37,38]. 20 mg of catalyst was taken as optimum dosage for degradation.

CONCLUSION

An ecologically innocuous method is projected to synthesize Ag-Co bimetallic nanoparticles from *Areva lanata* leaf extract. From UV-VIS spectal analysis it is proved that the particles are in nanoscale as per the positions of the Surface Plasmon Resonance (SPR) bands. FTIR data confirms the presence of secondary metabolites of phyto molecules as the bio reducing and capping agents of the formed nanoparticles. XRD, SEM andTEM analysis results evinced that Ag-Co BMNPs are in spherical morphology and cubic crystalline structure with size between 30-100 nm. The photocatalytic activity of these nanoparticles is examined under sunlight for degradation of MG dye which is environmental pollutant. The % photodegradation of MG dye changes with parameters such as contact time, concentration of MG dye, pH, photocatalyst dosage. From this research study on bimetallic Ag-Co BMNPs synthesized from *Areva lanata* leaf extract, the optimum conditions found in the degradation of MG dye is pH 8, weight of catalyst 20 mg, dye concentration of 10 ppm and contact time of 120 minutes. The degradation is obtained as 88.6% under these optimum conditions.

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INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

Biogenic Synthesis of Novel Ag-Ni Bimetallic Nanoparticles : Characterization and Photocatalytic Activity on Malachite Green

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ABSTRACT

An environmentally benign cost effective method was reported for green synthesis of Ag-Ni bimetallic nanoparticles by using *Aerva Lanata* leaf extract. The phyto molecules present in the extract are acting as reducing agents, stabilizing and capping agents for the biosynthesized nano particles. The characterization studies were done by UV-VIS , FTIR spectroscopies , SEM, XRD, EDX and HRTEM analyses. These nanoparticles were further applied as photocatalysts for degradation of Malachite Green dye by irradiation under sunlight. From this present study on bimetallic Ag-Ni nanoparticles synthesized from *Areva lanata* leaf extract, the optimum conditions found in the degradation of malachite green dye is pH **8**, weight of catalyst **30 mg**, dye concentration of **10 ppm** and contact time of **120 minutes**. A maximum photodegradation of **~ 85-89%** could be obtained under these optimum conditions.

KEY WORDS

Bimetallic nanoparticles (BMNPs), *Aerva Lanata*(AL), Malachite Green (MG), photo degradation.

INTRODUCTION

Nanotechnology involves the synthesis and application of materials having one of the dimensions in the range of 0.1-100 nm and acts as a channel between bulk materials and atomic or molecular structures.[1] In recent times bimetallic nanoparticles have gained considerable attention because of their importance for magnetic, optical and catalytic applications in multiple fields[2],[3]. Various physical, chemical and biological methods have been employed for the synthesis of bimetallic nanoparticles[4][5]. Several advantages of biological methods over physical and chemical methods are the biological process is environmentally benign, less time consuming, cost effective with almost negligible industrial waste without use of toxic chemicals[6]. Biosynthesized bimetallic nanoparticles are used in several contemporary fields viz. imaging, labeling, luminescence tagging, drug delivery and biomedical field because of their superior properties. Biosynthesized bimetallic nanoparticles additionally have been found to possess extraordinary catalytic activities[7].

Dyes are used widely in various fields such as cosmetics, leather, food and textile industries. The release of these industrial dye effluents into water shows adverse effect on quality of water[8]. One of such dye is malachite green (MG).

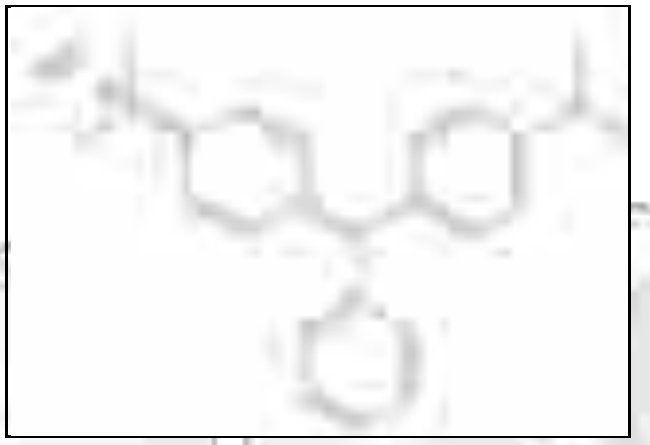

Figure 1 (a) : Structure of Malachite Green dye

Malachite green dye is a cationic green crystalline water soluble dye and belongs to triphenylmethane category[9] . (Figure 1.a). MG dye is a potential environmental contaminant and a peril to public health as it is a multi-organ toxin proved as by both experimental clinical observations [10] .So it is necessary to exterminate the MG dye effluents from water . Many chemical, physical and biological treatment methods including adsorption, precipitation filtration, electrodialysis, coagulation, oxidation and membrane separation are used in the treatment of dye effluents[11]. Dye removal via degradation using photocatalysts is the most scatheless and desirable among all methods because of its sustainable and ecofriendly technology[12][13]. Recent times nanotechnology has been extended in the waste water treatment and nanoparticles are used as photocatalysts for degradation of dyes due to their extensive surface area[14].

Herein, an effortless and robust green method for synthesis of Ag-Ni bimetallic nanoparticles (BMNPs) by using leaf extract of *Areva lanata* as a stabilizing,reducing and capping agent was reported and by using these BMNPs as catalysts for photodegradation of MG dye carried under solar light irradiation was also studied at various reaction conditions to observe the optimum conditions for the maximum photodegradation.

2. EXPERIMENTAL

2.1. Materials: Chemical reagents used (siver nitrate and nickel nitrate) in this study were of analytical grade. Deionised water was used to clean glassware, to prepare chemical solutions and for experimental procedure. Fresh leaves of *Aerva lanata* were collected from agricultural fields in S.Kota, Vizianagaram district, Andhra Pradesh state, India.

2.2. Preparation of *Aerva lanata* leaf extract: 100g of fresh leaves were weighed and thoroughly washed with running tap water to remove detritus on surface of leaves followed by deionised water to get rid of other contaminants from leaves and dried up under shade for 10 days. These leaves were cut into tiny pieces and made homogenized powder by using home blender. The procured powder placed in refrigerator at

4ºC which was kept in an air tight container. Now 200 mL deionised water was taken in 500 mL beaker to this 10g stored powder was weighed and added. The contents in the beaker heated for 30 minutes at 50ºC with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled concoction was filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4ºC. This was taken as leaf extract throughout the experiment (Figure : 1.(b)1(c)).

Figure 1(b): *Aerva Lanata* plant

Figure 1(c): *Aerva Lanata* leaf extract

2.3. Synthesis of Ag-Ni bimetallic nanoparticles:

Equimolar (25 mM) concentrations of silver nitrate and nickel nitrate aqueous solutions were prepared separately in 100 ml volumetric flasks by dissolving 0.4246 g, 0.7267 g weight of $AgNO_3$ and $Ni(NO_3)_2$ in deionized water respectively. Synthesis of Ag-Ni BMNPs was done by taking 100mL of $AgNO_3$ solution in a 500 mL beaker, to this 90ml of leaf extract, 100mL of $Ni(NO_3)_2$ solution were added by drop wise in simultaneous addition process. After this addition the beaker was placed on a magnetic stirrer for continuous agitation. This mixture was stirred at 70ºC for 60 minutes at pH 8 on magnetic stirrer. These synthesized BMNPs were separated out by doing centrifugation at 5000 rpm for 40 minutes. The obtained BMNPs were washed with deionised water 2 times to remove unwanted constituents and dried in oven at 80^0C for two hours. The resultant BMNPs particles were collected (figure: 1(d)) and used for characterization.

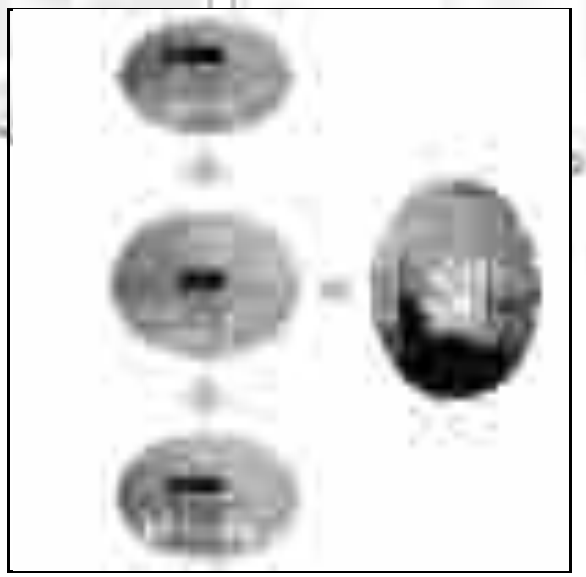

Figure : 1(d) . Synthesis of Ag-Ni BMNPs from precursor solutions

2.4. Characterization:

Formation of Ag-Ni BMNPs was confirmed by UV-Visible absorption spectra using UV-2450 SHIMADZU double beam spectrophotometer, FTIR using Bruker, SEM, EDX studies are done by using Hitachi S-3700N machine and the morphology of BMNPs was elucidated by HRTEM analysis with FEI Technai machine.

3. RESULTS AND DISCUSSION

3.1.UV-Visible spectral analysis:

UV-Visible spectrum of *Aerva Lanata* leaf extract is given in Figure2(a). UV-Visible absorption spectrum of Ag-Ni BMNPs is presented in Figure.2(b). The characteristic surface plasmon resonance (SPR) band at around 438 nm is observed in Ag-Ni BMNPs which confirms the nano size of the synthesized BMNPs[15] .

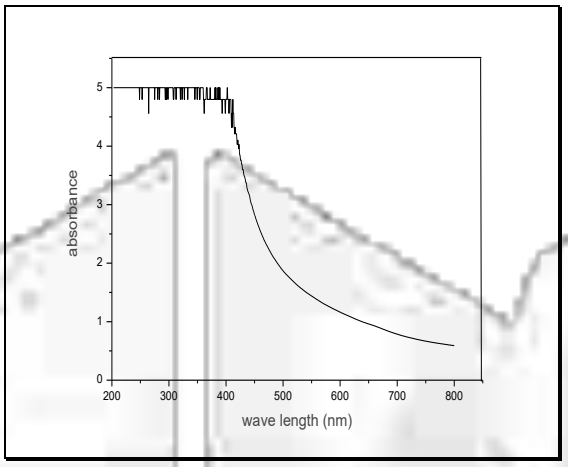

Figure 2 (a): UV-VIS Spectrum of *Aerva Lanata* leaf extract

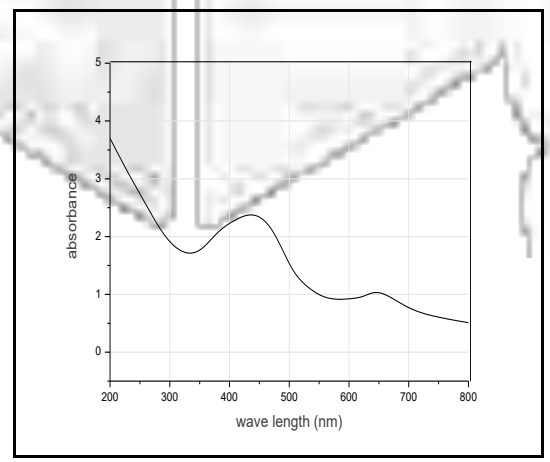

Figure.2(b): UV-Visible absorption spectrum of Ag-Ni BMNPs

3.2. FTIR spectral analysis:

FTIR spectral data is used to identify different functional groups present in biomolecules of leaf extract.These groups are responsible for the bioreduction of Ag^{+}, Ni^{+2}precursors and also for capping and stabilization of Ag-Ni BMNPs. The intense peaks were observed and compared with standard values to analyze the functional groups in *Aerva lanata* leaf extract and greensynthesized Ag-Ni BMNPs. FTIR spectra of *Aerva lanata* leaf extract and synthesized Ag-Ni BMNPs by using *Aerva lanata* leaf extract were shown in **Figure.3 (a)** and **Figure.3 (b)** respectively**.** The comparison of the FTIR spectra of both Ag-Ni BMNPs and leaf extract of *Aerva lanata* clearly indicates the existence of

the plant extract phytomolecules such as polyphenols ,terpenes, flavonoids, glycosides, tannins, sterols, amides, carbohydrates, amines the surface of the Ag-Ni BMNPs [16,17].

Figure.3 (a): FTIR spectrum of *Aerva lanata* leaf extract

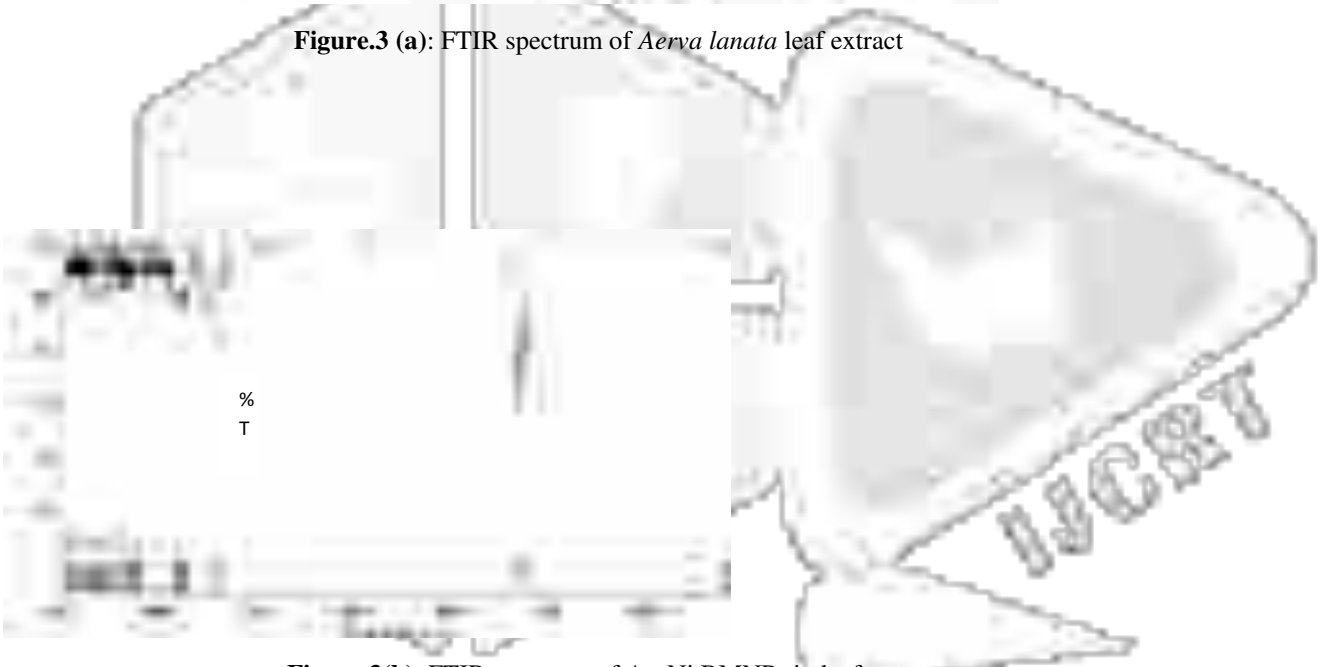

Figure 3(b). FTIR spectrum of Ag-Ni BMNPs in leaf extract

The strong intense peaks between 3200 cm^{-1} to 3950^{-1}may be due to N-H ,O-H stretching of 1^0 amines and polyhydroxy groups present in the extract.The strong absorption at 1643 cm^{-1}indicates the presence of C=O group of amides.This result gives us the evidence about the high protein content of the extract.The small peak at 2892 cm^{-1} is may be due to C-H symmetrical stretching of methylene groups.The peak position at 1519 cm^{-1} may be due to C=C stretch of aromatic ring.The peak at 598 cm^{-1} is denoting the presence of C-Cl group.

The FTIR spectrum of Ag-Ni BMNPs exhibits major peak positions at 3212 cm^{-1},3416 cm^{-1} and 3382 cm^{-1}which indicate the N-H stretching vibrations of amines and O-H stretching of hydroxyl groups of alcohols and phenols. Intense peak at 1640 $cm^{-1}$is due to C=O stretching of amide group. Very small peak at 602 cm^{-1} indicates the presence of C-Cl group.

FTIR analysis clearly confirms that all the aforementioned absorption peaks of the extract are barely shifted in the FTIR spectrum of Ag-Ni BMNPs as the phyto molecules of the extract act as bioreducing agents, capping and stabilizing agents for the synthesized nanoparticles. The existence of these IR bands also in the Ag-Ni BMNPs confirmed that the surface of the nanoparticles was covered by plant secondary metabolites such as carbohydrates, glycosides, Saponin, phytosterols, phenolic compounds, tannins, flavonoids, proteins, aminoacids, diterpenes, carboxylic acids, amides, ketones, alkyl halides.[18]

3.3.**SEM and EDX analysis:**

From energy dispersive X-ray analysis we can analyze all the elements present in the BMNPs prepared by *Aerva lanata* leaf extract. **Figure.4** and **Table.1** show EDX spectrum and elemental composition respectively. This indicates the presences of Ag and Ni which confirms the formation of Ag-Ni bimetallic nanoparticles. This is also supported by the EDX study which gives quantitative data of siver and nickel compositions in BMNPs **.** Scanning electron microscopic (SEM) images of Ag-Ni BMNPs with various magnifications are given in **Figure 5**. From this it can be clearly noted that the prepared Ag-Ni bimetallic nanoparticles are in the size range between 50 and 100 nm in diameter.

Table: 1. Quantitative results of Ag-Ni BMNPs

Element	Weight %	Atomic %
Ni L	16.17	16.95
Ag L	73.06	41.67
O K	10.76	41.38
Totals	100.0	100.0

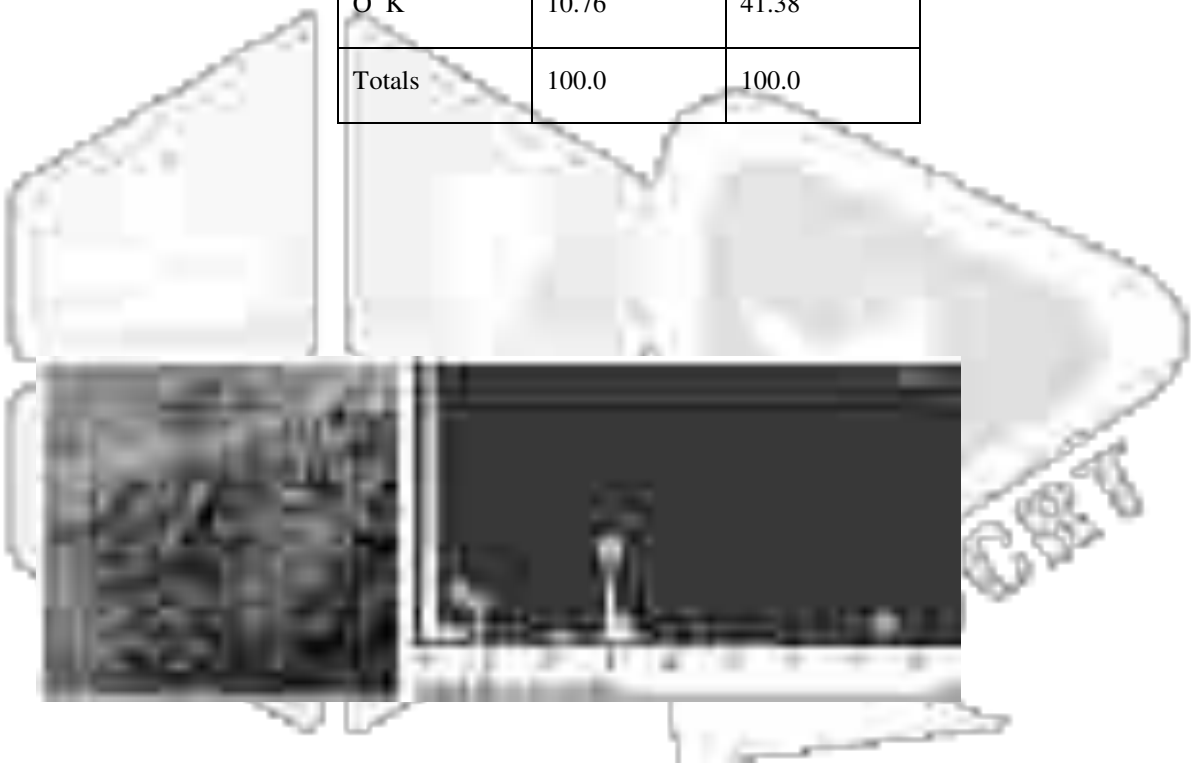

Figure 4 : EDX Analysis of Ag-Ni BMNPs

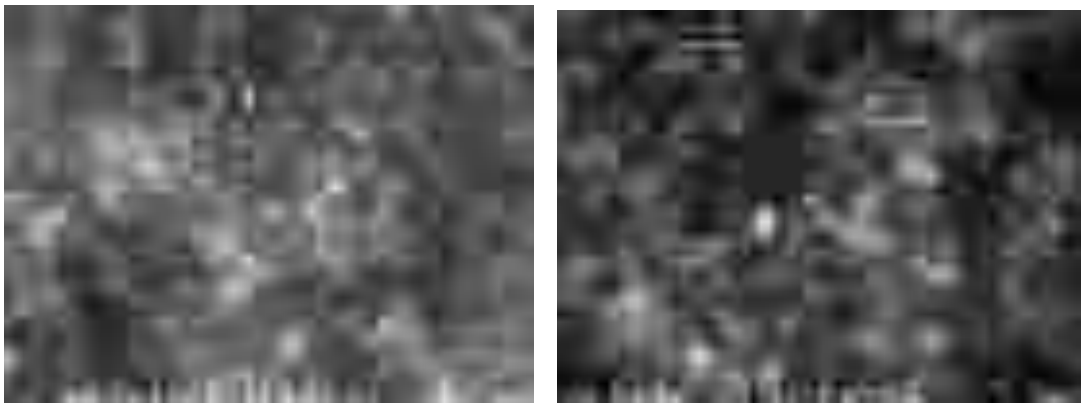

Figure.5 : SEM images of Ag-Ni BMNPs

3.4. **HRTEM analysis: Figure.6** shows the high resolution transmission electron microscopy (HRTEM) images for synthesized Ag-Ni BMNPs from *Areva lanata* leaf extract. From these images, it was observed that Ag-Ni BMNPs were formed with spherical morphology and crystalline structure below 100 nm in size. More specifically, the two metal nanospheres appear to be positioned adjacent to each other. It is also in strong accordance with the images from SEM analysis.

Figure .6 : HRTEM images of Ag-Ni BMNPs

3.5. XRD analysis :

The XRD spectrum of green synthesized Ag-Ni BMNPs from leaf extract is shown in **figure: 7.**
The peaks appeared at 2θ values of 38.20^0, 44.36^0, 64.40^0, 77.42^0 correspond to the Bragg's reflections of Ag(111), Ag(200), Ni(111), Ag(220), Ni(222) , Ag (311) planes respectively of face centered cubic crystal structure [19] as shown in the **Figure:7**

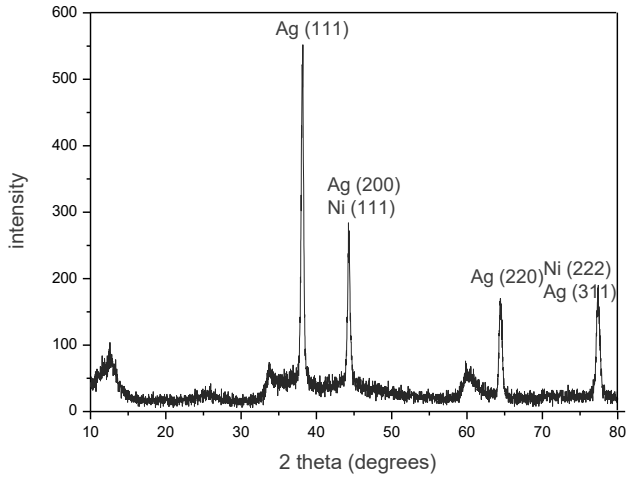

Figure:7 . XRD spectrum of Ag-Ni BMNPs

The average size D (in nm) of Ag-Ni bimetallic nanoparticles was calculated by using Debye-Scherrer equation (1).

$$D=\frac{K\lambda}{\beta\cos\theta} \quad \text{.....(1)}$$

D = crystalline size of Ag-Ni BMNPs

λ = wavelength of x-ray source (0.15406 nm) used in XRD

β = full width at half maximum (FWHM) of the diffraction peak

K = Scherrer constant = 0.9

θ = Bragg's angle

S.no.	2θ (degrees)	θ (radians)	Cos θ	β (radians)	D (nm)
1	38.20^0	0.3333	0.9450	0.00714	20.5495
2	44.36^0	0.3871	0.9260	0.00664	22.5503
3	64.40^0	0.5620	0.8462	0.00686	23.8855
4	77.42^0	0.6756	0.7803	0.00673	26.4031

The numerically calculated value of the synthesized Ag-Ni BMNPs materials corresponds to an average particle size of **23.3471 nm**.

4. Photodegradation studies on Malachite Green dye using Ag-Ni BMNPs :

The photo degradation experiments are carried on Malachite Green dye using green synthesized Ag-Ni BMNPs acting as a catalyst. Initially, 50 ppm of malachite green stock solution was prepared. Then reaction mixtures were prepared by adding certain amount of Ag-Ni BMNPs (10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg,70 mg,80 mg) to 100 mL of malachite green for distinct concentrations(5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 35 ppm). The pH of the reaction mixtures was maintained in the experiments at various values for both acidic and basic range (i.e. pH 3,4,5,6,7,8,9,10,11) by adding 0.1 N H_2SO_4 or 0.1 N NaOH solutions when required. Now this mixture was agitated for 20 minutes in dark condition to attain adsorption-desorption equilibrium between malachite green and Ag-Ni BMNPs. Sun light was used as irradiating source to reaction mixture for studying photodegradation during 11.00 am to 3.00 pm. At regular 30 minutes time intervals, aliquot part of the reaction mixture was taken, centrifuged to remove the photocatalyst particles and optical absorption properties were analyzed by using UV-Visible Spectrophotometer . The absorbance was observed by varying parameters like changing the time of contact between the catalyst and the dye, pH of the reaction mixture, concentration of the dye solution, dosage of the catalyst. Malachite green shows the highest absorption at 617 nm. [20].To determine the percentage degradation of MG dye solution following equation (2) was used.

$$\% \text{ degradation} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100 \quad \ldots \quad (2)$$

Where, A_0 is the initial absorbance of the MG solution at zero minutes and A_t is the absorbance of the degraded solution after time t minutes.

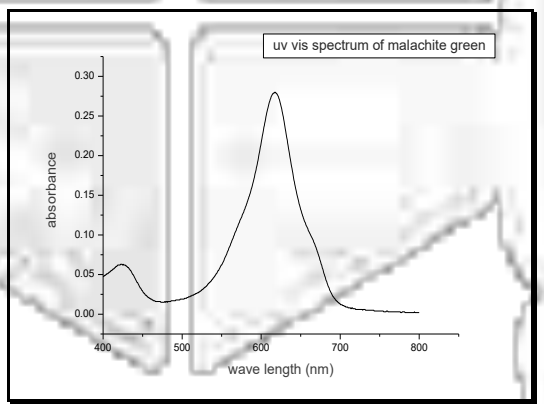

Figure 8(a): UV- VIS spectrum of Malachite Green

To study the photocatalytic activity of the Ag-Ni BMNPs on malachite green visible region of the light source was selected on UV-visible spectrophotometer . Absorption spectrum of 10 ppm malachite green solution was shown in **Figure 8(a).** Highest absorption peak at 617 nm was observed and this maximum absorption peak was considered to monitor the photodegradation reaction of MG dye for all further studies in this paper.

4 (a) . Probable Mechanism of Photocatalytic degradation of MG dye with Ag-Ni BMNPs

By absorbing suitable wavelength radiations MG dye goes to its first excited singlet state. Then by undergoing intersystem crossing (ISC) it enters into to the triplet state. In the intervening time Ag-Ni BMNPs also absorb the radiant energy to excite its electron from valence band to the conduction band. The hole abstracts an electron from H_2O to generate $\cdot OH$ radical and H^+. $\cdot OH$ radical oxidizes the MG dye to its leuco form degrades into colourless product. The electron will be abstracted by oxygen molecule generating superoxide anion radical ($\cdot O_2^-$). The formed anion radical reduce the MG dye to its leuco form, which on degradation forms products .

4.1. Effect of time of contact :

Photodegradation capacity of the Ag-Ni BMNPs on malachite green dye was studied in the presence of sunlight by batch mode experiments. The efficiency of BMNPs photocatalyst on degradation of MG is expected to be increased by increasing contact time. The effect of contact time was carried out by taking 10 ppm of 100 ml MG dye solution and 10 mg of BMNPs (pH at 7) as catalyst load which is shown in **Figure 8(b)** and **Figure 8 (c).** From the initial part of the graph i.e upto 120 min, % photodegradation was exhibiting rapid rise. However, above 120 min the slope is relatively less steeper although % photodegradation remains still considerably predominant thus shows higher values.

Thus, upto 120 min, the % photodegradation of the dye by using the BMNPs photocatalyst was found to be rapid, and above 120 min it becomes relatively less rapid although active even upto 180 min. This is due to strong adsorption forces that predominate between the dye and the BMNPs as the number of the reactive sites on the photocatalyst were largely vacant during initial periods of contact time. But after 120 minutes of contact period, equilibrium between the number of vacant sites of adsorption and the dye molecules appears to be established although at slower rate, hence exhibit slightly slower rise of % photodegradation. [21]

(1) 0 mins (2) 30 mins (3) 60 mins (4) 90 mins

(5) 120 mins (6) 150 mins (7) 180 mins

Figure 8(b): Colour change in MG dye after addition of Ag-Ni BMNPs at various time intervals

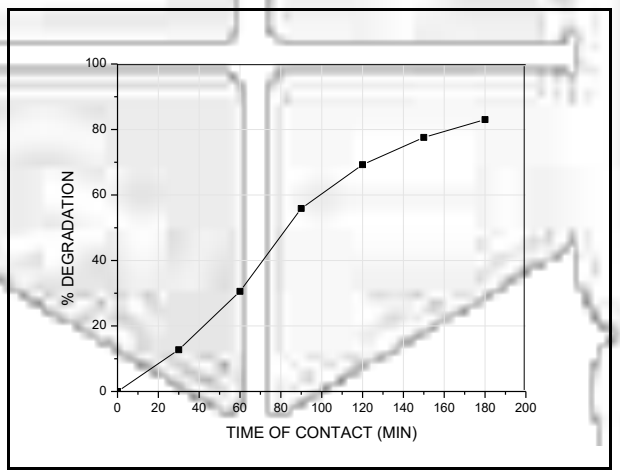

Figure 8(c): Effect of contact time on photo degradation

4.2. Effect of initial concentration of MG dye solution :

Initial concentration of MG dye solution is also expected to effect the rate of photo degradation. To investigate this fact, dosage of BMNPs nano catalyst was kept constant at 10 mg , maintaining the dye solution pH at 7 and the time of irradiation as 180 minutes. However one parameter the initial concentration of the MG dye solutions was varied at 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm , 30 ppm and 35 ppm. The rate of photodegradation can be represented graphically in **Figure 8(d).**

It can be observed from the figure that the maximum photodegradation is found at 5 ppm, then it decreases with further rise in the concentration of MG dye solution. Thus as the initial concentration of the dye increases beyond 5 ppm, % photodegradation decreases[22]. This observation can be explained by the fact that at the low initial concentration of the dye solution, large number of dye molecules could be adsorbed on the surface of BMNPs . Increasing the initial concentration of the dye can result competitive adsorption among the dye molecules while the area of active reaction sites on the catalyst is fixed. Consequently the % photodegradation of MG dye decreases.

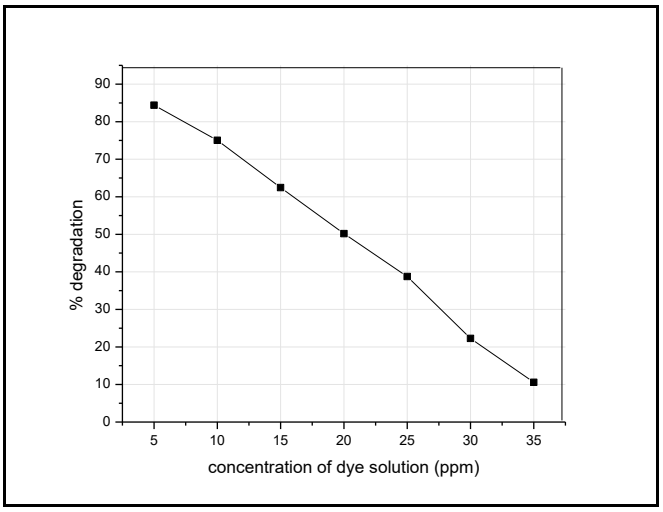

Figure.8(d): Effect of concentration of MG dye solution on photodegradation

4.3. Effect of pH:

pH of dye solution can influence the adsorption of dye on the photocatalyst. Keeping pH as variable, all other parameters were kept constant viz. the initial concentration of the malachite green solution was taken as 10 ppm , the concentrations of the photocatalyst as 10 mg and with time of irradiation 120 minutes. Different solutions of various pH values from 3 to 11 were prepared. Photodegradation efficiencies were compared which was shown in **Figure: 8(e).**

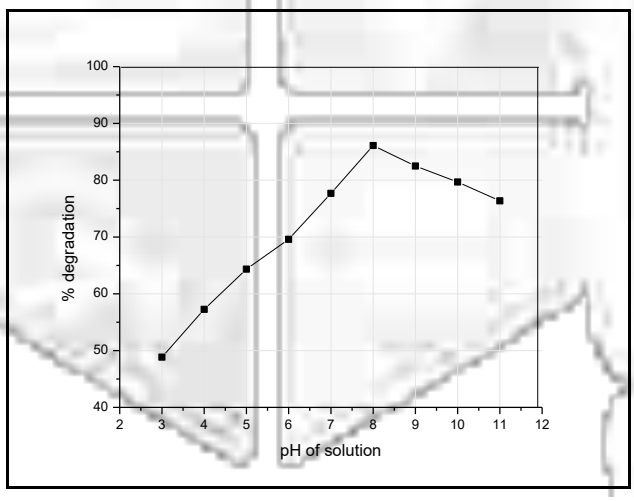

Figure:8(e): Effect of pH on photo degradation

It is observed that by increasing pH of the MG dye solution, the % photodegradation of MG dye on the photocatalyst enhances up to pH 8 and on further increase of pH, the rate of photodegradation was diminished. At pH less than 4 lower values of % photodegradation of the dye by the catalyst was observed. Solution pH may influence both solution chemistry and surface active sites of the adsorbent. At acidic pH , H^+ ions of the water molecules, may race with dye ions for the adsorption sites of the catalyst surface, there by hindering the adsorption of the dye. As the pH value increases, the number of H^+ ions of the aqueous solution reduces, hence the cationic dye molecules could approach the catalyst surface at a faster rate, thus exhibit an fast increase in the rate of % photodegradation. At pH 8 the adsorption is maximum, hence the photo degradation was found to be maximum. At pH >8, the malachite green dye may not be maintained in its cationic form due to high concentration of OH^- ions in the aqueous dye solution. Consequently, the electrostatic repulsion increases between the MG dye and negatively charged catalyst surface. As a result the photodegradation slowly decreases from pH 8 to 11 . [23]

4.4. Effect of dosage of photo catalyst:

In photodegradation process, one of the important parameters of decolourizing of dye solution is dosage of the photocatalyst. To avoid splurge of costly catalyst and attain the maximum absorption of photons optimization of the catalyst dosage is important. For this, dosage amount was varied from 10 mg to 80 mg taken in 100 ml of 10 ppm MG dye at pH 8 with contact time 120 minutes. The degradation of MG was shown in **figure 8(f).**

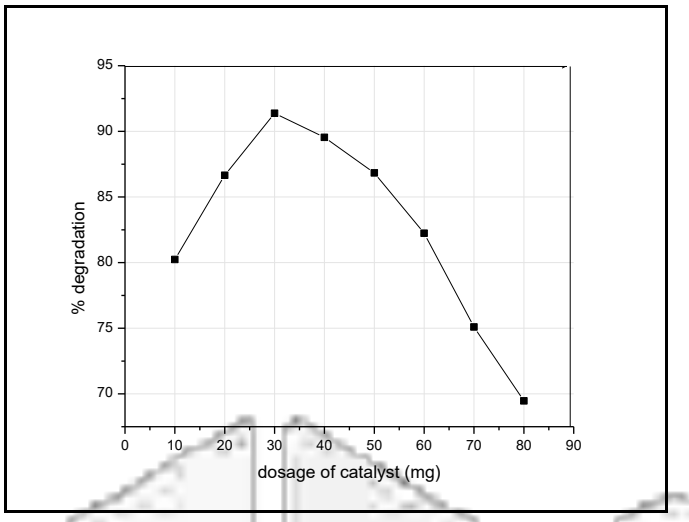

Figure 8(f): Effect of dosage of catalyst on photo degradation

From the graph it can be concluded that, by increasing dosage of catalyst from 10 mg to 80 mg in 100 ml dye solution, the % photodegradation of the MG dye shows an increasing trend upto 30 mg. This is because the increase in amount of catalyst upto 30 mg would increase the number of reactive sites that produce more reactive species [24]. On further increase of catalyst dosage, % photodegradation of MG dye decreases because the catalyst particles form more turbid suspensions at higher loadings, which increase the scattering of the solar light and reduce the penetration of light into the reaction mixture.

CONCLUSION

An ecologically safe method is projected in this report to synthesize Ag-Ni bimetallic nanoparticles from *Areva lanata* leaf extract. From UV-VIS spectral analysis it is confirmed that the particles are in nanoscale as per the positions of the Surface Plasmon Resonance (SPR) bands. FTIR data confirms the presence of secondary metabolites of phyto molecules that act as the bio reducing and capping agents of the formed nanoparticles. Results of XRD, SEM and TEM analyses proved that Ag-Ni BMNPs are in spherical morphology and cubic crystalline structure with size between 20-100 nm. The photocatalytic activity of these nanoparticles is examined under sunlight for degradation of MG dye which is an environmental pollutant. The % photodegradation of MG dye changes with parameters such as contact time, concentration of MG dye, pH, photocatalyst dosage. From this research study on bimetallic Ag-Ni BMNPs synthesized from *Areva lanata* leaf extract, the optimum conditions found in the degradation of MG dye is pH **8**, weight of catalyst **30 mg**, dye concentration of **10 ppm** and contact time of **120 minutes**. A maximum photodegradation of **~ 85-89%** could be obtained under these optimum conditions.

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Phyto Fabricated Green Synthesis of Novel Ag-Fe Bimetallic Nanoparticles : Characterization and Photocatalytic Activity on Malachite Green Dye

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Abstract - **An environmentally sound, facile and cost effective method was reported for green synthesis of Ag-Fe bimetallic nanoparticles by using Aerva Lanata leaf extract. The phyto molecules present in the extract are acting as reducing agents, stabilizing and capping agents for the biosynthesized nano particles. The characterization studies were done by UV-VIS , FTIR spectroscopies , SEM, XRD, EDX and HRTEM analyses. These nanoparticles were further applied as photocatalysts for degradation of Malachite Green dye by irradiation under sunlight to study optimum conditions for maximum photodegradation. It was observed that the maximum photodegradation 88.75% was achieved for 10 ppm of dye solution at pH 9 with a catalyst dose of 40 mg under irradiation for 120 minutes.**

keywords - **Bimetallic nanoparticles (BMNPs), Aerva Lanata(AL), Malachite Green (MG), photo degradation**

INTRODUCTION

Nanotechnology involves the synthesis and application of materials having one of the dimensions in the range of 0.1-100 nm[1] . Nanoscience and nanotechnology is about the precise and purposeful manipulation of matter at the atomic level. It embraces many different fields like biology, chemistry, physics, engineering and medicine[2].There are two types of methods generally adopted for fabricating nanomaterials: top-down methods and bottom-up methods. In top down method we remove material progressively from bulk substrate until the desired nanomaterial is obtained. Bottom-up methods work starting from the atomic or molecular precursors and by gradually assembling it until the desired structure is formed (**Figure.1**)[3].

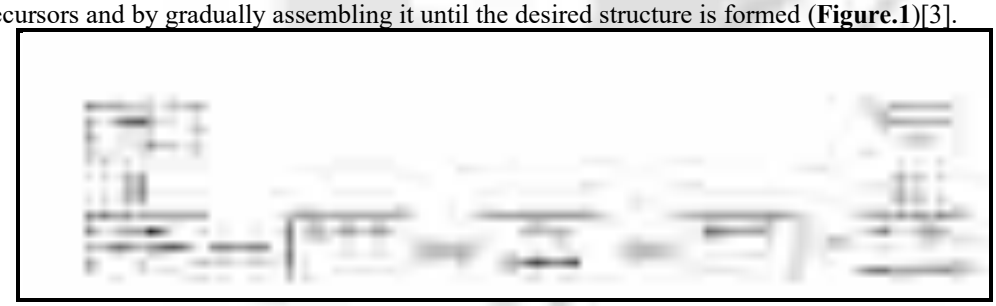


Figure.1: Different approaches for preparation of nanoparticles

Prodigious growth in nanotechnology has spread out its application in biomedical sciences, nutrition, energy sciences , nanobiotechnology, cosmetics, mechanics , optics , chemical industries, drug-gene delivery [4]. Alloying of different metals in nano size could enhance the properties of their respective monometallic nanoparticles. Bimetallic nanoparticles show greater stability, catalytic activity than monometallic nanoparticles [5]. Generally nanometals are synthesized by chemical reduction methods but they are hazardous and expensive. Green methods are ecofriendly, cheap and environmental benign methods for the synthesis of nanometals. Secondary metabolites present in plants acts as reducing and capping agents [6].Bimetallic alloy nanoparticles were synthesized in green method by using microorganisms such as yeast, bacteria and algae became one of the facile methods [7].

Dyes are used widely in various fields such as cosmetics, leather, food and textile industries. The release of these industrial dye effluents into water shows adverse effect on quality of water[8]. One of such dye is malachite green (MG). Malachite green dye is a cationic green crystalline water soluble dye and belongs to triphenylmethane category[9] . (**Figure 1.a**). MG dye is a potential environmental contaminant and a peril to public health as it is a multi-organ toxin as proved by both experimental and clinical observations [10] .So it is necessary to exterminate the MG dye effluents from water .

Figure 1 (a) : Structure of Malachite Green dye

Many chemical, physical and biological treatment methods including adsorption, precipitation filtration, electrodialysis, coagulation, oxidation and membrane separation are used in the treatment of dye effluents[11]. Dye removal via degradation using photocatalysts is the most scatheless and desirable among all other methods because of its sustainable and ecofriendly technology[12][13]. Recent times nanotechnology has been extended in the waste water treatment and nanoparticles are used as photocatalysts for degradation of dyes due to their extensive surface area[14].

Herein, an effortless and robust green method for synthesis of Ag-Fe bimetallic nanoparticles (BMNPs) by using leaf extract of *Areva lanata* as a reducing and capping agent was reported and by using these BMNPs as catalysts for photodegradation of MG dye under solar light irradiation was also studied at various reaction situations to observe the optimum conditions for the maximum degradation.

2. EXPERIMENTAL

2.1. Materials: Chemical reagents used (siver nitrate and ferrous sulphate) in this study were of analytical grade. Deionized water was used to clean glassware, to prepare chemical solutions and for experimental procedure. Fresh leaves of *Aerva lanata* were collected from agricultural fields in S.Kota, Vizianagaram district, Andhra Pradesh state, India.

2.2. Preparation of *Aerva lanata* leaf extract: 100g of fresh leaves were weighed and thoroughly washed with running tap water to remove detritus on surface of leaves followed by deionised water to get rid of other contaminants from leaves and dried up under shade for 10 days. These leaves were cut into tiny pieces and made homogenized powder by using home blender. The procured powder placed in refrigerator at 4$_o$C which was kept in an air tight container. Now 200 mL deionised water was taken in 500 mL beaker to this 10g stored powder was weighed and added. The contents in the beaker heated for 30 minutes at 50$_o$C with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled concoction was filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4$_o$C. This was taken as leaf extract throughout the experiment (**Figure : 1.(b)1(c)**).

Figure 1(b): *Aerva Lanata* plant

Figure 1(c): *Aerva Lanata* leaf extract

2.3. Synthesis of Ag-Fe bimetallic nanoparticles:

Equimolar (25 mM) concentrations of silver nitrate and nickel nitrate aqueous solutions were prepared separately in 100 ml volumetric flask by dissolving 0.4246 g, 0.6950 g weight of $AgNO_3$ and $FeSO_4$ in water respectively. Synthesis of Ag-Fe BMNPs was done by taking 100mL of $AgNO_3$ solution in a 500 mL beaker, to this 90ml of leaf extract and 100mL of $FeSO_4$ solution were added drop wise by simultaneous addition process. During addition process beaker was placed on a magnetic stirrer for continuous agitation. Now this mixture was stirred at 40$_o$C for 50 minutes at pH 7 on magnetic stirrer. These synthesized BMNPs were separated out by doing centrifugation at 5000 rpm for 40 minutes. The obtained BMNPs were washed with using deionized water for 2 times to remove unwanted constituents and dried in oven at 90$_0$C for two hours. The resultant BMNPs particles were collected (**figure: 1(d)**) and used for characterization.

Mechanism of green synthesis of Ag-Fe bimetallic nanoparticles :

Aerva Lanata leaf extract + Ag(I)-Fe(II) precursor salt solutions

↓ Reduction

Ag(0)-Fe(0) bimetallic nanoparticles

Growth and stabilization

Capped Ag(0)-Fe(0) bimetallic nanoparticles

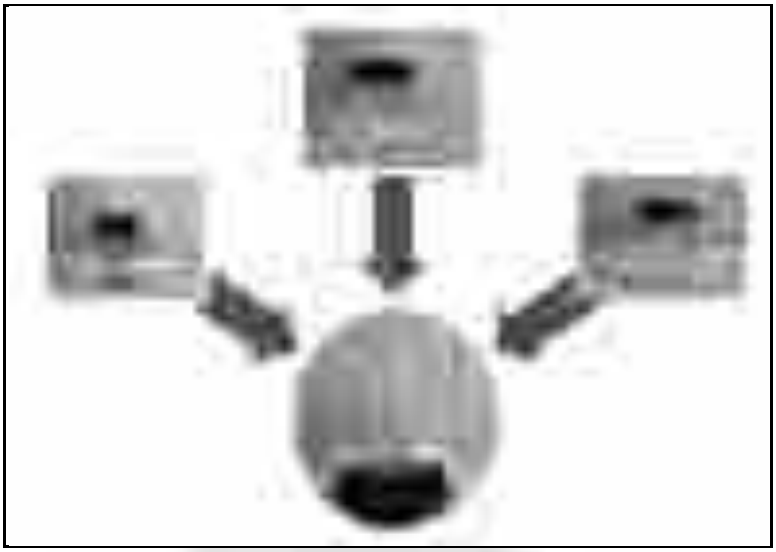


Figure : 1(d) . Synthesis of Ag-Fe BMNPs from precursor solutions

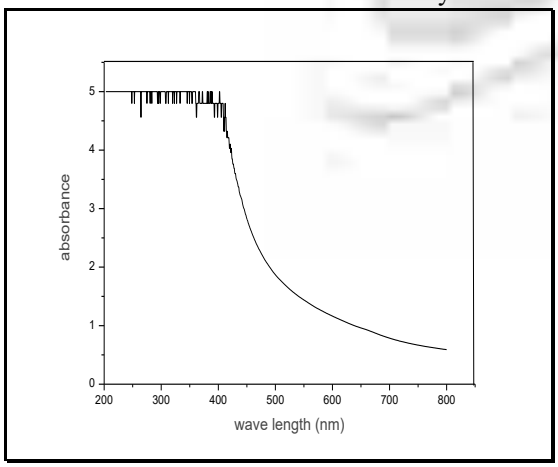
2.4. Characterization:

Formation of Ag-Fe BMNPs was confirmed by UV-Visible absorption spectra using UV-2450 SHIMADZU double beam spectrophotometer, FTIR using Bruker, SEM, EDX studies are done by Hitachi S-3700N machine and the morphology of BMNPs was elucidated by HRTEM analysis with FEI Technai machine.

3. RESULTS AND DISCUSSION

3.1.UV-Visible spectral analysis:

UV-Visible spectrum of *Aerva Lanata* leaf extract is given in **Figure2(a)**. UV-Visible absorption spectrum of Ag-Fe BMNPs is revealed in **Figure.2(b)**. The characteristic surface plasmon resonance (SPR) band at around 436 nm is observed in Ag-Fe BMNPs which confirms the nano size of the synthesized paricles [15] .



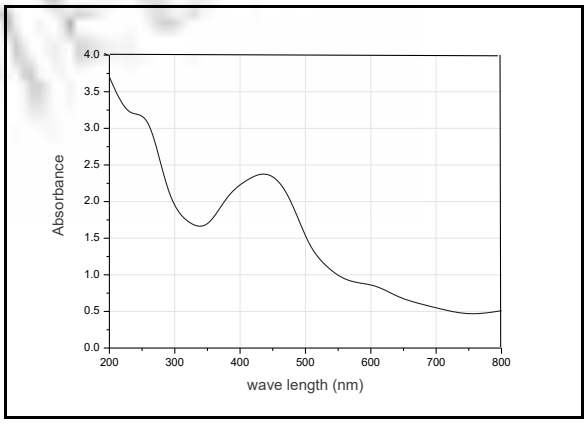


Figure 2 (a): UV-VIS Spectrum of *Aerva Lanata* leaf extract **Figure.2(b):** UV-Visible absorption spectrum of Ag-Fe BMNPs

3.2. FTIR spectral analysis:

FTIR spectral data is used to identify different functional groups present in biomolecules of leaf extract. These groups are responsible for the bioreduction of Ag_{+}, Fe_{+2} precursors and also for capping and stabilization of Ag-Fe BMNPs. The intense peaks were observed and compared with standard values to analyze the functional groups in *Aerva lanata* leaf extract and green synthesized Ag-Fe BMNPs. FTIR spectra of *Aerva lanata* leaf extract and synthesized Ag-Fe BMNPs by using *Aerva lanata* leaf extract were shown in **Figure.3 (a)** and **Figure.3 (b)** respectively**.** The comparison of the FTIR spectra of both Ag-Fe BMNPs and leaf extract of *Aerva lanata* clearly indicates the existence of the plant extract phytomolecules such as polyphenols ,terpenes, flavonoids, glycosides, tannins, sterols, amides, carbohydrates, amines the surface of the Ag-Fe BMNPs [15].

Figure.3 (a): FTIR spectrum of *Aerva lanata* leaf extract **Figure 3(b)**. FTIR spectrum of Ag-Fe BMNPs in leaf extract

The strong intense peaks between 3200 cm^{-1} to 3950^{-1}may be due to N-H ,O-H stretching of 1$_0$ amines and polyhydroxy groups present in the extract. The strong absorption at 1643 cm^{-1}indicates the presence of C=O group of amides. This result gives the evidence about the high protein content of the extract. The small peak at 2892 cm^{-1} is may be due to C-H symmetrical stretching of methylene groups. The peak position at 1519 cm^{-1} may be due to C=C stretch of aromatic ring. The peak at 598 cm^{-1} is denoting the presence of C-Cl group.
The FTIR spectrum of Ag-Fe BMNPs exhibits major peak positions at 3214 cm^{-1},3417 cm^{-1}, 3622 , 3905 cm^{-1} and 3935 cm^{-1} which indicate the N-H stretching vibrations of amines and O-H stretching of hydroxyl groups of alcohols and phenols. Intense peak at 1641 $cm^{-1}$is due to C=O stretching of amide group. Very small peak at 588 cm^{-1} connotes the presence of C-Cl group [16],[17].
FTIR analysis clearly confirms that all the aforementioned absorption peaks of the extract are barely shifted in the FTIR spectrum of Ag-Fe BMNPs as the phyto molecules of the extract act as bioreducing agents, capping and stabilizing agents for the synthesized nanoparticles. The existence of these IR bands also in the Ag-Fe BMNPs confirmed that the surface of the nanoparticles was covered by plant secondary metabolites such as carbohydrates, glycosides, Saponin, phytosterols, phenolic compounds, tannins, flavonoids, proteins, aminoacids, diterpenes with functional group phenols, carboxylic acids, amides, ketones, alkyl halides.[18]

3.3.**SEM and EDX analysis:**
From energy dispersive X-ray analysis we can analyze all the elements present in prepared BMNPs by *Aerva lanata* leaf extract. **Figure.4** and **Table.1** show EDX spectrum and elemental composition of Ag-Fe BMNPs respectively. EDX study which gives the quantitative data of silver and Iron elemental compositions in the synthesized BMNPs **.** Scanning electron microscopic (SEM) images of Ag-Fe BMNPs with various magnifications are given in **Figure 5**. From this it can be clearly noted that the prepared Ag-Fe bimetallic nanoparticles are within the size range of 50 to 100 nm in diameter.

Table: 1. Quantitative results of Ag-Fe BMNPs

Element	Weight %	Atomic %
Fe L	33.93	49.80
Ag L	66.07	50.20
Totals	100.0	100.0

Figure 4 : EDX Analysis of Ag-Fe BMNPs

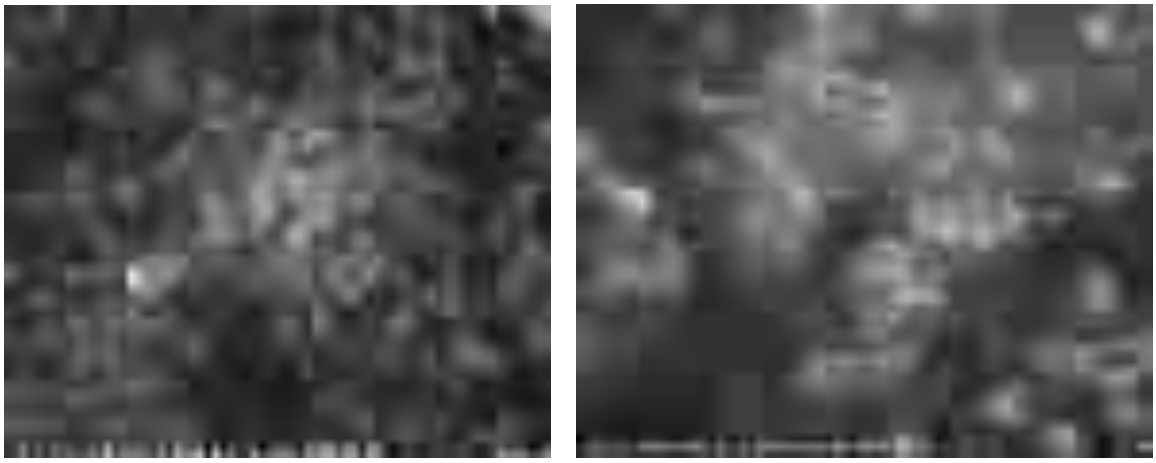


Figure.5 : SEM images of Ag-Fe BMNPs

3.4. **HRTEM analysis:** **Figure.6** shows the high resolution transmission electron microscopy (HRTEM) images for synthesized Ag-Fe BMNPs from *Areva lanata* leaf extract. From these images, it was observed that Ag-Fe BMNPs were formed with spherical morphology and crystalline structure below 100 nm in size. More specifically, the two metal nanospheres seem to be positioned side by side, providing an overall bilobal structure. It is also in strong accordance with the images from SEM analysis.

Figure .6 : HRTEM images of Ag-Fe BMNPs

3.5. XRD analysis :

The XRD spectrum of green synthesized Ag-Fe BMNPs from leaf extract is shown in figure: 7. The peaks appeared at 2θ values of 37.96$_0$, 44.22$_0$, 64.32$_0$, 77.26 $_0$ corresponding to the Bragg's reflections of Ag(111), Ag(200), Fe(110), Ag(220), Fe(220) , Ag (311) planes respectively of face centered cubic crystal structure [19] as shown in the **Figure:7**

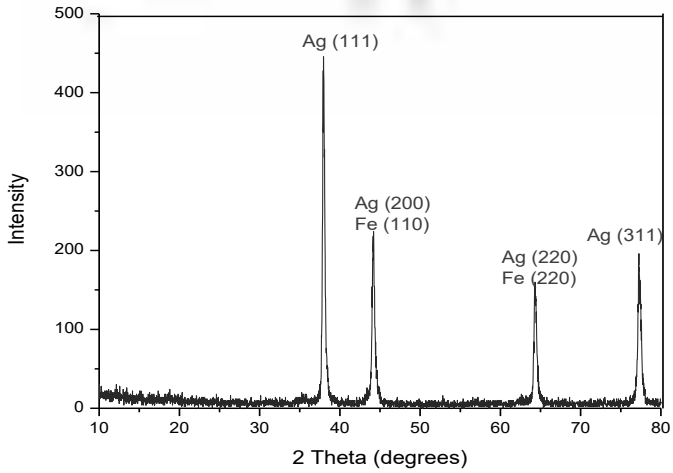


Figure:7 . XRD spectrum of Ag-Fe BMNPs

The average size D (in nm) of Ag-Ni bimetallic nanoparticles was calculated by using Debye-Scherrer equation (1).

$$D=\frac{K\lambda}{\beta\cos\theta}.....(1)$$

D = crystalline size of Ag-Fe BMNPs
λ = wavelength of x-ray source (0.15406 nm) used in XRD
β = full width at half maximum (FWHM) of the diffraction peak
K = Scherrer constant = 0.9
θ = Bragg's angle

S.no.	2θ (degrees)	θ (radians)	Cos θ	β (radians)	D (nm)
1	37.96_0	0.2876	0.9589	0.005501	26.2856
2	44.22_0	0.3859	0.9264	0.005864	25.5235
3	64.32_0	0.5613	0.8466	0.005658	28.9462
4	77.26_0	0.6742	0.7812	0.005642	31.4584

The numerically calculated value of the synthesized Ag-Ni BMNPs materials corresponds to an average particle size of **28.0534 nm**.

4. Photodegradation studies on Malachite Green dye using Ag-Fe BMNPs :

The photodegradation experiments are carried on Malachite Green dye using green synthesized Ag-Fe BMNPs acting as a photocatalyst. . Initially, 50 ppm of malachite green stock solution was prepared. Then reaction mixtures were prepared by adding certain amount of Ag-Fe BMNPs (10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg,70 mg, 80 mg) to 100 mL of malachite green for distinct concentrations(5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 35 ppm). The pH of the reaction mixtures was altered (for pH 3,4,5,6,7,8,9,10,11) by adding 0.1 N H_2SO_4 or 0.1 N NaOH solutions when required. Now this mixture was agitated for 20 minutes in dark condition to attain adsorption-desorption equilibrium between malachite green and Ag-Fe BMNPs. Sun light was used as irradiating source to reaction mixture for studying degradation during 11.00 am to 3.00 pm. At regular 30 minutes of time intervals , aliquot part of the reaction mixture is taken, centrifuged to remove the photocatalyst particles and optical absorption properties were analyzed by using UV-Visible Spectrophotometer . The absorbance was observed by varying parameters like changing the time of contact between the catalyst and the dye, pH of the reaction mixture, concentration of the dye solution, dosage of the catalyst. Malachite green shows the highest absorption at 617 nm. [19].To determine the percentage degradation of MG solution following equation (2) was used.

$$\%\ \text{degradation} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100 \quad \ldots \quad (2)$$

Where, A_0 is the initial absorbance of the MG solution at zero minutes and A_t is the absorbance of the degraded solution after time t minutes.

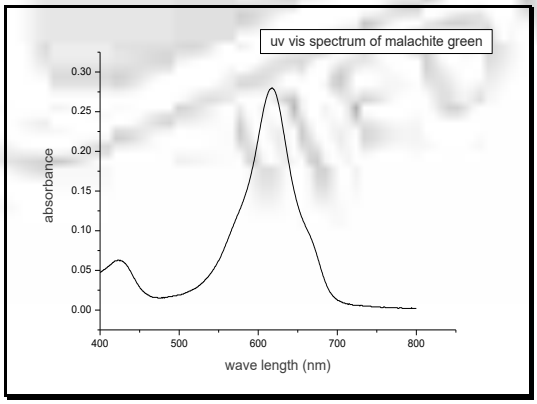


Figure 8(a): UV- VIS spectrum of Malachite Green

To study the photocatalytic activity of the Ag-Fe BMNPs on malachite green visible region of the light source was selected on UV-visible spectrophotometer was used. Absorption spectrum of 10 ppm malachite green solution was shown in **Figure 8(a).** Highest absorption peak at 617 nm was observed and this maximum absorption peak was considered to monitor the photodegradation reaction of MG dye for all further studies in this paper.

4 (a) . Probable Mechanism of Photocatalytic degradation of MG dye with Ag-Fe **BMNPs**

By absorbing suitable wavelength radiations MG dye goes to its first excited singlet state. Then by undergoing intersystem crossing (ISC), it enters into to the triplet state. In the intervening time Ag-Fe BMNPs also absorb the radiant energy to excite its electron from valence band to the conduction band. The hole abstracts an electron from H_2O to generate $^{\cdot}OH$ radical and H^{+}. $^{\cdot}OH$ radical oxidizes the MG dye to its leuco form degrades into colourless product. The electron will be abstracted by oxygen molecule generating superoxide anion radical ($^{\cdot}O_2^{-}$). The formed anion radical reduce the MG dye to its leuco form, which on degradation forms products.

4.1. Effect of time of contact :

Efficiency of photodegradation of the Ag-Fe BMNPs on malachite green dye was studied by batch mode experiments. The efficiency of BMNPs on degradation of MG is expected to be increased by increasing contact time. The effect of contact time was carried out by taking 10 ppm of 100 ml MG dye solution and 10 mg of BMNPs (at pH 7) as catalyst load (**Figure 8(b)** and **Figure 8 (c)**). Initially, degradation of dye by using BMNPs was found to be rapid and then it almost settles down as % degradation attains more or less constant value with this increase in contact time. This is due to strong adsorption forces predominate between the dye and the BMNPs and as the number of the reactive sites on the catalyst were vacant during initial periods of contact time. But after 120 minutes of contact time, percent degradation gradually approached a constant value to some degree when equilibrium was almost reached because the number of vacant sites available for further dye adsorption is inevitably diminishing[20].

(1) 0 mins (2) 30 mins (3) 60 mins (4) 90 mins (5) 120 mins (6) 150 mins (7) 180 mins

Figure 8(b): Colour change in MG dye after addition of Ag-Fe BMNPs at various time intervals

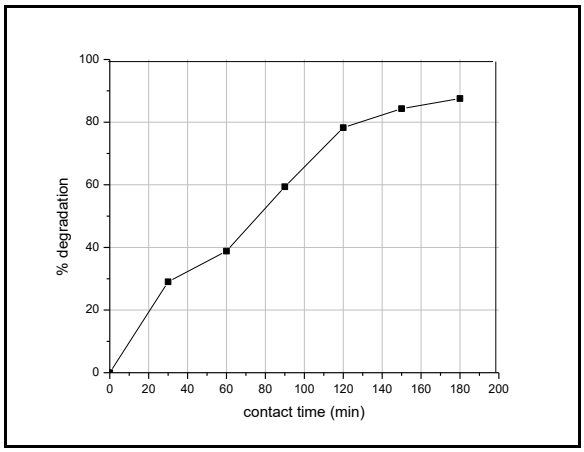


Figure 8(c): Effect of contact time on photo degradation

4.2. Effect of initial concentration of MG dye solution :

Initial concentration of MG dye solution is also expecting to effect the rate of photo degradation. To investigate this fact, dosage of BMNPs nano-catalyst was kept constant at 10 mg , keeping solution pH at 7 and the time of irradiation was maintained as 180 minutes. Whereas the initial concentrations of the MG dye solutions were varied at 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm , 30 ppm and 35 ppm. The rate of photodegradation can be represented graphically in **Figure 8(d)**. From the figure it can be observed that the highest degradation is located at 5 ppm, and the photodegradation decreases gradually with increase in the concentration of MG dye solution. This finding can be explained by the fact that as the concentration of the dye increases, the dye continues to act as a barrier for the incident light and does not allow the light intensity to touch the BMNPs surface and thus the %photodegradation decreases.[21]

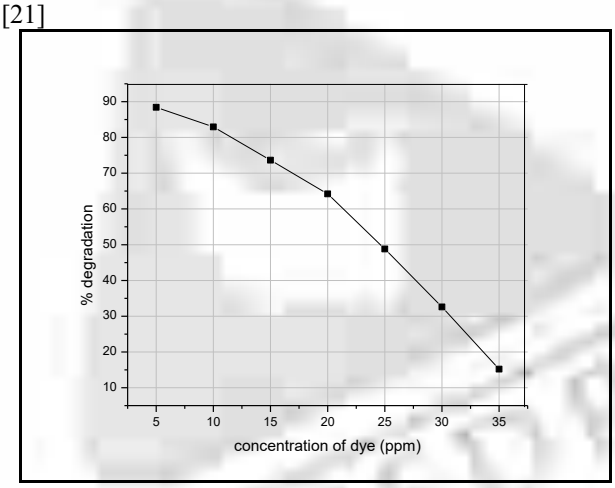


Figure.8(d): Effect of concentration of MG dye solution on photodegradation

4.3. Effect of pH:

pH of dye solution can influence the adsorption of dye on photocatalyst. The initial concentration of malachite green solution 10 ppm and the concentrations of the photocatalyst 10 mg were kept constant with time of irradiation 120 minutes. Different solutions of various pH values of 4 to 11 were prepared. Degradation efficiencies were compared which was shown in **Figure 8(e).**

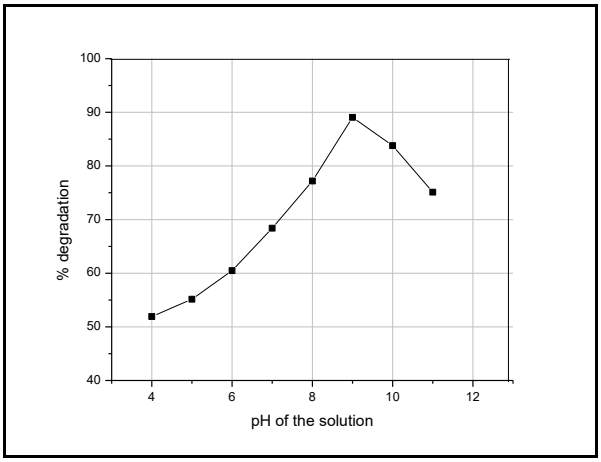


Figure.8(e): Effect of pH on photo degradation

It was observed that at initial pH values the degradation was minimum .This is due to the fact that at low solution pH Fe may undergo corrosion to form iron oxides (Fe_2O_3, Fe_3O_4) and hydroxides on the surface of Fe nanoparticles. So MG dye undergoes less degradation. Also in acidic condition the H^+ ions were made to compete with cationic MG dye and hence the adsorption of dye was decreased. In contrast at pH > 9 in the more alkaline medium, the surface of the catalyst contains a huge number of –OH ions that may provide negative charge to the adsorbent resulting to a high efficiency for the removal of MG dye.[22]

4.4. Effect of dosage of photo catalyst:

In photodegradation process, one of the important parameters of decolourizing of dye solution is photocatalyst dosage. To avoid unnecessary wastage of expensive catalyst and attain the maximum absorption of photons optimization of the catalyst dosage is essential. For this, dosage amount was varied from 10 mg to 70 mg taken in 100 ml of 10 ppm MG dye at pH 8 with contact time 120 minutes. The degradation of MG was shown in **figure 8(f).**

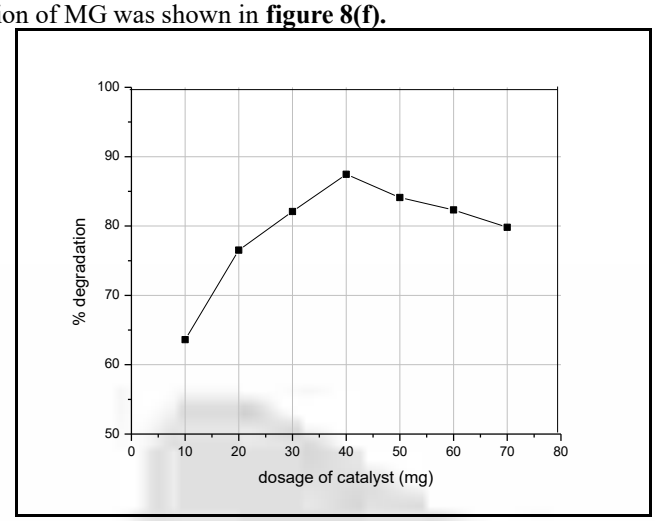


Figure 8(f): Effect of dosage of catalyst on photo degradation

It was concluded that, by increasing dosage of catalyst from 10 mg to 80 mg in 100 ml, the degradation of MG dye enhances because the increase in amount of catalyst upto 40 mg would increase the reactive sites that produce more reactive species. On further loading of catalyst ,% degradation of MG dye decreases. This can be rationalized by the fact that, the addition of excess catalyst results in more turbid suspensions and decelerates the light penetration into the solution . Due to particle-particle interactions (agglomeration) and sedimentation of the catalyst there will be significant decrease in the active surface area and hence the degradation falls down.[23]

CONCLUSION

An ecologically innocuous method is projected to synthesize Ag-Fe bimetallic nanoparticles from *Areva lanata* leaf extract. From UV-VIS spectal analysis it is confirmed that the particles are in nanoscale as per the positions of the Surface Plasmon Resonance (SPR) bands. FTIR data confirms the presence of secondary metabolites of phyto molecules as the bio reducing and capping agents of the formed nanoparticles. Results of XRD, SEM and TEM analyses evinced that Ag-Fe BMNPs are in spherical morphology and cubic crystalline structure with size between 20-100 nm. The photocatalytic activity of these nanoparticles is examined under sunlight for degradation of MG dye which is environmental pollutant. The % photodegradation of MG dye changes with parameters such as contact time, concentration of MG dye, pH, photocatalyst dosage. From this research study on bimetallic Ag-Fe BMNPs synthesized from *Areva lanata* leaf extract, the optimum conditions found in the degradation of MG dye is pH **8**, weight of catalyst **40 mg**, dye concentration of **10 ppm** and contact time of **120 minutes**. The maximum degradation is obtained as **88.75%** under these optimum conditions.

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Isolation and Identification of Mercury Tolerant Bacteria from Mercury Contaminated Areas

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Abstract: *Bacteria posses distinctive characteristics, hence they were exploited by different scientists for various applications. In this piece of work, bacteria were isolated from mercury contaminated soils, and were further screened for superior mercury resistant bacteria for using them in bioremediation. Initially, mercury tolerant bacterial strains were isolated from mercury contaminated soils, and labelled them as HG 1, HG 2, HG 3 and HG 4. In order to derive maximum mercury tolerance, all the four isolates were inoculated on nutrient broth, supplemented with a vast range of concentrations of mercuric chloride (100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm and 600 ppm). All the isolates were found adaptable to the concentration of mercury up to 300 ppm. Among them, only one isolate (HG 2), due to its potential mercury resistance and high Minimum Inhibitory Concentration (MIC) values, was found to be the relatively most tolerant and was selected for molecular characterization. Further phylogenetic analysis using the MEGA 6.0 software revealed that the selected isolate HG 2 belongs to the genus Brevundimonas. These mercury tolerant bacterial strains would be of immense use in the bioremediation of metal contaminated soils, in future research and biotechnological development.*

Key Words: *Bioremediation, mercury, phylogenetic analysis, contaminated soils.*

1. INTRODUCTION:

As it is a known fact that, industrial growth is directly proportional to the rise in heavy metal contamination in agricultural soils. Among all the heavy metals, mercury is identified as the most toxic metal. It is ubiquitous and can be transported through the atmosphere as it is very volatile in nature (1). Mercury can easily enter the water bodies through the process of precipitation. Subsequently it enters the food chain of aquatic animals, and ultimately reaches the human beings, which leads to bioaccumulation. Biomagnification is evident from the fact that the high concentration of mercury is found in carnivorous fish (2, 3 & 4). Mercury is the most toxic to all organisms including plants and animals due to the fact that they exhibit a strong affinity for thiol groups of proteins which are present in organisms (5 & 6). Mercury contamination adversely effects the growth and development of plants. It causes inhibition in germination (7), results in decrease of biomass production (8), and hinders protein function (9 & 10*)* shows negative impact on DNA (11) and inhibits photosynthesis in many plants (12). Considering the above ill effects caused by the mercury, we should prioritize the need of the sustainable techniques to mitigate its hazardous effects. Many methods are being used but the bioremediation is quite efficient, environment friendly and cost effective in decontaminating the environment (13, 14 &15). Several studies have shown that extensive variability of micro organisms (bacteria, fungi, yeast and algae) has distinct and natural capacities to absorb toxic heavy metals (16, 17 & 18). Bacterial bioremediation provides us certain advantages such as it is easy to culture; they multiply faster and can accumulate metals efficiently in various conditions (19 & 20).

Bacteria evolved to exist in metal contaminated areas (21). Various studies affirm that bacteria acquire resistance towards heavy metals (22 & 23), to survive in extreme environmental stress condition (24). In this connection, bacterial application in bioremediation of heavy metals needs to be studied and high mercury resistant bacteria needs to be isolated.

The aim of this study is to identify the efficient mercury resistant bacteria, isolated from high mercury contaminated soils by 16S rRNA gene sequencing analysis. Construction of phylogenetic tree for taxonomic classification of bacteria using 16S rRNA gene was initially performed by various researches (25).

2. Methods and materials:

Sample Collection: Mercury resistant bacteria isolated from soil collected near pharmaceutical industries in Visakhapatnam.

Bacterial isolation: Mineral salts medium (MSM) supplemented with 100 ppm mercuric chloride, was used for isolation and characterization of mercury tolerant bacteria. The MSM has the following composition in (g/L): KH_2PO_4, 4.8; K_2HPO_4, 1.2; NH_4NO_3, 1.0; $MgSO_4.7H_2O$, 0.2; Ca $(NO_3)_2.4H_2O$, 0.04; and Fe $(SO_4)_3$, 0.001 with pH 7.0. Nutrient Agar medium was used for pure culture maintenance. The composition of the media are as follows (g/L); sodium chloride 5.0; peptone 1.5; yeast extract 1.5; agar 15.0; (pH-7.4).

Culture enrichment and isolation of mercury tolerant bacteria from soil samples:

Five samples were serially diluted and third dilution was inoculated into MS media which contains 100 ppm of $HgCl_2$ and 0.25% of glucose as carbon source and incubated in orbital shaker for three days at 37^0C and 240 rpm. Then 100 µl of samples from the above treatments were transferred into fresh tube containing MS media with 250 ppm $HgCl_2$ and 0.5% of glucose and incubated in orbital shaker for three days at 37^0C and 240 rpm. Then from the above treatments 100 µl of sample was transferred into test tube containing MS media with 500ppm of $HgCl_2$ and 0.75% of glucose and incubated in orbital shaker for three days at 37^0C and 240 rpm. Finally 100 µl of sample was inoculated into test tube containing MS media with 1000 ppm of $HgCl_2$ and incubated in orbital shaker for three days at 37^0C and 240 rpm. After incubation, 100 µl from each treatment were spread separately onto mineral agar plates supplemented with $HgCl_2$ were incubated at 37^0C for 24 h.

Identification of the colonies: Based on variation in physical appearance and morphological characters, few of the bacterial colonies were selected and were inoculated onto nutrient agar plates. The bacteria were allowed for biochemical and molecular characterization

Biochemical characterization of isolated bacteria: The individual bacterial colonies that grew on the medium were sub cultured onto mineral agar containing chlorpyrifos of the same concentration until pure cultures were obtained. Bacterial isolates were subjected to morphological and biochemical studies.

Selection of metal tolerant bacteria: In order to isolate potential mercury tolerant bacteria, the selected bacterial isolates (HG 1, HG 2, HG 3 and HG 4) were grown in nutrient broth supplemented with different concentrations of Hg such as 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm and 600 ppm. Control bacterial cultures maintained without metal ion. 5% inoculums were added in every tube. The inoculated tubes were incubated at 37^0C for 48 hours in orbital shaker incubator at 180 rpm. Culture medium was taken as a blank for every reading. The results were presented in Figure 3. Growth kinetics was observed after 48 hours of incubation using a spectrophotometer at 600 nm OD for the conformation of the bacterial density.

Molecular characterization of specific isolate: The strains used in this study were grown in shaking flasks containing Nutrient broth at 37°C.

Isolation of chromosomal DNA: Chromosomal DNAs were isolated by a versatile quick-prep method for genomic DNA of bacteria (26) with some modifications. Mycelia (1–2 ml) grown in a GYM broth cultures were centrifuged, rinsed with TE and resuspended in 0.4 ml SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris, pH 7.5). Lysozyme was added to a concentration of 1 mg/ml and incubated at 37°C for 30 min–1 h. Then 0.1 vols of 10% SDS and 0.5 mg Proteinase K ml^{-1} were added and incubated at 55°C with occasional inversion for 2 h. One-third volume 5 M NaCl and 1 vol. chloroform were added and incubated at room temperature for 30 min with frequent inversion. The mixture was centrifuged at 4500 rpm for 15 min and the aqueous phase was transferred to a new tube using a blunt-ended pipette tip. Chromosomal DNA was precipitated by the addition of 1 vol. 2-propanol with gentle inversion. The DNA was transferred to a new tube, rinsed with 70% ethanol, dried under vacuum and dissolved in a suitable volume (about 100 ml) of distilled water. The dissolved DNA was treated with 20 mg RNase-A ml^{-1} at 37°C for 1 h. Samples were extracted in the same volume of phenol/chloroform/isoamyl alcohol (25: 24 : 1) and precipitated with 2.5 vols cold ethanol and 0.1 vols 3 M sodium acetate. The pellets were washed with 70% ethanol, dried and dissolved in TE or distilled water.

PCR Amplification and sequence analysis of 16S rRNA gene: Potential mercury tolerant bacterial isolate (HG 2) selected for molecular study, to identify the species. Bacterial genomic DNA was extracted from the selected isolate (HG 2). With the above isolated genomic DNA, the PCR amplification of the 16S rRNA gene was performed using 16S rRNA specific two universal primers, 27F (5'AGAGTTTGATCMTGGCTCAG3') and 492R (5'TACGGYTACCTTGTTACGACTT3'). PCR reaction mixture of 25 µl total volume, containing 1/10 volume 10× *Taq* buffer, 2 mm $MgCl_2$, 1 unit *Taq* DNA polymerase, 0.2 mM dNTP, 20 pmol forward primer, 20 pmol reverse primer and 100 ng DNA. DNA amplification was carried out in a Biorad Mini thermocycler with the following PCR conditions: an initial denaturing step at 94°C for 5 min; 40 cycles for 1 min at 94°C (denature), 1 min at 48°C (annealing), 2 min at 72°C (extension) and a final elongation step at 72°C for 5 min. PCR products were separated by electrophoresis on 1.5% agarose gel containing 0.5 µg/ml ethidium bromide, and photographed. The standard DNA samples (100 bp DNA ladder marker) were used as molecular size marker. The purified PCR products were subjected to Sanger's dideoxy sequencing, in both forward and reverse directions, using Big Dye terminator v 3.1 cycle sequencing kit on ABI Prism 3700 DNA Analyzer (Applied Biosystems Inc., USA) as per manufacturer's instructions. Sequencing of the cloned 16S r RNA gene of strain HG 2 resulted in a virtually complete 1417 bp long sequence. The

resulting 16S r DNA gene sequence (1417 bp) was used to search in the Gen Bank/ EMBL/DDBJ database with the BLAST program to determine the relative phylogenetic positions. Phylogenetic analysis was conducted using MEGA 6.0 (27) by first generating a complete alignment of 16S r DNA gene sequences of the isolates and type strains of all valid species. A phylogenetic tree was inferred using neighbour-joining tree algorithms (28). 16S r DNA datasets were cooperatively analyzed using MEGA 6.0 (27) which was used to calculate evolutionary distances and similarity values. Topography of the constructed tree was evaluated by bootstrap analysis with 1000 replicates (29).Only nodes with bootstrap values over 50% were considered to be significant.

Phylogenetic analysis: Multiple sequences were aligned using CLUSTAL W version 1.8 software packages (30). The resultant sequence alignment can then be used for the neighbour-joining (29), maximum-parsimony (31) and maximum-likelihood (32) methods using the MEGA 6.0 (27) package; all were implemented within the server. The alignment can be exported for use by external programs including MEGA 6.0 (27) and BioEdit (version 7.2.0) (33). An evolutionary distance matrix was generated for the neighbour-joining as described by (28).The resultant tree topology was evaluated by a bootstrap analysis (34), with 1000 re samplings from the neighbour-joining dataset using Seqboot and Consense from the Phylip package (35)

3. RESULT DISCUSSION:

Isolation and identification of mercury tolerant bacteria:

The bacterial isolates were characterized on the basis of morphological and biochemical assays.

Morphological characterization of the bacterial isolates: Colony morphology of all the isolates was examined in this study. Characteristics including colour, shape, elevation and colony surface were studied (Table 1). The colonies (HG 1 and HG 2), which were selected visually based on differences with naked eye, were identified as white in colour, while the remaining two were observed to be yellow coloured. It was observed that the shapes of the three colonies of bacterial isolates (HG 1, HG 2 and HG 3) were mostly circular, whereas HG 4 is in irregular form. The surface characteristics of bacterial isolates HG 1 and HG 2 were found to be smooth, while surface was found to be rough in the case of HG 3 and HG 4. In the case of the HG 3 and HG 4 flat margins were observed, however in HG 1 and HG 2, there was a visible elevated growth of the colony.

Biochemical characterization of bacterial isolates:

Biochemical characteristics of the bacteria isolated from the soil samples were shown in Table 2.

Grams staining: Following the protocols of standard Gram staining, it was found that, all the four selected isolates belong to Gram negative bacteria. Based on the previous reports it was known that Gram negative bacteria are more resistant to heavy meals than Gram positive bacteria (36).

Starch hydrolysis: Among the four isolates, HG 3 showed positive result for starch hydrolysis (capacity to utilize starch) and remaining (HG 1, HG 2 and HG 4) showed negative test results.

Citrate test: HG 1 and HG 3 exhibited positive test results for citrate utilization, where as other isolates HG 2 and HG 4 found to be negative.

Nitrate test: All the three bacterial isolates (HG 1, HG 2 and HG 4) except HG 3 have the ability to reduce nitrate to nitrite, and provided positive results for nitrate reductase test but HG 3 has no ability to reduce nitrate and hence it was negative.

Methyl red test: No isolate showed positive to methyl red test.

Voges proskauer test: Volkes Prosker test is positive for bacteria which form acetoin. This
Test was positive for HG 1 and HG 4. The other HG 2 and HG 3 are not able to synthesize acetoin hence they are negative for the test.

Catalase test: Isolates HG 1 and HG 2 were found to be catalase positive as they showed bubble formation on addition of H_2O_2. The bacterial isolates HG 3 and HG 4 were found to be catalase negative as no bubbles were formed.

Salt tolerance: Bacterial isolates HG 1, HG 3 and HG 4 were identified as salt sensitive isolates, where as HG 2 noticed as salt tolerant strain.

Gelatin hydrolysis: Only HG 2 was observed as negative to gelatin hydrolysis test, where as remaining three isolates (HG 1, HG 3 and HG 4) were positive for the test.

Growth kinetics of bacterial isolates:

The present study was designed to evaluate the adaptation capabilities of soil bacteria under high mercury concentrations. HG 1, HG 2, HG 3, and HG 4, were the bacterial isolates used for the experiment. The growth of these bacterial isolates, were screened on nutrient broth supplemented with various concentrations ranging from 100 ppm to 600 ppm of $HgCl_2$. All the bacterial strains were capable of withstanding up to 300 ppm of $HgCl_2$ concentration. Mercury has no significant effect on growth of bacteria at low concentrations, but higher concentrations of mercury negatively influence the growth of bacterial colony (37). The growth of bacterial strains steadily decreased with increase

in mercury concentration. The Minimum Inhibitory Concentration all the isolates in nutrient broth was 600 pp of $HgCl_2$. From the figure 1, it was inferred that bacterial growth was inversely proportional to concentration of mercury. The first notable difference observed at the lowest concentration of mercury (100 ppm), at which HG 4 and HG 2 exhibited the highest growth, but at the increasing concentration (400 ppm), growth of HG 4 decelerated and HG 2 showed the highest tolerance even at 600 ppm of $HgCl_2$. When HG 1 was moderately tolerant, HG 3 was more susceptible and ceased to grow at 400 ppm of mercury chloride. The analysis of growth kinetics of selected bacterial isolates cultured in different mercury concentrations were presented in figure 1, demonstrate that one bacterial isolate (HG 2) possess more efficient mechanisms of resistance in comparison to the other isolates. It showed growth up to 600 ppm mercury chloride concentration in growth medium. From these results we can deduce that isolate HG 2 was the most tolerant bacterial isolate. It was observed that strain HG 2 could resist up to 600 ppm of $HgCl_2$ concentration, which revealed the strong mercury resistance ability. Therefore molecular study was performed on this strain.

Identification of Mercury tolerant bacterial strain by 16S rRNA analysis:

The strain HG 2 was selected for further phylogenetic analysis of 16 S r RNA gene sequence. The DNA of mercury tolerant bacterial strain HG 2 was amplified with primers 1492R and 27F. The 16S rDNA gene sequence of bacterial isolate HG 2 was PCR amplified and products were detected by 0.8% agarose gel electrophoresis with ultraviolet (UV) light. The length of object fragment is about ~1417 bp (Figure 2). Sequence analysis of the 16S r RNA gene has been considered a fast and accurate method to identify the phylogenic position of bacteria. The 16S r RNA gene sequence (Figure 3) was deposited in NCBI Genbank with an accession number MT072131.Comparative analysis of the sequences with already available database showed that the strains were closed to the members of genus *Brevundimonas* and it was classified in the branch of *Brevundimonas sp*. A phylogenetic tree was constructed using MEGA 6.0 software which showed 97.47% homology with the genus *Brevundimonas.* Hence the bacterium which shows highest tolerance to mercury was identified as *Brevundimonas sp.* The occurrence of *Brevundimonas sp* in mercury contaminated areas was confirmed by the earlier reports (38) and reports of several authors (39 & 40) suggested that Brevundimonas accumulates high amounts of mercury by removing it from the surrounding medium. The mercury resistant bacteria have various remediation mechanisms of heavy metals which help to convert them to less toxic forms may provide the way to check the entry into the food chain. In this way it might lessen the hazardous metals to affect the health of human beings and other organisms. It is obvious from the earlier reports that bacteria that tolerate high concentrations of mercury could serve to effectively remediate mercury pollution (41 & 42).

4. RESULTS:

Table 1: Morphological characters of the HgCl2 tolerant bacterial isolates.

Morphological characters	Bacterial isolates			
	Hg1	Hg2	Hg3	Hg4
Colony Colour	White	White	Yellow	Yellow
Colony shape	Circular	Circular	Circular	Irregular
Elevation	Raised	Raised	Flat	Flat
Colony Surface	Smooth	Smooth	Rough	Rough

Total 4 bacteria were isolated from the above samples studied. The biochemical characters were mentioned below

Isolate	Hg 1	Hg 2	Hg 3	Hg 4
Grams staining	Negative rods	Negative rods	Negative cocci	Negative rods
Starch hydrolysis	-	-	+	-
Citrate test	+	-	+	-
Nitrate test	+	+	-	+
Methyl red test	-	-	-	-
Voges proskauer test	+	-	-	+
Catalase test	+	+	-	-
Salt tolerance	-	+	-	-
Gelatin	+	-	+	+

Table 2: Biochemical characters of the HgCl2 tolerant bacterial isolates.

+: positive test; - : Negative test

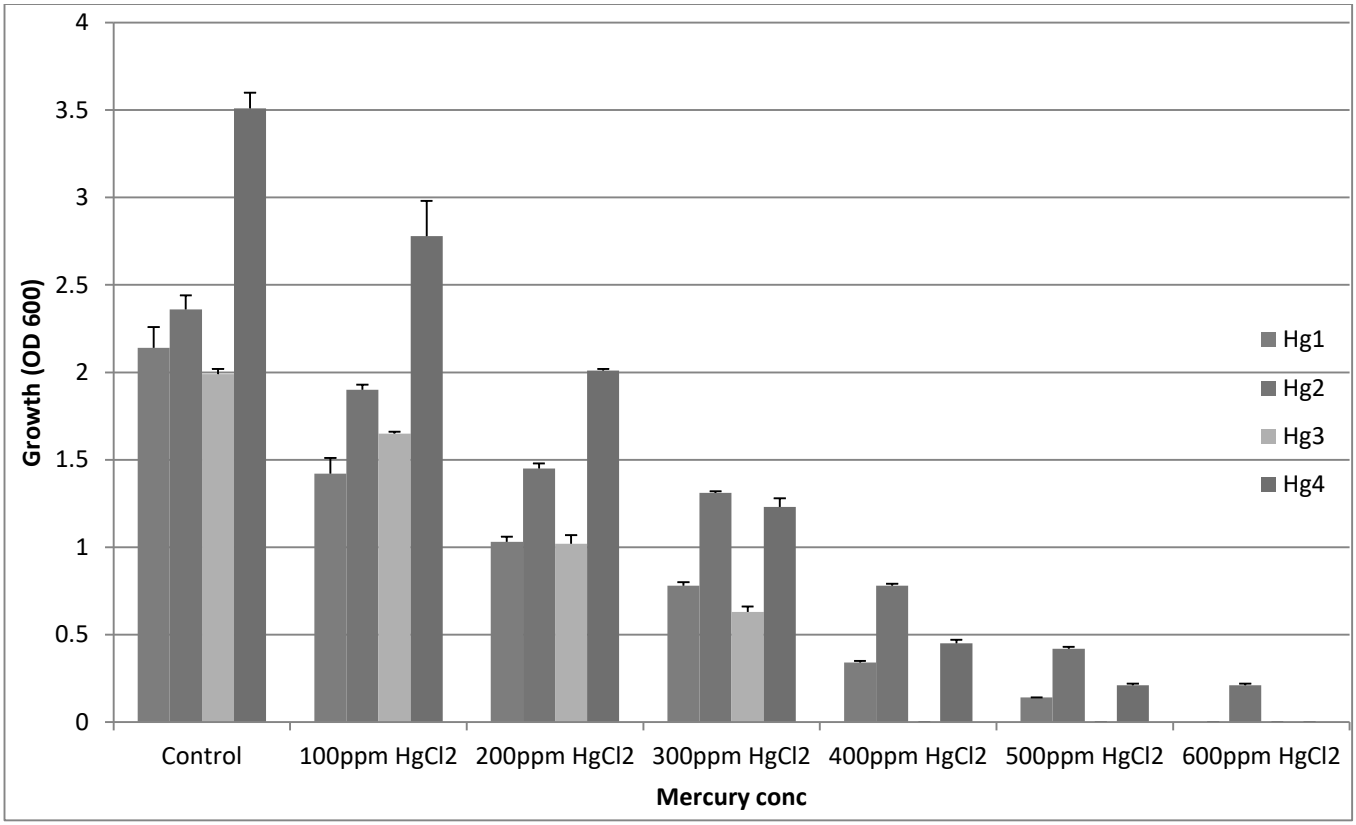
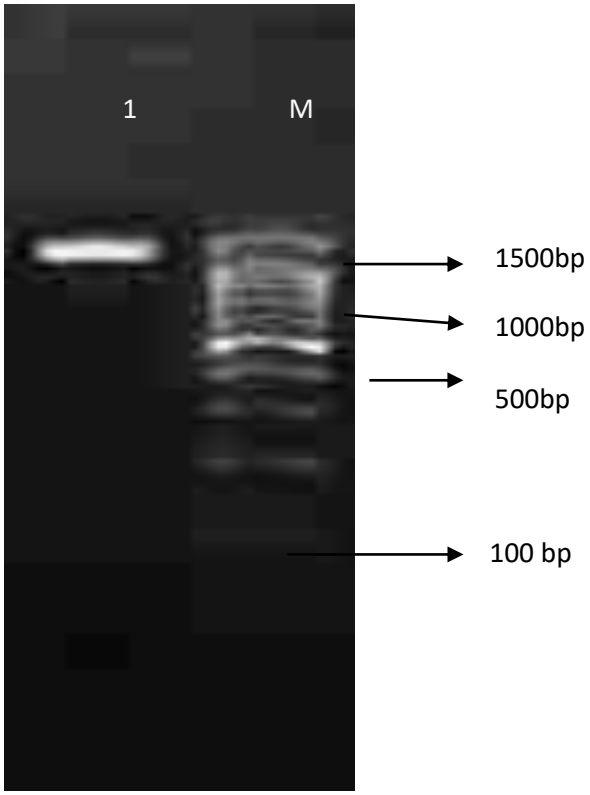


Figure 1: Growth characterization of four bacterial isolates at different mercury concentrations.

Molecular Characterization:



1: lane 1 M: Lane M

Figure 2: Agarose gel electrophoresis for 16s rRNA gene amplified product.
Lane M: 100 bp Ladder marker
Lane 1: DNA of bacterial isolate HG 2

Based on the molecular characterization the highest $HgCl_2$ tolerant isolate (HG 2) belongs to *Brevundimonas spp.*
16 S ribosomal RNA sequence of Hg 2 isolate:

AGCGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGACCCTTCGGGGTTAGTGGCGGACGGG
TGAGTAACACGTGGGAACGTGCCCTTTGGTTCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGAATGT
GCCCTTCGGGGGAAAGATTTATCGCCATTGGAGCGGCCCGCGTCTGATTAGCTAGTTGGTAGTGTAACG
GACTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGC
CCAAACTCCTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATGGGCGAAAGCCTGACGCAGCCATGCC
GCGTGAATGATGAAGGTCTTAGGATTGTAAAATTCTTTCACCGGGGACGATAATGACGGTACCCGGAGA
AGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAATTA
CTGGGCGTAAAGGGAGCGTAGGCGGACATTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCTCGGAAT
TGCCTTTGATACTGGGTGTCTTGAGTATGAGAGAGGTATGTGGAACTCCGAGTGTAGAGGTGAAATTCG
TAGATATTCGGAAGAACACCAGTGGCGAAGGCGACATACTGGCTCATTACTGACGCTGAGGCTCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTTGGG
AAGTTTACTTCTCGGTGACGCAGCTAACGCATTAAGCAATCCGCCTGGGGAGTACGGTCGCAAGATTAA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCA
GAACCTTACCACCTTTTGACATGCCCGGACCGCCACAGAGATGTGGCTTTCCCTTCGGGGACTGGGACA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTCGCCATTAGTTGCCATCATTTAGTTGGGAACTCTAATGGGACTGCCGGTGCTAAGCCGGAGGAAGGT
GGGGATGACGTCAAGTCCTCATGGCCCTTACAGGGTGGGCTACACACGTGCTACAATGGCGACTACAG
AGGGTTAATCCTTAAAAGTCGTCTCAGTTCGGATTGTCCTCTGCAACTCGAGGGCATGAAGTTGGAATC
GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CATGGGAGTTGGTTCTACCCGAAGGCGGTGCGCTAACCAGCAATGGAGGCAGCCGACCACGGTAGGGT
CAGCGACTGGGGTGAAGTCGTAACAAGGTGCCGTAGGGGAACCTGC

Figure 3 Complete sequence of HG 2 isolate

Phylogenetic tree:

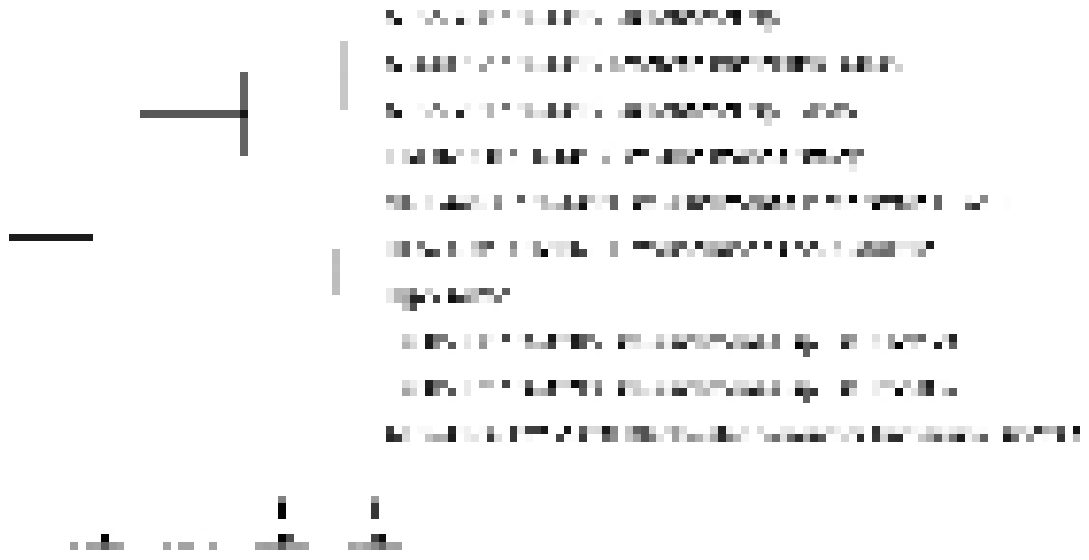


Figure 4: Phylogenetic tree based on 16S r DNA gene sequence showing the relationship with the most closely related bacterial strains.

5. CONCLUSION:

Despite the alarming present scenario of degrading ecological systems, there is a ray of hope from microbes as an emerging efficient technique in remediation. The selected bacterial strain (*Brevundimonas*) was capable of withstanding high concentration of mercury. Hence it is necessary to study its possible *in situ* application in reclaiming environments contaminated with heavy metals. It is crucial to study the mechanisms and the operating pathways

enabling bacteria to survive in metal rich areas and genes that are controlling them for better utilization in cleaning the hazardous metals present in the environments.

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INHIBITION OF PROLIFERATION OF K-562 HUMAN BLOOD CANCER CELL DUE TO *OPUNTIA ELATIOR* FRUIT EXTRACT

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Abstract : -- The anticancer potential of the ethanolic extract of Opuntia elatior was investigated in vitro against the human blood cancer cell line K-562. With the use of the MTT test and flow cytometric analysis, the cytotoxicity of the ethanolic extract was determined. Using the MTT assay, it was discovered that the Opunria elatior curde extract had an IC50 value of 74.32 l/ml, indicating that it was cytotoxic to a human blood cancer cell line when tested. The flow cytometry analysis reveals that the cells have been halted in the G2/M and S stages. The percentage of cells arrested as a result of Opuntia elatior extract is greater than that of the positive control Cisplatin. As a result, Opuntia elatior fruit extract has the potential to be exploited as an effective source of anticancer compounds in the treatment of human blood cancer.

Key word: Opuntia elatior, MTT, Flow cytometry, LC-MS/MS.

Introduction

Cancer is beginning to rank among the major causes of death in the globe, and only little progress has been made in terms of lowering the morbidity and mortality associated with this illness (Aziz et al., 2016). As reported by the American Cancer Society, 7.6 million people died as a result of cancer-related causes worldwide in 2007. In 2050, it is estimated that there will be an extra 27 million cancer diagnoses and 17.5 million cancer deaths throughout the world (Thun et al., 2009). Considering that plants have been used in cancer therapy for a very long time, it's worth noting that more than 60% of the anti-cancer medications now available are derived from natural sources.

In the genus Opuntia, succulent shrubs are found in abundance. They are native to the New World, but they are also widely cultivated in arid climates around the world for their distinctive appearance and attractive flowers. Opuntia is a broad genus of succulent shrubs that are native to the New World. According to El-Mostafa et al. (2014), Opuntia extracts have been used as food and medicine for thousands of years, and their therapeutic efficacy has been shown in scientific investigations conducted in vitro and in vivo throughout the years (Hail, 2005). Other than for food, Opuntia ficus-indica is used to cure diabetes, whooping cough, prostate issues, dentistry, rheumatism, nosebleed, and other conditions across Central and South America (El-Mostafa et al., 2014). As a result, it was determined that it would be desirable to investigate the anticancer potential of O. elatior fruits in the current research.

Material and Method

For MTT assay - K562 (Leukemia) cell line, RPMI Medium 1640 (Cat No-11875-093), Antibiotic – Antimycotic 100X solution (Thermo Fisher Scientific) - Cat No-15240062, K562 (Leukemia) cell line, RPMI Medium 1640 (Cat No-11875-093), K562 (Leukemia) cell line, RPMI Medium 1640 (Cat No-11875-093, K562 (Leukemia) The IC 50 of substances was estimated with the help of graph Pad Prism Version 5.1.

For Flow cytometry analysis - Cell line K562, 70% ethanol (in DI water), phosphate-buffered saline (PBS), PI/RNase staining solution, BD Biosciences (Catalog no. 550825), Cytomics FC500 Flow cytometer, Beckman Coulter, USA, and PI/RNase staining solution, BD Biosciences (Catalog no. 550825). FlowJo X 10.0.7 is the analysis programme used.

For LC-MS/MS – QTOF/6500 series Q-TOF B.05.01 (B5125.3) LC-MS apparatus from the QTOF/6500 series. A total of five precursor ions were chosen in Q1 with an isolation width of two dimensions and fragmented in the collision cell using a slope of collision energies ranging from five to forty-five electron volts. With a collision RF of 150/400 Vpp, a transfer time of 70 ms, pre-pulse storage of 5 ms, a pulse frequency of 10 kHz, and a spectral rate of 1.5 Hz for collision-induced dissociation (CID) of in-source fragment ions, the energy of the in-source CID was increased from 0 to 100 V. The product ions were identified with a collision RF of 150/400 Vpp, Accurate mass spectra were obtained in the m/z range of 50–1000 at a rate

of 2 spectra per second, resulting in a m/z range of 50–1000.

Extraction of plant material

The fruits of Opuntia elatior utilised in this research were obtained from the drier portion of the Solapur district and were employed in the experiment. It was decided to extract the fruits of Opuntia elatior by continuous extraction in Soxhlet apparatus for 12 hours, with ethanol (40 - 45 oC boiling range) serving as the solvent (Figure 1). (Horwitz, 1980). A rotary evaporator was used to evaporate the solvent used in the extraction. After drying over anhydrous sodium sulphate, the powder was kept at -4oC for further analysis. The varied concentrations of ethanolic extracts were generated in preparation for the subsequent investigation.

MTT assay-

A density of roughly 5103 cells/well was seeded in a 96-well flat-bottom microplate and the cells were kept at 370C with 95 percent humidity and 5 percent CO2 for an overnight period. The samples were treated at various concentrations (400, 200, 100, 50, 25, 12.5 l/mL) and times. It took another 48 hours before the cells were ready to be used. A total of 20 mL of the MTT staining solution (5 mg/ml in phosphate buffer solution) was applied to each well of the plate, and the plate was incubated at 370C for 30 minutes after each wash with phosphate buffer solution was completed. To dissolve the formazan crystals, 100 L of dimethyl sulfoxide (DMSO) was put into each well and the absorbance was measured at 570 nm using an absorbance metre and microplate reader after 4 hours (Ghagane et al., 2017 and Bhat et al., 2018).

Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100.

For Flow cytometry analysis –

Cells were seeded onto 6-well plates and cultured at 37°C for 24 hours before being removed from the plate. Cells were treated for 16 hours with the IC 50 concentration of the 64 percent alcoholic Opuntia elatior fruit extract at room temperature before being trypsinized and transferred to 15ml tubes. To prepare the cells, they were washed with 1X DPBS twice, fixed in cold 70% ethanol (-20oC), rinsed with 1X DPBS twice more, resuspended in 400 microliters of PI-RNase solution per million cells, and transferred to 1.5 microliter tubes. The samples were well mixed and examined using a Cytomics FC500 Flow cytometer from Beckman Coulter in the United States (Pozarowski and Darzynkiewicz, 2004).

LC-MS/MS Analysis

The alcoholic extract of the fruit Opuntia elatior was investigated using a TOF/6500 series Q-TOF B.05.01 (B5125.3) LC-MS equipment from the 6200 series TOF/6500 series Q-TOF series. A total of five precursor ions were chosen in Q1 with an isolation width of two dimensions and fragmented in the collision cell using a slope of collision energies ranging from five to forty-five electron volts. Nitrogen is used as a When the collision RF was 150/400 Vpp, the transfer time was 70 ms, the pre-pulse storage time was 5 ms, the pulse frequency was 10 kHz, and the spectra rate was 1.5 Hz for collision-induced dissociation (CID) of in-source fragment ions, the collision gas and product ions were identified. The in-source CID energy was increased from 0 to 100 V. Accurate mass spectra were obtained in the m/z range of 50–1000 at a rate of 2 spectra per second, resulting in a m/z range of 50–1000. Internal calibration was performed in positive mode with signals at m/z 121.0509 (protonated purine) and 922.0098 (protonated hexakis (1H,1H,3H-tetrauoropropoxy) phosphazine) in the presence of protons. Mass Hunter Workstation software was used to process both the raw HPLC–QTOFMS (Agilent 6540 UHD QTOF LC-MS) complete single MS and MS/MS data, as well as the data mining based on molecular formulas estimates and fragment patterns for the data mining (Qualitative Analysis). With the molecular features extraction approach, ions with similar elution patterns and corresponding m/z values (indicating various isotopes of the same drug) were recovered from complete single MS data using the same algorithm used for full single MS data (MFEs).

Results

Cell Viability

An MTT test was used to determine the cytotoxicity of an alcoholic fruit extract of O. elatior (DP) in vitro in this study. Cell viability was determined to be 95.381, 72.690, 59.400, 45.786, 20.259, and 15.073 at 12.50, 25.00, 50.00, 100.00, 200.00, and 400.00 l/ml for 48 hours at the concentrations of 12.5 to 400 l/ml for 48 hours, and the concentrations of 12.5 to 400 l/ml for 48 hours were found to be cytotoxic. Extract at a concentration of 12.50 l/ml and a lower concentration of DP had no impact on the viability of K-562 cells, and the extract's inhibitory concentration (IC 50) was 74.13 l/mL. (See Fig. 1) The in vitro cytotoxicity of O.

elatior's alcoholic fruit extract was investigated (DP). The cells were tested against the K562 human cancer cell line, and the vitality of the tumour cells was determined using the MTT method. A concentration dependent effect of the alcoholic extract of O. elatior on cell survival when used against the K 562 cancer cell line

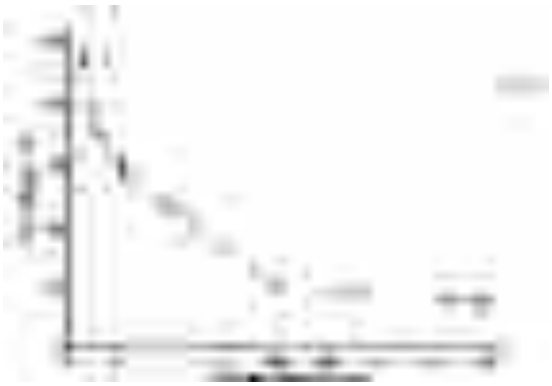
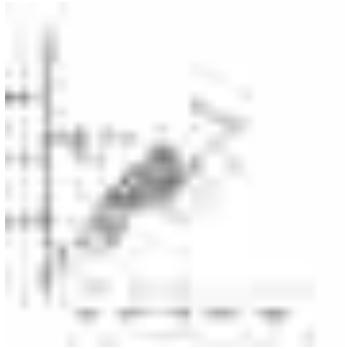


Fig. 1. Cytotoxicity Assessment by MTT assay in K-562 cells following exposure of various concentrations of Alcoholic fruit extract of Opuntia elatior.

Flow Cytometry analysis:

The flow cytometry analysis of untreated, positive control, and Opuntia elatior (OP) cells revealed that the percentage of cells in different phases of the cell cycle as compared to untreated cells revealed that the fruit extract of Opuntia elatior inhibited cell growth in the G0/G1 phase when compared to untreated cells. It is followed by and causes an increase in DNA damage in the cell. Sub G1 population was increased as a result of the Opuntia elatior extract and the proliferating cell population G2/M was dramatically lowered as a result of the extract.. (Table 1 and 2) (Fig.1, 2).



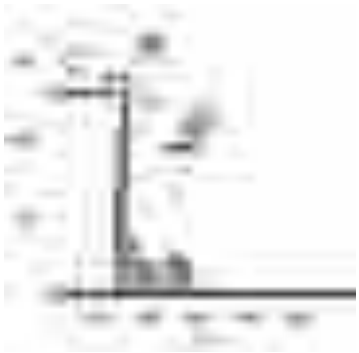


Fig 2. A) *Flow cytometric analysis of untreated cell K562 10000 cell (Ungated)* ***B)*** *Cell cycle untreated K562 9679 cell* ***C)*** *Cell cycle Untreated K562 9484 single cell.*

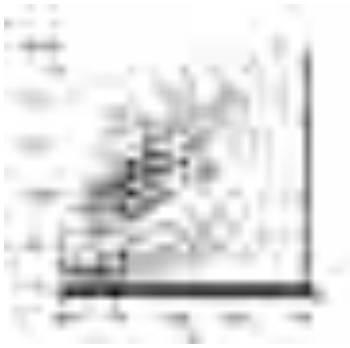


Fig 3. *Flow cytometric analysis of Cisplatin* ***A)*** *Cell cycle Cisplatin K562 10000 cell (Ungated)* ***B)*** *cell cycle Cisplatin K562 9170 cell* ***C)*** *Cell cycle Cisplatin K562 single cell*

Fig.4. *Flow cytometry analysis of O. elatior fruit extract* ***A)*** *cell cycle sample DP K562 10000 cell (ungated)* ***B)*** *Cell cycle DP K562 9608 cell* ***C)*** *Cell cycle sample DP K562 single cell.*

Table

Table No.1. Cell arrested in different phases of cell cycle by using Flow cytometric analysis. (Untreated sample, Positive control Cisplatin, DP sample)

Phase of cell cycle	Untreated sample		Positive control Cisplatin		DP Sample	
	Count of cell	Frequency of %	Count of cell	Frequency of %	Count of cell	Frequency of %
Single cell	9484	98.0	8859	96.6	9358	97.4
Sub G1	182	1.92	1953	22.0	263	2.81
GO/ G1	4866	51.3	2796	31.6	5215	55.7
S	2566	26.8	2711	30.6	2305	24.6
G2/ M	1894	20.0	1380	15.6	1562	16.7

Table. No.2. Effect of *Opuntia elatior* fruit extraction different phases of cell cycle K562 human blood cancer cell line.

Sr.No.	Sample Name	% of cells in different phases of cell cycle			
		SubG1 (Damaged DNA)	G0/G1	S	G2/M
1	Untreated	1.92	51.3	26.8	20.0
2	Positive control (Cisplatin) – 20 µg/mL	22.0	31.6	30.6	15.6
3	Sample DP – 74.13 µL/mL	2.81	55.7	24.6	16.7

LC-MS/MS analysis-

The different bioactive substances found by LC-MS/MS analysis in the fruit extract of Opuntia elatior include mostly alkaloids, flavonoids, amino acids, and plant hormones, with alkaloids being the

most abundant (Fig. 5). One of the substances found in this was aqueous alcoholic extract. Sulfabenzamide belongs to the sulfonamide group, Arecoline and Chlorpromazine belong to the alkaloid group, Kinetin belongs to the amino purines group, Leucine belongs to the amino acid group, Eseroline belongs to the pyrroloindole group, Rhamentin belongs to the flavonoid group, and Bilirubin belongs to the normal catabolite cell pathway. Sulfabenzamide is a chemical that has anti-cancer activities in the colorectal, renal, and cervical cancer areas (Gupta et al., 1988, and Lee et al., 2007). Arecoline, a compound derived from the plant Areca catechu, has been shown to have anti-cancer characteristics in the treatment of breast cancer, blood cancer, and oral cancer (Tsai et al., 2008, and Chen and Chang, 2011, Lin et al., 2015). Kinetin, a compound derived from Nicotiana tabacum, has been shown to suppress the growth of breast cancer cells (Mehrzad and Rajabi, 2011). Leucine, derived from the plant Craterostigma plantagineum, was shown to have inhibitory characteristics in the liver, kidney, urinary bladder, and breast tumour xenograft Chinese hamster models (Gong et al., 2015, Kato et al., 1979, Zhao et al., 2006, Tobey and Ley, 1971 and Xiao et al., 2016). Chlorpromazine, derived from Hyptis martiusii, has been shown to have anti-cancer activities in the colon, kidney, and cervical cancers (Lee et al., 2007, and Gupta et al., 1988). Eseroline, derived from the plant Desmostachya bipinnata, has anti-cancer potential against myeloma cancer (Rickardson et al., 2006). Rhamentin, which has been isolated from Pisum sativum and Vicia faba, has been shown to have anti-Ehrlich ascites carcinoma and anti-breast cancer characteristics (Ertekin et al., 2016, and Lanet al., 2018). Bilirubin revealed that Strelitzia Nicolai was discovered to have cancer-inhibiting characteristics in colon cancer and stomach cancer (Keshavan et al., 2004, and Rao et al., 2006).

Fig. 5: LC-MS/MS chromatogram of alcoholic extract of *Opuntia elatior*.

Discussion

Development of resistance to common chemotherapeutic anticancer drugs is a serious issue that occurs as a result of the ATP binding site (ABC) superior family of drug transporters, which is responsible for active efflux anticancer drug transport (Baguley, 2010). The numerous mechanisms of cell death produced by a cytotoxic substance vary depending on the cancer cell line being studied. Cisplatin primarily promotes apoptosis, which reduces the amount of cancer cells (Siddik, 2003 and Watanabe et al., 2008). Meanwhile, Lim et al. (2010) found that Ursodeoxycholic acid might cause apoptosis in HepG2 cells that had been exposed to Cisplatin-induced necrosis to flip to apoptosis in response to Ursodeoxycholic acid. Different chemicals were discovered in Opuntia elatior extract that have been linked to a variety of bioactivities by other studies, such as sulfabenzamide, which shown anticancer action against breast cancer (Mohammadpour et al., 2012). Breast, leukaemia, and oral cancer were all affected by arecoline's cytotoxic properties (Tsai et al., 2008, Chen and Chang, 2011 and Lin et al., 2015). Kinetin shown anti-cancerous action against breast cancer cells (Mehrzad and Rajabi, 2011). Xiao et al., 2016) and Chlorpromazine against colorectal, kidney, and cervical cancer (Lee et al., 2007 and Gupta et al., 1988), Eseroline demonstrated anticancer activity on Ehrlich's ascites carcinoma and breast cancer (Rickardson et al., 2006), Rhamentin demonstrated anticancer activity on Ehrlich's ascites carcinoma and breast cancer (Rickardson (Ertekin et al., 2016 and Lan et al., 2018). Bilirubin is effective against colon and stomach cancer (Keshavan et al., 2004 and Rao et al., 2006). Several researchers have also reported on the involvement of arecoline in human kertinocyes in response to the modulation of the inflammatory process in peripheral blood mononuclear cells (Hsu et al., 2001 and Jeng et al., 2003). Apoptosis (the death of lymphocytes) is caused by this alkaloid, which has immunosuppressive properties (Selvan and Rao, 1993). According to the findings of Chan and Chang (2011), tumour necrosis factor (TNF) and tumour necrosis factor 2 (TNF2) play an important role in arecoline death cell proliferation and that the reduction in proliferation of arecoline treated cells was mediated through the TNRF2 pathway as a molecular mechanism responsible for arecoline induced immuno separation and death cell proliferation. In this work, the alcoholic fruit extract of O. elatior was shown to have antitumor cytotoxic effect against the K-562 human leukaemia cell line, which was previously undiscovered. Further research on the mechanism of action of DP extracts, as well as the risks and advantages associated with the plant-based bioactive chemical, is now underway.

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IN VITRO AND IN SILICO EVALUATION OF *CITRUS LIMON* (L.) BURM. F. ETHANOLIC FRUIT EXTRACT FOR ITS ANTICANCER ACTIVITY

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Abstract: ***It was decided to conduct this investigation to evaluate the anti-cancerous activity of ethanolic concentrate of Citrus limon plants grown in two different locations in Southern Tamil Nadu (Site I Nagercoil of Kanyakumari district and Site II Kudunkulam of Tirunelveli district). The anti-cancerous activity was determined using the MTT assay, tetrazolium reduction assay in MCF-7 cells, and in silico studies. Using the Raw 264.7 macrophage cell line, the percentage of cell viability was determined and reported. In the first experiment, the ethanolic fruit extract of Citrus limon from plants at Site I generated an IC 50 value of 218.72 g/ml, while the second experiment yielded an IC 50 value of 65.08 g/ml for the ethanolic fruit extract of Citrus limon from plants at Site II. According to the findings, the ethanolic fruit extract of Citrus limon from Site II displayed greater action against MCF-7 (Human Breast Adenocarcinoma) cell lines, which was manifested as a suppression of cancer cell growth. This research shown that when exposed to MCF-7 cell lines culture, all of the tested extracts reduced cancer cell viability only in a concentration – dependent way, regardless of the kind of extract studied. A molecular docking approach was used to evaluate the binding capability of two bioactive compounds, Indole-2-one-2,3-di hydro-N-hydroxy-4-methoxy-3, 3-di methyl from an ethanolic fruit extract of Citrus limon from Site I and Urs-12-en-3-ol, acetate, (3.beta) from an ethanolic fruit extract of Citrus limon from Site II, with cyclin dependent kinase 4. (CDK4). With the target Arg 200 sharing two hydrogen bonds with the oxygen atoms of CD1, the compound Indole-2-one, 2,3-di hydro – N-hydroxy -4 – methoxy -3, 3-dimethyl (CD1) possessed the lowest binding energy of -5.46, while the compound Urs-12-en-3-ol, acetate, (3.beta)- (CD2) possessed the least binding energy of -7.17. In contrast to the control medication, 5-flurouracil, both of the compounds demonstrated higher binding affinity for the target molecule than the control drug. The fact that CD1 displays less binding energy during docking indicates that the connection between the protein and the ligand is stronger. This discovery has cleared the path for future research into medication creation employing components derived from common plants such as Citrus limon in order to combat the ever-increasing number of cancer cases for the benefit of all humanity.***

Key Words: ***Citrus limon, Anticancerous activity, Breast Cancer cell line MCF– 7, RAW 264.7 macrophage cells. Docking, Protein- ligand, Discovery studio (DS) visualizer.***

Introduction:

As a member of the Rutaceae family, citrus limon is believed to have natural chemicals that have a variety of health advantages. Commercial citrus varieties such as sweet orange, grapefruit, lime, and lemon are believed to contain natural chemicals that also have a number of health benefits. Citrus fruits are one of the most important fruits with significant medicinal potential, and they have long been used as the basis of traditional treatments in a number of Asian nations, particularly in the Philippines. Apart from being used as medicine, citrus fruits are also employed in the preparation of salad dressings, sauces, jams and vinegar, as well as fresh fruits in their full form. Large-scale scientific study has been carried out on these compounds because of their medicinal and economic importance, and the results have provided a comprehensive knowledge of their chemical composition and bioactivities. There is widespread recognition that citrus fruits contain significant levels of bioactive substances. According to Wang et al. (2018), such fruits include folate, vitamin C, dietary fibre, and bioactive chemicals such as flavonoids, which are found in both the pulp and the peel. Despite the fact that flavonoids are found in high quantities in fragrant plants such as mint and tea, they are also found in large concentrations in citrus fruits and their peels. In spite of the fact that it includes carotenes, essential oils, pectin, and a variety of polyphenolic chemicals, citrus peel has significant untapped potential as a source of therapeutic substances (Tsitsagi et al., 2018). According to Cirmi et al. (2016), epidemiological research have revealed that a high intake of fruits and vegetables (>400 g/d) may lower cancer risk by a factor of around 20%. The Mediterranean diet is high in fruit pulp and juice, and the high consumption of fibre, antioxidants, and polyphenol chemicals that is associated with it has been related to a decreased risk of cancer in certain studies (Smeriglio et al 2019).

Materials and Methods

Cell culture and MTT assay:

For the cell cytotoxicity experiment, RAW 264.7 macrophage cells were used. These cells were obtained from NCCS (Pune, India) and cultured in Dulbecco's Modified Eagle Medium (DMEM; Himedia, India), which was supplemented with 10% foetal bovine serum (FBS), 100 units per millilitre of penicillin and 100 nanograms per millilitre of streptomycin, and incubated at 37°C under 5% CO2. Cells from RAW macrophages were co-treated with 1 g/mL of LPS and various doses (ranging from 25 to 500 g/mL) of the test chemical. The vitality of the cells was determined after 12 hours of treatment, and the Griess reaction was performed to determine the amount of nitrite present in the cell culture media. The MTT test was carried out in order to measure cell viability. The RAW cells were treated with MTT solution at a final concentration of 0.5

mg/mL for 4 hours before being washed with PBS. A microplate reader was used to measure the absorbance at 570 nm after the supernatant was removed and the formazan crystals were dissolved in 100 millilitres of DMSO (Alere AM 2100, India). It was necessary to plate the Breast Cancer cell line (MCF-7) separately in 96 well plates at a density of 1 104 cells/well in EMEM media supplemented with 1 X Antibiotic Antimycotic Solution and 10 percent foetal bovine serum (Himedia, India) in a CO2 incubator at 37 degrees Celsius with 5 percent CO2. A total of 200 mL of 1X PBS was used to wash the cells, after which they were cultured for 24 hours with different test concentrations of the chemical in serum-free medium. At the conclusion of the treatment period, the media was aspirated from the cells. 0.5 mg/mL MTT produced in 1X PBS was added and incubated at 37 °C for 4 hours in a CO2 incubator before the results were obtained. The media containing MTT was removed from the cells and washed twice with 200 mL of PBS after the incubation time was completed. The crystals that were formed were dissolved in 100 mL of DMSO and thoroughly mixed together. At a wavelength of 570nm, the growth in colour intensity was seen. The colour of the formazan dye changes to a purple blue. With a microplate reader, the absorbance was measured at 570 nm at a certain wavelength.

Docking Studies

Retrieval of protein and ligands

RCSB Protein Data Bank (https://www.rcsb.org/) provided the three-dimensional crystal structure of the target CDK4 (PDB ID: 6PH8), which was downloaded for this study.

Preparation of protein and ligands

In order to perform effective docking, high-quality receptor and ligand coordinates are required. In order to do this, the protein was separated from the solvent and non-standard residue, a polar hydrogen atom was added to the protein, and the force field was repaired by adding a Gasteiger charge to the protein. The Molecular format of the ligand coordinates is transformed into the.pdb format by utilising the OpenBabel chemical toolbox (O'Boyle et al., 2011), which is a chemical toolbox. Molecular representations in AutoDock are simplified representations of the molecules that are saved in a modified PDB file format, known as PDBQT.

Molecular docking

Grid-based molecular docking using Autodock was performed on the compounds from C. limon that were chosen for further study and evaluation (Huey and Morris 2008). The grid boxes were created using the dimensions X=127; Y=127; Z=127 as the starting point. The docking operation was carried out with the help of the Lamarckian genetic algorithm for a total of 100 runs. One of the finest conformations created by autodock was chosen from a pool of ten possible conformations. The complex structures with the lowest binding energy and ligand efficiency, as well as the highest number of hydrogen bonds, were chosen for their superior performance.

Analysis of docking

A visualisation tool called Discovery studio (DS) was used to analyse and depict interactions between protein-ligand complexes, including their amino acid location in relation to bond lengths and the kinds of bonds that were involved (BIOVIA, 2021). Discovery studio visualizer is a powerful molecular modelling programme that allows you to see and analyse large amounts of macromolecular data. DS generates maps of protein-ligand interactions on its own. It generates visual representations of protein-ligand interactions for a specified protein-ligand interaction database (PDB). Hydrogen bonds are shown by dashed lines connecting the atoms involved, while hydrophobic interactions are represented by an arc with spokes extending towards the ligand atoms, they touch in the DS representation. The atoms that have been touched are portrayed with spokes that radiate back.

Results

Cytotoxicity and Anticancer Activity of citrus lemon ethanol extract

As a member of the Rutaceae family, citrus limon is believed to have natural chemicals that have a variety of health advantages. Commercial citrus varieties such as sweet orange, grapefruit, lime, and lemon are believed to contain natural chemicals that also have a number of health benefits. Citrus fruits are one of the most important fruits with significant medicinal potential, and they have long been used as the basis of traditional treatments in a number of Asian nations, particularly in the Philippines. Apart from being used as medicine, citrus fruits are also employed in the preparation of salad dressings, sauces, jams and vinegar, as well as fresh fruits in their full form. Large-scale scientific study has been carried out on these compounds because of their medicinal and economic importance, and the results have provided a comprehensive knowledge of their chemical composition and bioactivities. Some of the chemical constituents in citrus fruits, such as vitamins, dietary fibre, carotenoids, flavonoids, lipids, and essential oils, may be responsible for their health-promoting properties. High quantities of monoterpenes and flavanones found in citrus fruits are assumed to be responsible

for the fruit's anticancer effects, which are expected to cut the incidence of digestive system cancer by half, according to research. These chemicals reduce the likelihood of developing cancer in two ways. The drugs block inflammation, which is a critical stimulant for cancer cell growth, and they also interfere with a number of processes that are necessary for cancer cell growth, making tumour formation more challenging. Citrus has a wide range of compounds that are not found in other plants, and some of these chemicals have been demonstrated to be effective against a number of cancer cell lines in studies.

The MTT test (3- (4,5 dimethylthiazol-2-yl) 2,5 diphenyltetrazolium bromide) tetrazolium reduction assay) was used to assess the cell viability of ethanolic fruit extract of Citrus limon obtained from two different chosen sites (Site I and Site II). The percentage of cell viability against the Raw 264.7 macrophage cell line was determined at five different concentrations (Fig. 1 & 2).

It was discovered that the cell viability of treated cells was 86.36 percent at 500 g/mL, 90.48 percent at 250 g/mL, and 92.99 percent at 100 g/mL. 50 g/ml yields 95.71 percent cell viability of Raw 264.7 cell lines cultures; however, 25 g/ml yields 99.43 percent cell viability when exposed to the ethanol extract of Citrus limon fruits from site II (Fig A: Plate 1). When employing an ethanol extract of Citrus limon fruits from site II at a concentration of 500 g/ml, cell viability was found to be 83.84 percent. After being exposed to the ethanol extract of Citrus limon fruits from site I, 87.93 percent cell viability is achieved at 250 grammes per millilitre, 90 percent at 100 grammes per millilitre, 97.10 grammes per millilitre at 50 grammes per millilitre, and 97.90 grammes per millilitre at 25 grammes per millilitre (Plate 1).

In the study of anticancer activity of ethanol extracts of Citrus limon fruits extracts from plants from sites I and II against MCF 7 cells, it was shown that the concentration of fruit extract increased significantly with increasing concentration of fruit extract (Fig. 1). Cell viability of cancer cells was reduced to 18.43 percent when the fruit extract from plants from site I was used at 500 g/ml, 30.82 percent when the extract was used at 250 g/ml, and 43.81 percent when the extract was used at 100 g/ml. Cancer cell viability was 57.70 percent at 50 g/ml and 65.56 percent at 25 g/ml when the concentrations were used (Fig C: Plate 1). It was discovered that the viability of cancer cells increased when the concentration of the extract was lowered. Cancer cell viability was only 27.79 percent at 500 g/ml of the fruit extract from plants at site II, 44.41 percent at 250 g/ml, and 66.77 percent at 100 g/ml of the fruit extract from plants at site II. MCF-7 cells were found to have roughly 82.18 percent viability when 50g/ml of fruits extract was used, whereas 25g/ml of fruits extract exhibited 91.84 percent cancer cell viability when used (Fig 3& 4: Plate 2).

Docking Studies

Results of this study revealed that the chemicals Indole-2-one, 2, 3-dihydro-4-methoxy-3, 3-dimethyl- (CD1) and Urs-12-en-3-ol acetate, (3.beta.)- (CD2), derived from the chosen plant, have significant anti-cancer activity. 5-flurouracil was used as a control agent in the in silico tests, and both compounds demonstrated higher binding affinity for the target molecule than this medication (Table 1). The residues ILE117 (4.18 and 4.31) and GLU115 (1.9) were found to interact with CD1; ARG29 (2.16), ARG123 (5.31), LEU287 (5.17), HIS65 (5.48 and 4.30), PHE127 (4.84), and PHE284 (5.0) were found to interact with CD2 (Fig.). ILE117 (4.18 and 4.31 Hydrogen bonding interactions, alkyl interactions, and pi-orbital interactions are some of the most important interactions that contribute to excellent binding affinity.

Table 1: Docking results of the selected phytochemicals with CDK4

Ligand	Binding Energy	Ligand Efficiency	Inhibition Constant	Electrostatic Energy	RefRMS
5-Fluorouracil	-2.88	-0.32	7.79 mM	-0.2	72.68
Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-(CD1)	-3.51	-0.23	2.66 mM	-0.05	72.62
Urs-12-en-3-ol, acetate, (3.beta.)-(CD-2)	-5.59	-0.16	5.53	-0.19	60.24

Fig. 5- A) 3D structure of CDK-4 protein, 2D structure of ligands B) 5-Fluorouracil, C) Urs-12-en-3-ol, acetate, (3.beta.) (CD-2), D) Indole-2-one, 2, 3-dihydro-N-hydroxy-4-methoxy-3, 3-dimethyl- (CD-1)

Fig. 6- Interaction of 5-fluorouracil (control) with CDK4. A) 5-flurouracil and CDK4 complex. The protein is represented in pink and green represents the ligand. B) 2D plot of the complex generated by DS visualizer.

Fig. 7- Interaction of CD1 with CDK4. A) CD1-CDK4 complex. The protein is represented in pink and green represents the ligand. B) 2D plot of the complex generated by DS visualizer.

Both CD1 and CD2 exhibited high binding affinity for the target CDK4, which was seen in both compounds. The lower the binding energy of the protein, the better the interaction between the protein and the ligand. When comparing the binding energy of the compound and the control, it is obvious from Table 1 that the compound demonstrated more binding affinity than the control. CD1 and GLU115 establish a typical hydrogen bond, as shown by the docked result of the complex shown in Fig. 5. The interatomic distance between CD1 and GLU115 is within the desired range. CD1 and ILE-117 also share two alkyl hydrophobic bonds, which is a rare occurrence. CD2 has the lowest binding energy of -5.59 kcal/mol. ARG29 and the oxygen atoms of CD2 form hydrogen bonds that are shared by both molecules. Furthermore, this shared Alkyl and pi-Alkyl interactions with the molecule were discovered. In the presence of CD2, the residues ARG123 and LEU287 established alkyl connections with one another. It has been shown that the aromatic ring interacts with the positively charged arginine (ARG123) and histidine (HIS65) residues, as well as with LEU287, which is typical of aromatic ligands.

Fig. 8- Interaction of CD2 with CDK4. A) CD2-CDK4 complex. The protein is represented in pink and green represents the ligand. B) 2D plot of the complex generated by DS visualizer. Black labelled residues

Discussion

Anticancer Activity

The majority of cancer medications are supplemented with a degree of natural improvements. Therapeutic plants have a positive influence on cancer patients' financial well-being. Natural remedies are used into a few therapies to enhance the overall quality of life. A broad range of regular compounds derived from plants, such as flavonoids, terpenes, alkaloids, and other phytochemicals, have received considerable attention in recent years because of their major pharmaceutical capabilities, which include cytotoxic and malignant growth chemo preventive effects (Shoeb, 2006). The discovery and development of vinca alkaloids such as leurocristine and vincaleukoblastine, as well as the isolation of the cytotoxic naphtho, dioxol-6(5aH)-one, marked the beginning of the hunt for disease specialties derived from plant sources in the 1950s. It has been shown that natural chemicals derived from restorative plants have a significant influence on the treatment of illness (Cho et al., 2003). Plant-based medication has undoubtedly been found to perform a role in sickness recovery (chemotherapy), and the component of interaction between many phytochemicals and malignant development cells has been studied extensively. In particular, there is growing interest in the pharmacological evaluation of many plants that are used in Indian customary treatment arrangements. There are more than 270,000 higher plants on our planet, which is a significant number. However, only a little amount of photosynthesis research has been conducted. As a result, it is anticipated that plants would provide potential bioactive mixes for the development of novel pushes to combat malignant infections (Oskoueian et al., 2011). Mavundza et al. (2010) conducted a phytochemical screening of lemon and discovered the presence of a variety of material combinations, including alkaloids, flavonoids, glycosides, phenolics, steroids, tannins, and terpenoids. Therapeutic plants may include a variety of various types of substance segments, and their organic activities are not always attributed to a single moiety or a single compound. A research was conducted to determine the cytotoxic activity of fruit ethanol extract from C. limon plants grown in two different locations around Tamil Nadu. A study was conducted to determine the cytotoxic potential of C. limon organic product ethanolic concentrate against the Raw 264.7 and MCF-7 cell lines.

Citrus fruits' positive properties, according to Abudayeh et al.,2019, may be attributed to their antioxidant properties. Polyphenolic compounds

are made up of a variety of bioactive chemicals, including flavonoids, limonoids, coumarins, phenolic acids, terpenoids, tannins, stilbenes, lignans, and carotenoids. Flavonoids are the most abundant kind of bioactive component in plants. The high concentrations of monoterpenes and flavanones found in these fruits are likely to contribute to their anticancer qualities, which are expected to cut the incidence of digestive system cancer by around half. Several of these compounds reduce the likelihood of developing cancer in two different ways: either by inhibiting inflammation, which is an important stimulant for cancer cell growth, or by interfering with several of the processes required for cancer cell growth, thereby making tumour development more difficult. Citrus has a wide range of compounds that are not found in other plants, and some of its constituents have been demonstrated to be effective against a number of cancer cell lines.

Cell viability of RAW cell lines grown in medium containing ethanolic fruit extract of C. limon plants grown at two separate locations (Site I and Site II) was determined in the present investigation. Cell viability was found to be concentration dependent, with the highest cell viability observed at 500 g/ml of ethanolic fruit extract of C. limon fruits from site I, which came to be 86.36 percent, and the highest cell viability observed at the same concentration of the extract from site II, Cell growth was inhibited in MCF 7 cell cultures when they were cultured in media containing ethanolic fruit extracts from plants from sites I and II, according to the findings. Only 18.43 percent and 27.79 percent cell viability were seen in the fruits extract from plants from sites I and II, respectively, at 500 g/ml, with a maximum of 65.56 percent and 91.84 percent cell viability observed at 25 g/ml of the extract, respectively. It was discovered that the viability of the MCF-7 cell line rose when the concentration of the extract was lowered. The cytotoxic impact of each extract varied depending on the concentration used. This might be owing to the bioactive mixes and range of phytochemicals such as phenolics and flavonoids present in the cells, which could cause the cytotoxic activity of this restorative plant. Mavundza and colleagues (2010). Oskoueian and colleagues (2011) revealed the ability of flavonoids and phenolic combinations to act as anticancer specialists in a variety of cancers. The degrees of activity of ethanolic fruit extracts from various geographical regions on MCF -7 cell lines are variable. A combination of environmental conditions and extract chemicals may be responsible for this action, or it may be a tissue-specific response to a single extract chemical. The contribution of the samples to MCF-7 proliferation is assessed in terms of percent cell viability. Plants at site I produced an ethanol fruit extract of C. limon that had an IC 50 value of 218.72 g/ml, while plants at site II produced an ethanol fruit extract that had an IC 50 value of 65.08 grammes per millilitre (g/ml). Because of the phytochemical components present in the fruit extract, including flavonoids and terpenoids, the fruit extract has demonstrated anticancer activity against breast cancer cell lines. These phytochemical components are also responsible for the possible cytotoxic effect against breast cancer cell lines. This study confirms previous research on the phytochemical contents of citrus fruits, which found the presence of flavonoids and terpenoids, which is further supported by the results of the present study.

According to Sak (2012), nanovesicles derived from C. limon were shown to inhibit malignant cell proliferation in a variety of tumour cell lines. As an added bonus, the nanovesicles from C. limon smother chronic myeloid leukaemia (CML) tumour growth in vivo by specifically reaching the tumour location and activating TRAIL-interceded apoptotic cell measures (Sak K, 2012). Because of the aglycones and glycosides of limonoids and flavonoids included in the Citrus concentrate, it is possible that it will serve as a chemo preventive specialist for breast cancer in the future (Atjanasuppat et al., 2009).

The degrees of activity of ethanolic fruit extracts from various geographical regions on MCF -7 cell lines are variable. A combination of environmental conditions and extract chemicals may be responsible for this action, or it may be a tissue-specific response to a single extract chemical. The contribution of the samples to MCF-7 proliferation is assessed in terms of percent cell viability. In the first study, the ethanol fruit extract of C. limon from site I produced an IC 50 value of 218.72 g/ml, whereas the second study produced an IC 50 value of 65.08 g/ml using the same extract from the second site. C. limon fruit extract from site II was discovered to exhibit significant anti-tumour action against MCF -7 (Human Breast Adenocarcinoma) cell lines, as well as the ability to promote cell growth. The vast majority of chemotherapy medications are not only cytotoxic to cancer cells, but they are also poisonous to healthy cells and have immune suppressing side effects as well. As a result, the development of new chemicals that are not only cytotoxic to cancer cells but are also non-toxic to healthy cells and have the ability to modulate the immune response has emerged as a major research objective in the biomedical sciences (Manassero et al., 2013). The results of the current investigation shown that all of the tested extracts significantly reduced cell viability in a concentration-dependent way in both MCF-7 and Raw 264.7 cell lines, as well as in MCF-7 cells. Citrus peels have been shown to have

anticancer action in the form of single compounds or as a combination of chemicals, depending on the study (Wang et al., 2014; Rawson et al., 2014). Essential oils from lemon and grapefruit peel, for example, have been shown to have medium-to-weak cytotoxicity against human prostate cancer cell lines PC-3, lung cancer cell lines A549), and breast cancer cell lines MCF-7 (Zu et al., 2010; Diab et al., 2015). Furthermore, the ethanolic extract from orange peel and its fractions were shown to have a modest to moderate cytotoxic effect on HL-60 cells when tested (Raimondo et al., 2015). Our findings imply that the presence of limonoid may be responsible for the increased cytotoxicity of the ethanol extracts. The phytochemical components included in the fruit extract, particularly flavonoids and terpenoids, are thought to be responsible for the probable cytotoxic action on breast cancer cell lines, in accordance with these findings. This study confirms previous research on the phytochemical contents of citrus fruits, which found the presence of flavonoids and terpenoids, which is further supported by the results of the present study.

The increased mortality linked with cancer, along with the high expense of cancer therapy, necessitates ongoing research into new anticancer medications that are more effective at treating cancer while also having fewer adverse effects. Secondary metabolites such as phenols, flavonoids, alkaloids, and tannins have all been proven to have anticancer activities in animal and human studies (Kim et al., 2012). An investigation into the anticancer effects of the ethanolic extract of lemon against the human cancer cell lines MCF7 (breast) and Raw 264.7 was carried out. It was discovered that the extracts had cytotoxic effects on the cell lines in a concentration dependent manner (Kooti et al., 2017) and that the concentration dependent pattern was seen. Also in agreement with the results of this research is a report by Rawson, Ho, and Li (2014), who discovered that citrus peel extracts inhibited the formation of PC3 xenograft tumours in immune-deficient mice, even at modest doses of citrus peel extract. When compared to the standard curcumin, the IC50 values of the petroleum ether and ethyl acetate rind extracts in prostate and breast cancer cells, respectively, were not statistically significant.

The observed cytotoxicity, yet low selectivity for breast cancer cells due to significant cytotoxicity to normal cells, could be due to trace amounts of organic solvents in the extracts. This could explain the observed cytotoxicity, yet low selectivity for breast cancer cells due to significant cytotoxicity to normal cells. According to Jamalzadeh et al. (2016), certain organic solvents, although effective in enhancing solubility, may cause some harmful interference in cellular-based tests, despite their usefulness in raising solubility. Furthermore, the reduced selectivity for MCF-7 cells when compared to Raw 264.7 cells might be related to the fact that breast cancer cells have a usually lower cytotoxicity (Badisa et al., 2006). It is possible that this is due to the fact that MCF-7 is a hormone-dependent cell line, while Raw 264.7 is not. Certain phytohormones have been proven to stimulate the development of cancer cells that are reliant on hormones. The phytoestrogen Genistein, according to a research conducted by Kwon (2014), increased the development of estrogen-dependent MCF-7 cells in vitro when administered at modest dosages, but had no influence on the growth of hormone-independent breast cancer cells MDA-MB231. Naringenin, which is present in certain citrus fruits and is 79 regarded as a predecessor to genistein, was shown to have anti-tumour potential, according to a study published in 2017 by Ganna and colleagues (Garg, 2015). However, according to a research conducted by Zhang et al. (2016), naringenin has the ability to suppress the development of breast cancer cells by reducing the release of Transforming Growth Factor TGF-1, which is a protein that is responsible for cell growth and proliferation. In conclusion, our work has shown that a variety of C. limon components have anticancer action in vitro.

In order to better understand the anti-cancer properties of citrus phytochemicals, a molecular docking technique was performed using the selected ligands and the cyclin dependent kinase, CDK4, as the target enzyme. CDK4 is an essential CDK cell cycle regulator that regulates the passage of the cell cycle from the G1 to the S phase by forming a complex with cyclin-D during the G1 phase (Ingham and Schwartz, 2017; Bloom and Cross, 2007). Several malignancies, including non-small cell lung cancer, colorectal cancer, melanomas, and breast cancers, have low amounts of endogenous CDK inhibitors, but high levels of CDK4 in their cells (Sher et al., 2016; Lim et al., 2016). Aside from that, cyclin D1, a CDK4-related cyclin, is frequently up-regulated in a wide range of cancer types including breast and lung cancers as well as bladder and gastro intestinal tract cancers (VanArsdale et al., 2015). CDK4 inhibition has been found as a promising method for the targeted therapy of a variety of malignancies by arresting the cell cycle during the G1 phase, as previously reported (Parylo et al., 2019).

Both compounds had favourable interactions with the target, which was similar to the control medication. Their contact with the target is strengthened because they share a single hydrogen bond with an interatomic distance in the optimal range. Furthermore, they have weak contacts, such as alkyl hydrophobic interactions, which are similar to those of the control drug, and this plays a

significant role in defining the structure and function of proteins. It has been discovered that the aromatic structure of CD2 forms pi-alkyl interactions with particular residues of the target. There have been many reports of the interaction of pi-cation with proteins and ligands in protein-ligand complexes, including X-ray crystallography and computational investigations (Biot and colleagues, 2003; Zacharias and Dougherty, 2002).

Summary and Conclusion

Proteomics is becoming more important in research in this post-genomic age. Explorations in both experimental and computational approaches are being made to generate and analyse enormous amounts of information produced from 3D protein structures, with the ultimate objective of making a scientific and economic breakthrough in drug development. Cancer is a complex illness in which the key causal variables include a certain genetic background, extended exposure to a variety of environmental exposures, and an inadequate nutritional intake. Molecular alterations or mutations of essential proteins caused by any of these risk factors will eventually result in the development of cancer. CDK4, which is a cyclin dependent kinase, has been discovered to be overexpressed in the majority of malignancies. Therefore, there is a need for effective inhibitors of the Cyclin D1/CDK4-mediated pathway. Because citrus extract had significant anticancer action, we investigated the anticancer potential of the two phytochemicals that were chosen.

It is obvious from the docking data that the compounds had the lowest binding energy with the target when compared to the control medication. The sort of interaction that was observed, as well as the residues with which it interacted, indicated that they had a high binding affinity for the target protein. As a result, the Citrus compounds CD1 and CD2 might be regarded as a potential source for the isolation, identification, and development of effective anticancer medicines in the future. However, more experimental validation is required, which will be carried out in the near future via molecular biology studies that will take into account the structural aspects that may make it possible for the molecules to be used as anti-cancer lead molecules in the future.

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INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed Refereed Journal

IN VITRO ANTIBACTERIAL STUDIES OF PHYTOFABRICATED Ag-Co HYBRID NANOPARTICLES

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ABSTRACT

An ecologically benign green strategy is presented for the synthesis of Ag-Co hybrid nanoparticles taking a medicinal plant, *Aerva lanata* for the phyto reduction of the precursor salt solutions. The formed nanoparticles are characterized by instrumental techniques such as UV-Visible, FTIR, EDX, FESEM and HRTEM. These particles are found to possess significant antibacterial efficacy against both Gram-positive and Gram-negative bacteria, which is investigated by Well Diffusion method.

KEY WORDS

Hybrid nanoparticles (HNPs), *Aerva lanata (*AL), antibacterial, Gram-positive, Gram-negative, zone of inhibition.

INTRODUCTION

Nanotechnology involves the manipulation of materials having one of the dimensions in the range of 0.1-100 nm [1]. It has its applicability in different fields like biology, chemistry, physics, engineering and medicine [2]. There are two types of strategies generally adopted for fabricating nanomaterials, namely top-down methods and bottom-up methods. In top down method we transform material progressively from bulk substrate until the desired nanomaterial is obtained. Bottom-up strategies are employed starting from the atomic or molecular precursors and by gradually assembling it until the preferred structure is formed (**Figure. 1(a), 1(b)**) [3].

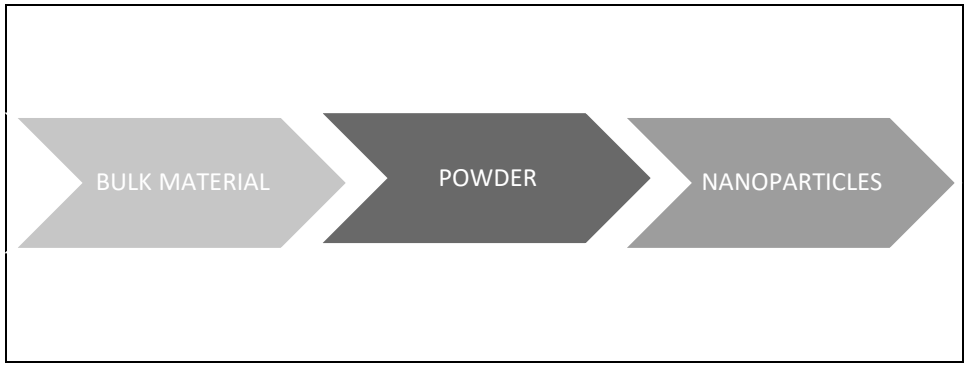

Figure: 1(a). Top-Down approach

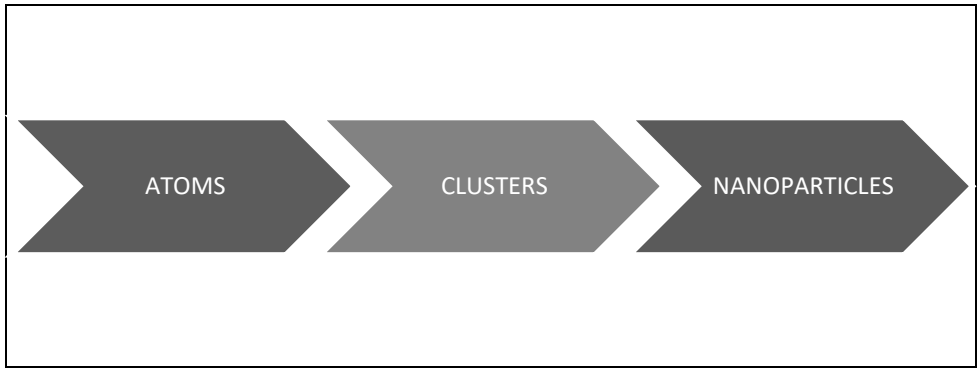

Figure 1(b): Bottom-Up approach

Remarkable expansion of nanotechnology has spread out its application in multiple fields such as biomedical sciences, nutrition, energy sciences, nano-biotechnology, cosmetics, mechanics, optics, chemical industries, drug-gene delivery [4]. Alloying of two different metals in nanosize may enhance their characteristics than that of their corresponding monometallic nanoparticles. These hybrid nanoparticles show greater stability, mechanical strength and catalytic activity than monometallic nanoparticles [5]. Generally nanometals are synthesized by physical or chemical reduction methods which are hazardous and expensive. In contrast, plant mediated green methods are eco-friendly, cheaper and benign for the synthesis of nanometals. Secondary metabolites present in plant extract will act as bio-reducing and capping agents [6].

Antibacterial agents are very important in the textile industry, water disinfection, medicine, and food packaging. Organic compounds used for disinfection have some disadvantages including toxicity to the human body. Therefore the interest in inorganic nanoparticles has been increased as they are benign to a greater extent [7]. Nanoparticles are increasingly used to target bacteria as an alternative to antibiotics [8]. Traditional methods like herbal extracts used to the synthesize nanometal particles have shown extensive consent in medicine. These synthesized nanometal particles have great bactericidal activity than bulk metals because of their ease of adsorption at bacterial surface [9]. Nanometals like silver, copper, gold, etc., are assumed to be able to participate in sub-cellular reactions as their size is comparable to biological molecules [10]. Bimetallic nanoparticles composed of two different metals have drawn greater interest than the monometallic nanopaticles as the properties differ from pure elemental particles include unique size dependent, optical, electronics, thermal, catalytic and biological effects. Hence they are being used as antimicrobial agents. Plant mediated green synthesized hybrid nanoparicles have increased attention towards their antimicrobial properties as they contain bioactive phytochemicals as stabilizing and capping agents.

In this present study, an effortless and robust green method is reported for the synthesis of Ag-Co hybrid nanoparticles (HNPs) by using leaf extract of *Areva lanata* as a reducing and capping agent. The synthesized HNPs are studied for their antibacterial activity against Gram-positive, Gram- negative bacteria.

2. EXPERIMENTAL

2.1. Materials:

Chemicals and apparatus required

- Silver nitrate and cobalt nitrate of analytical grade.
- Digital weighing balance
- Magnetic stirrer
- Centrifuge machine
- Hot air oven
- pH papers
- Whatmann-1 filter papers
- Leaves of *Aerva lanata*
- 0.1 N HCl
- 0.1 N NaOH

Deionized water is used to clean glassware, to prepare chemical solutions and for experimental procedure. Fresh leaves of *Aerva lanata* are collected from agricultural lands of S.Kota, Vizianagaram district, Andhra Pradesh state, India.

2.2. Preparation of *Aerva lanata* leaf extract: 100 g of fresh leaves are weighed and thoroughly washed with running tap water to remove detritus on surface of leaves followed by deionized water to get rid of other contaminants from leaves and dried up under shade for 10 days. These leaves are cut into tiny pieces and made homogeniozed powder by using home blender. The procured

powder is placed in refrigerator at 4 ºC which is kept in an air tight container. Now 200 mL deionised water is taken in 500 mL beaker to this 10 g stored powder is weighed and added. The contents in the beaker are heated for 20 minutes at 60 ºC with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled concoction is filtered two times with Whatman No.1 filter paper and reserved in refrigerator at 4 ºC. This is taken as leaf extract throughout the experiment (**Figure: 2(a), 2(b)**).

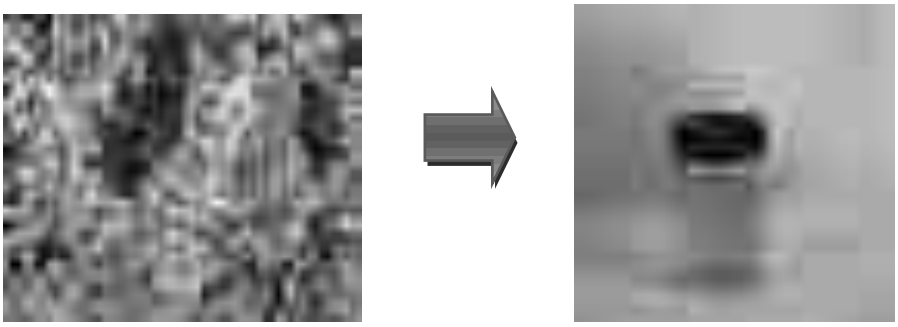

Figure 2(a): ***Aerva lanata*** **plant** **Figure 2(b): leaf extract**

2.3. Synthesis of Ag-Co bimetallic nanoparticles:

Equimolar (20 mM) concentrations of silver nitrate and cobalt nitrate aqueous solutions are prepared in deionized water respectively. Synthesis of Ag-Co BMNPs is done by taking 100 mL of $AgNO_3$ solution in a 500 mL beaker, to this 100 mL of leaf extract, 100 mL of $Co(NO_3)_2$ solution are added by drop wise in simultaneous addition process. After this addition the beaker is placed on a magnetic stirrer for continuous agitation. This mixture is stirred at 75 ºC for one hour at pH 8 on magnetic stirrer. These synthesized HNPs are separated out by centrifugation at 5000 rpm for 30 minutes. The obtained HNPs are washed with deionizer water twice to remove unwanted constituents and dried in oven at 80 ºC for two hours. The resultant HNPs particles are collected and used for characterization.

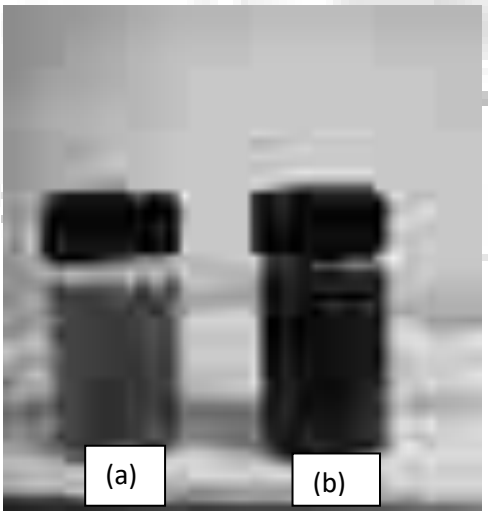

Figure 3: Precursor solutions (a) before (b) after the bioreduction

2.4 Characterization

The synthesized HNPs are characterized by various instrumental techniques. UV-Visible analysis (Figure 4) indicates the formation of HNPs by SPR band at band at around 436 nm [11]. The FTIR spectrum of Ag-Co HNPs exhibits major peak positions at 3212 cm^{-1}, 3416 cm^{-1} and 3382 cm^{-1}which indicate the N-H stretching vibrations of amines and O-H stretching of hydroxyl groups of alcohols and phenols. Intense peak at 1640 cm^{-1} is due to C=O stretching of amide group. Very small peak at 602 cm^{-1} indicates the presence of C-Cl group.

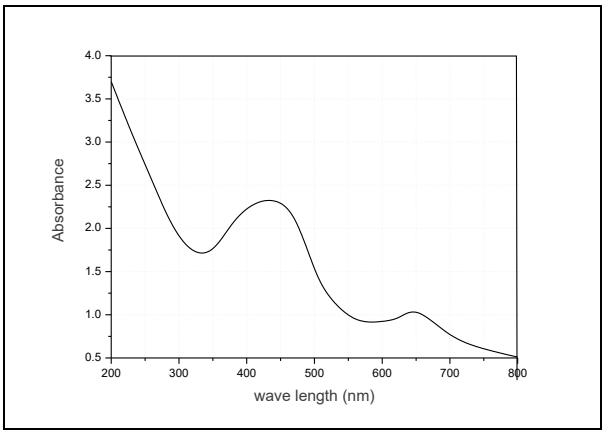

Figure 4: UV-Visible spectrum of Ag-Co HNPs

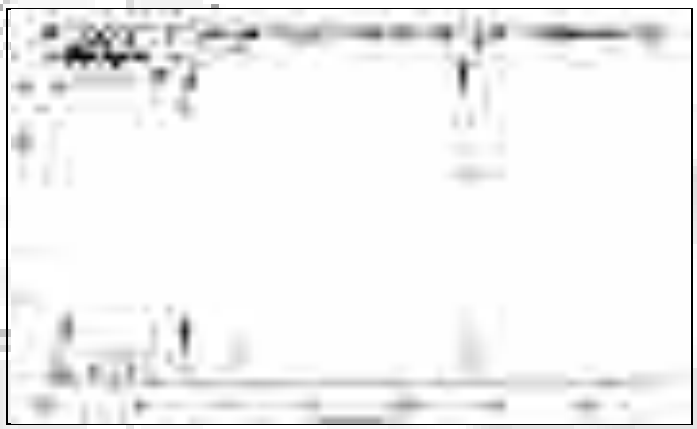

Figure 5: FTIR spectrum of Ag-Co HNPs

From energy dispersive X-ray analysis (EDX), we can analyze all the elements present in the HNPs which indicate the existence of Ag and Co which confirms the formation of Ag-Co hybrid nanoparticles. This is also supported by the EDX study which gives quantitative data of silver and cobalt compositions in HNPs**.** By Field Emission Scanning electron microscopic (FESEM) images of Ag-Co HNPs, it can be clearly noted that the prepared Ag-Co hybrid nanoparticles are in the size range between 50 and 100 nm in diameter.

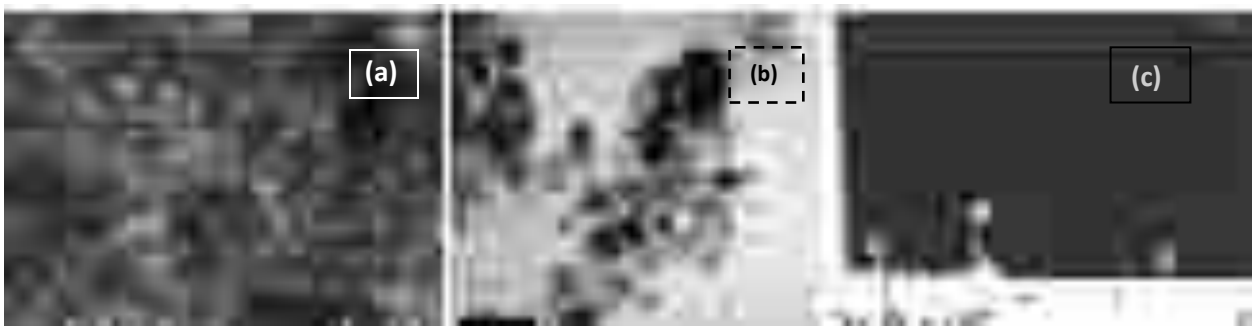

Figure 6 : (a) FESEM image (b) HRTEM image (c) EDX spectrum

HRTEM shows that Ag-Co HNPs are figured with spherical morphology and crystalline structure below 100 nm in size. More specifically, the two metal nanospheres are shown and spoted adjacent to each other. It is also in strong agreement with the micrographs from FESEM analysis. Powder XRD analysis (Figure 7) confirms that HNPs have FCC crystal structure with average particle diameter of 24.4 nm.

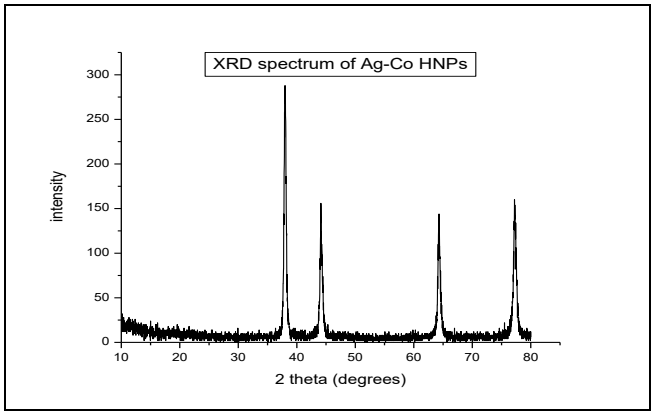

Figure 7: Powder XRD spectrum

DETERMINATION OF ANTIBACTERIAL ACTIVITY

Reagents and Materials

Microorganisms used are obtained from IMTECH, Chandigarh, India

1) *Bacillus subtilis* MTCC211
2) *Escherichia coli* MTCC443
3) *Staphylococcus aureus* MTCC6908
4) *Pseudomonas aeruginosa* MTCC2581

Experimental Determination

Antibacterial activity of the HNPs is evaluated by agar-well diffusion method [12]. The standardized cultures of test bacteria are first evenly spread onto the surface of Mueller Hinton Agar plates using sterile cotton swabs. Five wells (6 mm diameter) are made in each plate with sterile cork borer. Twenty microlitres of each of the compound and positive control is added in wells. Streptomycin (10 µg) is used as reference antibiotic. Diffusion of compounds, antibiotic and DMSO are allowed at room temperature for 1 hour. All of the plates are then covered with lids and incubated at 37 ^{o}C for 24 hours. After incubation, plates are observed for zone of bacterial growth inhibition. The size of inhibition zones is measured and antimicrobial activity of the compounds is expressed in terms of the average diameter of zone of inhibition in millimeters. Those compounds that are unable to exhibit inhibition zone (inhibition zone diameter less than 6 mm) are considered non-active. The compound is tested in triplicate with two independent experiments and the average values of diameters of inhibition zones are considered.

RESULTS AND DISCUSSIONS

Ag-Co HNPs are studied for their antimicrobial activity against two gram positive bacteria, two gram negative bacteria. In case of gram positive bacteria the test organisms were *Staphylococcus aureus* and *Bacillus subtilis.* The nano compound shows significant antibacterial activity against these two bacteria in all the four concentrations studied. HNPs demonstrate high activity against the two selected gram positive bacteria, 22 mm against *S. aureus* and 28 mm against *B. subtilis.*

In case of gram negative bacteria the test organisms were *Pseudomonas aerugisona* and *Escherichia coli.* The HNPs show substantial antibacterial activity against these two bacteria in all the four concentrations studied and demonstrate high activity against the two selected gram positive bacteria, 22 mm against *P. aeruginosa* and 27 mm against *E. coli* at 1mg concentration.

Table 1: Antibacterial activity of nanocompound against gram positive bacteria

S. No	Compound Name	Zone of inhibition (mm)							
		Gram positive *(Staphylococcus aureus)*				Gram positive *(Bacillus subtilis)*			
		1mg	0.75mg	0.5mg	0.25mg	1mg	0.75mg	0.5mg	0.25mg
1	**Ag-Co HNPs**	22	21	18	14	28	26	22	19
2	**Streptomycin**	31				32			

Table 2: Antibacterial activity of nanocompound against gram negative bacteria

S. No	Compound Name	Zone of inhibition (mm)							
		Gram negative *(Pseudomonas aerugisona)*				Gram negative *(Escherichia coli)*			
		1mg	0.75mg	0.5mg	0.25mg	1mg	0.75mg	0.5mg	0.25mg
1	**Ag-Co HNPs**	22	19	13	12	27	25	22	13
2	**Streptomycin**	28				30			

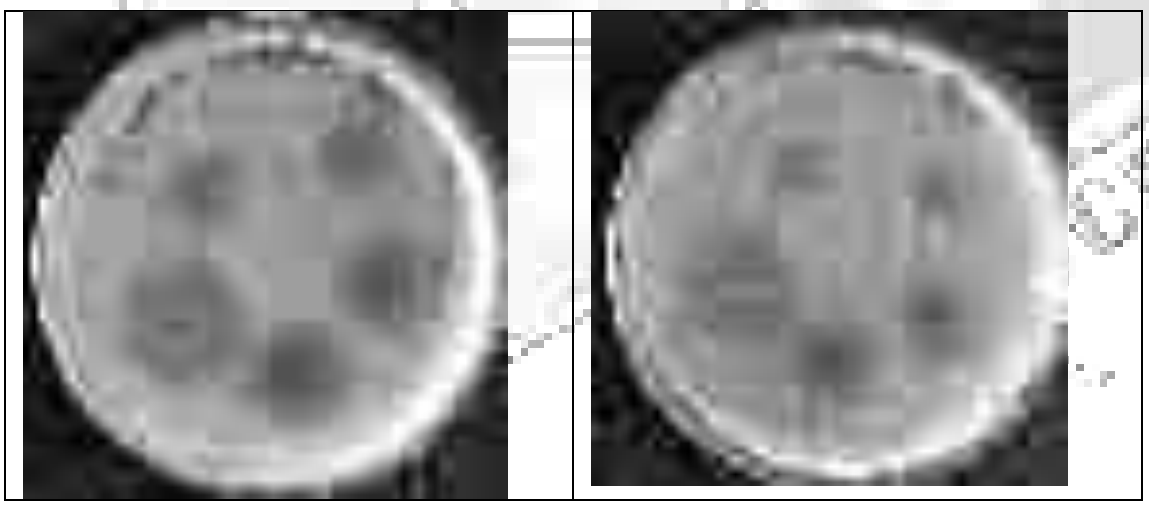

Figure 8: Antibacterial activities of Ag-Co HNPs against Gram-positive bacteria

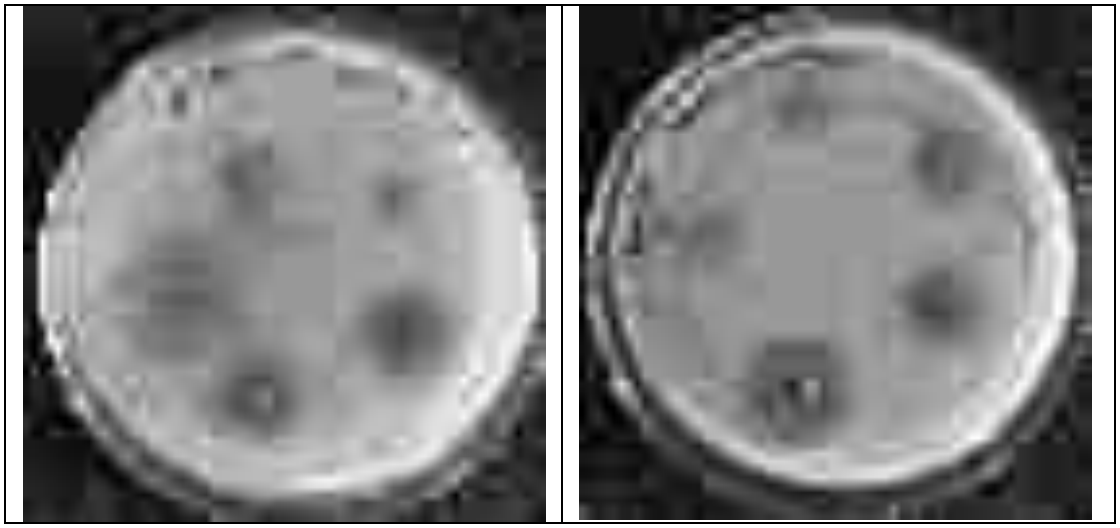

Figure 9: Antibacterial activities of Ag-Co HNPs against Gram-negative bacteria

CONCLUSIONS

An ecologically innocuous method is projected to synthesize Ag-Co bimetallic nanoparticles from *Areva lanata* leaf extract. From UV-Visible analysis it is proved that the particles are in nanoscale as per the positions of the Surface Plasmon Resonance (SPR) bands. FTIR data confirms the presence of secondary metabolites of phyto molecules as the bio reducing and capping agents of the formed nanoparticles. XRD, SEM and HRTEM results evince that Ag-Co HNPs are in spherical morphology and cubic crystalline structure with size between 30-100 nm. The findings of the well diffusion method demonstrate that *Aerva lanata* leaf mediated green synthesized Ag-Co hybrid nanoparticles are found to possess significant antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) respectively. This antimicrobial activity is attributed to the capped plant secondary metabolites that are present on the surface of the hybrid nanoparticles.

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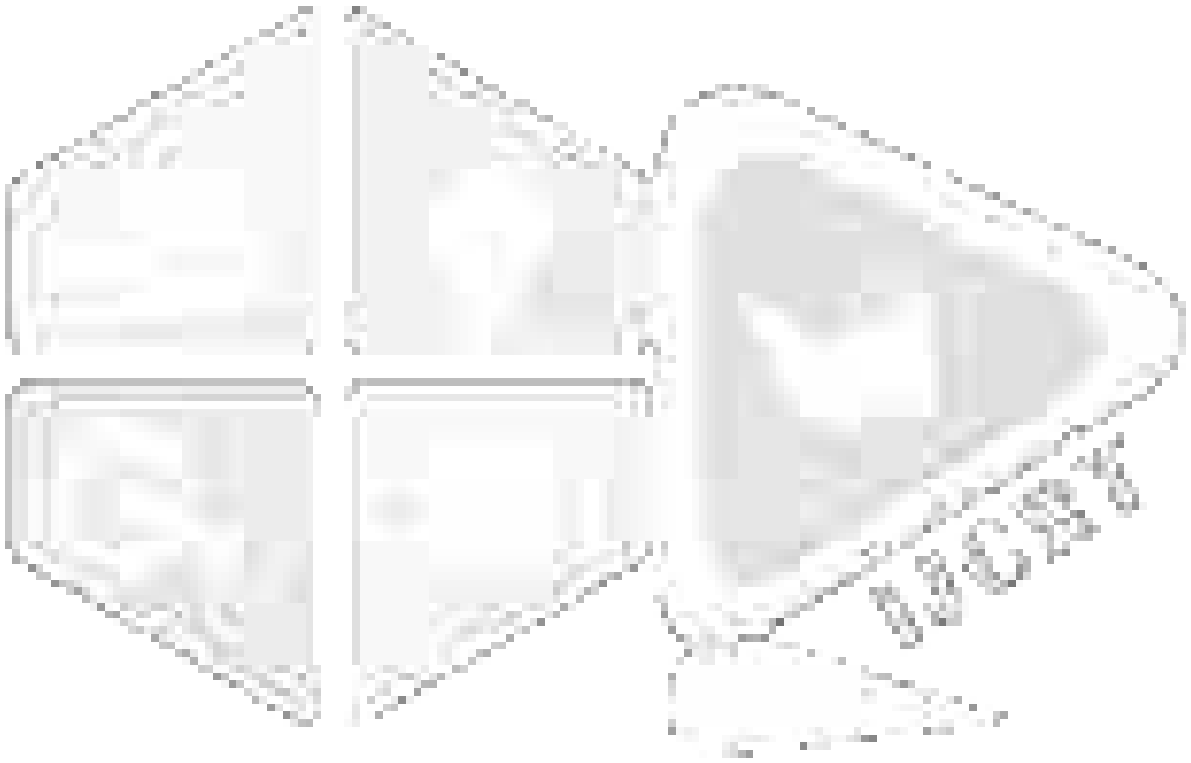

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

ASSESSMENT OF MERCURY TOXICITY ON SEED GERMINATION, SHOOT AND ROOT GROWTH OF Cajanus cajan (L.) MILLSP.

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Abstract:

The present study was carried out to test the phytotoxic effect of mercury on germination of seed and seedling growth of pigeon pea. The seeds of pigeon pea were treated with different concentrations (10, 50, 100, 150, 200, 250 and 300 ppm) of mercuric nitrate (Hg $(NO_3)_2$) solution. The germination percentages were calculated after two days (48 hrs.) and growth parameters like root length, shoot length, fresh weight and dry weight were calculated after seven days. In the present study it was observed that mercury at low concentrations (< 100 ppm) was found to show no significant effect on seed germination. However increasing concentrations of mercury (>100) decreases germination percentages significantly. It was observed that seed germination was completely inhibited at Hg concentrations above 250 ppm. Root length, shoot length and dry weight were found to be decreased with increasing concentrations of Hg $(NO_3)_2$. The inhibitory effect of mercury on shoot and root of seedling was more pronounced at 200 ppm.

Index terms:

Cajanus cajan, Mercuric nitrate Hg $(NO_3)_2$.

Introduction:

Heavy metal contamination caused by either natural processes or by human activities is one of the most serious eco-toxicological problems. Since, photosynthetic plants function as the primary and principal entry point of heavy metals into the food chain leading to animals and ultimately to man. These heavy metals enrich, bios accumulate, and bio magnifies in the food chain and ultimately a significant amount of these heavy metals are found in the animal body. Heavy metal toxicity in plants has been established by various authors exhibited from morphological to molecular levels of organization [1and 2]. Most studied aspect of heavy metal toxicity is the damaging effect on seed germination and seedling growth of different plant species. Several authors reported in different plant species that plants showed reduction in growth and recorded decrease in rates of seed germination when they are exposed to the heavy metals [3, 4, 5, 6, 7 and 8].

Mercury

Of all the heavy metals, mercury is found to have significant environmental concern. Once mercury is introduced to the soil, it lasts for a long time because of its indestructible and non-degradable nature and therefore causes potential risk for ecosystems [9 and10]. Mercury is not essential for any of the biological functions; rather it is toxic to both plants and animals. In living organisms, mercury is thought to interfere with the mode of enzyme action and protein synthesis by binding with the sulfhydryl groups due to its strong affinity for sulphur [11, 12 and 13]. Mercury is known to be the most hazardous heavy metal. Global release of mercury in the atmosphere has been raising three to four folds. However, the anthropogenic origin of mercury at local level is much more. Several researchers have studied the effect of mercury on growth, physiology and metabolism of several plant species including *Vigna ambacensis* [14], *Vigna radiata* [15], *Solanum melongena* [16], *Arabidopsis thaliana* [17], *Sesbania drummondii* [18], *Medicago sativa* [19], *Arachis hypogaea* L. [20], *Vigna unguiculata* [21], *Zea mays* [22], *Vigna radiata* (L.) [23]. Hg inhibits seed germination not only due to unavailability of sugars to embryo by impairing the solubilization of starch but may also be due to damage caused to embryos produced by Hg treatment [24].

MATERIALS AND METHODS

A pulse crop, *Cajanus Cajan*, (L) Millsp. (Pigeon pea) was selected for the present study. Pure line uncontaminated seeds of Pigeon pea were obtained from the seed market of Visakhapatnam. Seed treatment was not done by any seed dressing chemical and by any antifungal chemicals to avoid any interference during the experiments. Healthy seeds were hand shorted and selected for the experiments.

GERMINATION STUDIES:

During this study, 5 test solutions with different concentrations of Hg $(NO_3)_2$ (10, 50, 100, 150, 200, 250 and 300 ppm) were used to investigate the effect of mercury on germination of seed and seedling growth. Deionized water was served as control. The healthy and uniform seeds of Pigeon pea were collected and thoroughly washed with the same test solution. Germination experiments were carried out in sterilized petri dishes lined with a single layer of sterilized filter paper. Always 10 no of seeds and 10 ml treatment test solutions were utilized for single treatment. The seeds were arranged on the petri plates and the toxicant was poured into the petri plates. The seeds were set under different $32 \pm 2/ 25 \pm 2\ ^0$ C day / night temperatures for 7 days. Each test was carried out in three replicates. Seedlings were removed from filter paper with the help of forceps on the 7th day of treatment. The length root and shoot of seedling was measured on the 7th day of seedling with the help of a scale. The germination percentages were recorded after 48 hours and root and shoot length of seedlings were measured after 168 hours (7 days). Plant tissues were oven dried at $80\ ^0$ C for 24 hours to determine the dry weight. In all sets, the seeds were allowed to germinate in normal photo-inductive cycles and the illumination was maintained at 2400 ± 200 Lux, normal humid atmosphere and at a temperature of 28 ± 2^0 C for 48 hours. After 48 hours of normal soaking and incubation, all seedlings were transferred to the growth chamber (Culture rack). To calculate the percentage of germination, periodical observations were made at an interval of 24 hours up to 168 hours. First emergence of coleorhizae (Referred as Root) about 2 mm. in length was considered as germination. Visual screening was carried out based on the growth and the percentage of seed germination and percentage of seedling establishment was also calculated.

STUDY OF SEEDLING GROWTH (PETRI PLATE CULTURE):

In petri plate culture, first the petri plates are cleaned with tap water then with chromic acid and then tap water and finally with double distilled water, and dried in an oven. Ten soaked seeds were sown in petri plates at uniform distance in all the sets. All petri plates were kept at room temperature and normal photo-inductive cycle was maintained by providing light from fluorescent tubes in daytime. For all other studies, seeds were grown up to the seedling establishment stage (up to 7th day of exposure to the toxicant), inside petri plates in different concentrations of the mercury toxicant including control set, as it was done in germination studies. On each 7th day of exposure, seedlings were harvested for respective experimental analysis.

RESULTS AND DISCUSSION

Table-1. Changes in seed germination, seedling establishment, Shoot & Root length, ratio value and percent change value, when compared to control, of 168 h old pigeon pea seedlings, grown in different concentrations of the mercury in petri plate culture. Data calculated from the mean of the samples ± standard deviation.

Concentration of mercury	Percentage of seed germination	Percentage of seedling establishment	Shoot length in (cm)	Root length in (cm)	Shoot Fresh wt. (gm)	Root Fresh wt. (gm)	Shoot Dry wt. (gm)	Root Dry wt. (gm)
Control (0 ppm)	100	100	7.93±0.218	3.38±0.19	1.69±0.0886	0.7148±0.0182	0.171±0.004	0.0372±0.0013
10 ppm	100	100	7.08±0.149	2.84±0.19	0.938±0.005	0.1715±0.0115	0.169±0.004	0.0367±0.0017
50 ppm	100	100	6.18±0.035	2.38±0.16	0.879±0.0056	0.1366±0.0092	0.157±0.004	0.0305±0.0013
100 ppm	100	100	5.27±0.031	1.97±0.13	0.778±0.009	0.0969±0.0064	0.103±0.004	0.0161±0.0018
150 ppm	96	88	3.18±0.092	1.84±0.18	0.403±0.003	0.0859±0.0151	0.049±0.01	0.0104±0.0016
200 ppm	76	68	1.02±0.31	0.94±0.14	0.139±0.18779	0.0161±0.0086	0.029±0.01	0.0013±0.0019
250 ppm	28	0	0	0	0	0	0	0
300 ppm	0	0	0	0	0	0	0	0

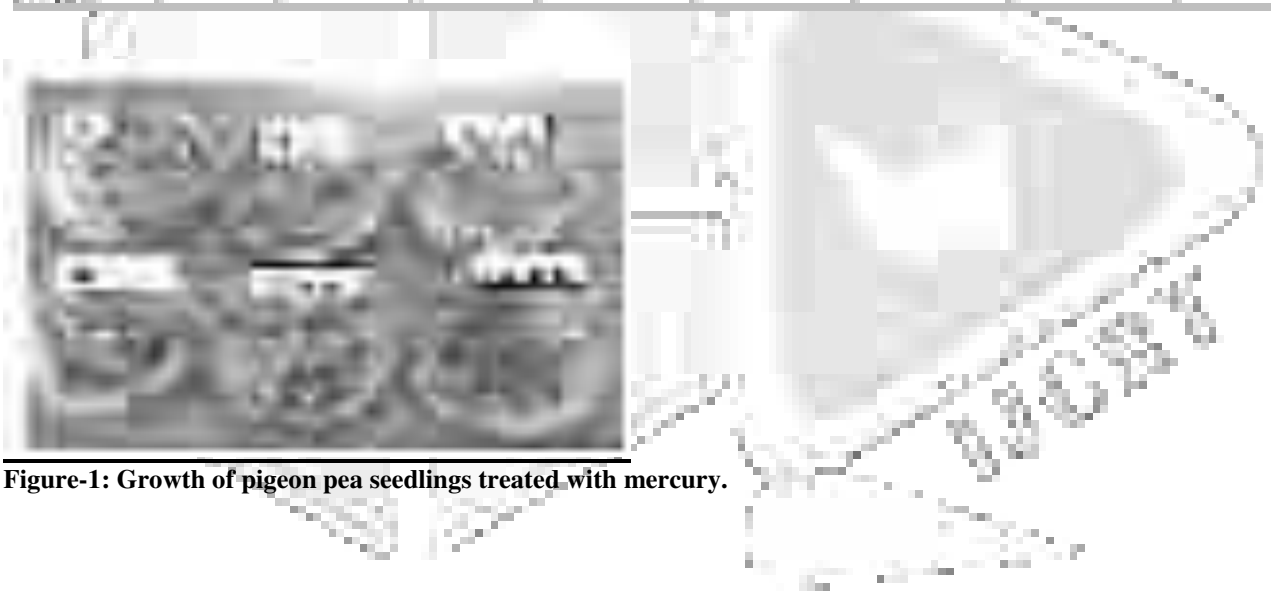

Figure-1: Growth of pigeon pea seedlings treated with mercury.

Figure-2: Growth of pigeon pea seedlings treated with mercury after 7 days.

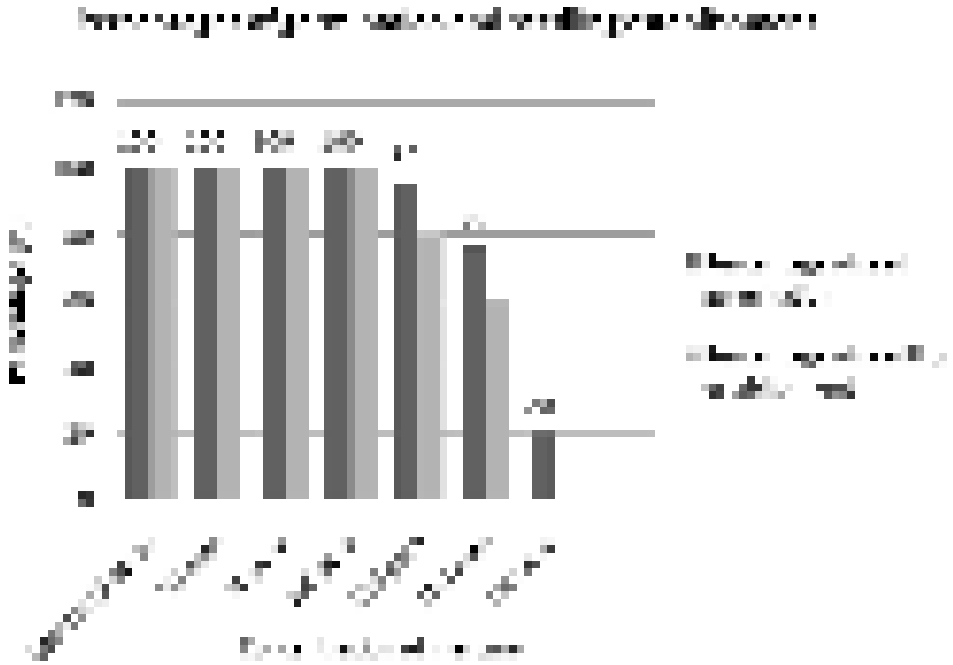

Figure 3: Showing percentage of seed germination and seedling establishment of pigeon pea in control and selected concentrations of mercury.

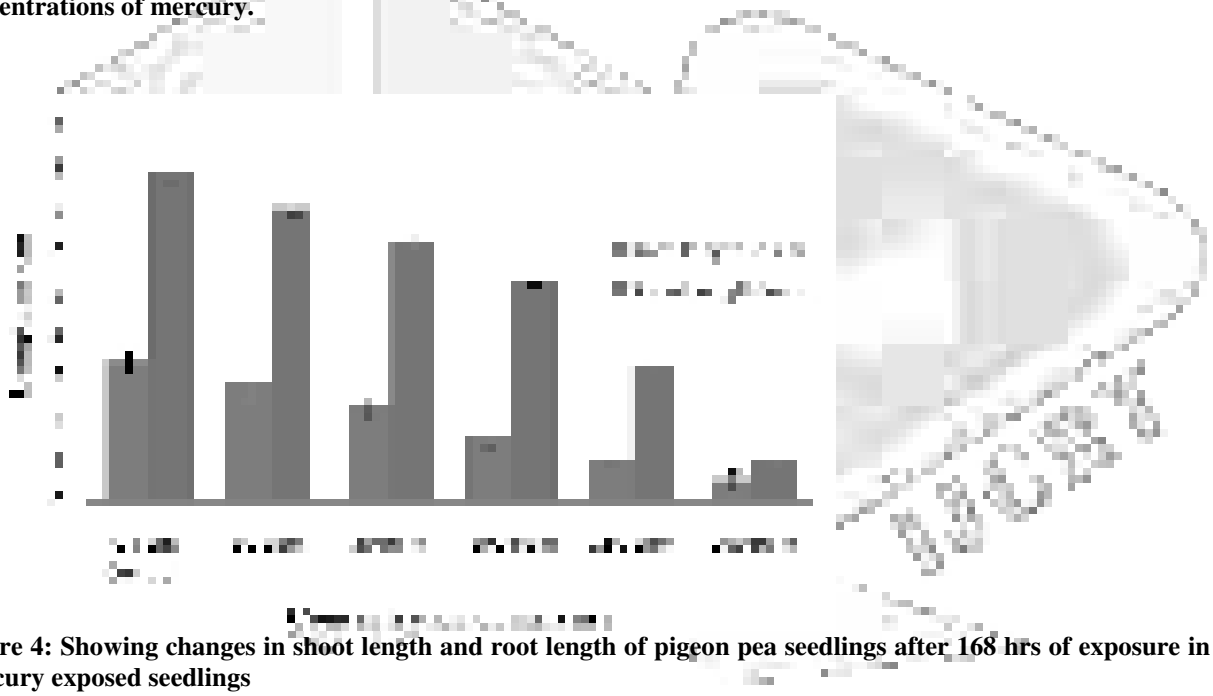

Figure 4: Showing changes in shoot length and root length of pigeon pea seedlings after 168 hrs of exposure in control and mercury exposed seedlings

Figure-5: Percent change in length of root and shoot of pigeon pea seedlings treated with mercury after 7 days.

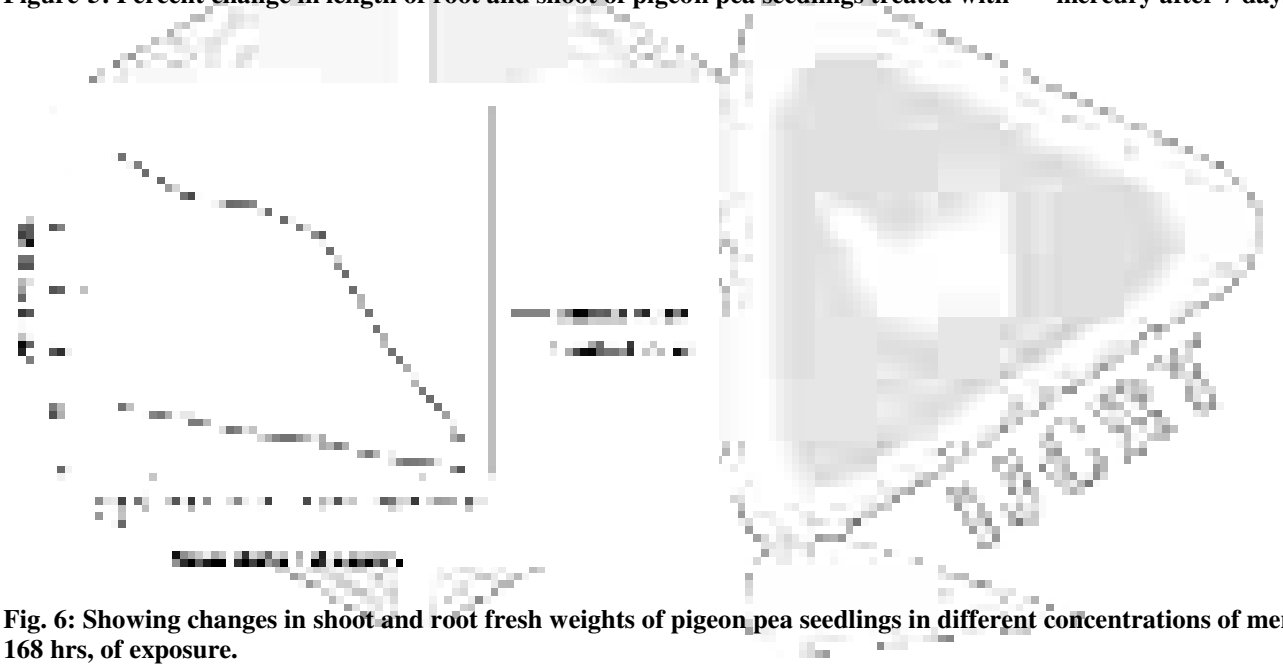

Fig. 6: Showing changes in shoot and root fresh weights of pigeon pea seedlings in different concentrations of mercury after 168 hrs, of exposure.

Fig. 7: Showing changes in shoot and root dry weights of pigeon pea seedlings in different concentrations of mercury after 168 hrs, of exposure.

To conduct experiments in the seed germinator the selected mercury concentrations were: 10 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm. In the control set, 100% seed germination and 100% seedling establishment was observed in petri plate culture. All the seeds were germinated and 100% seedlings established up to 100 ppm mercury concentrations. In the 150 ppm mercury treatment, 100% seeds germinated, out of which only 96% seedlings established. The rest of the germinated seedlings died because of the toxicant. In 150 ppm of mercury concentration, seeds swelled and the seeds started germinating only 96% seeds germinated, the rest could not reach to 2 mm size to be counted as germinated, and only 80% seedlings were established. At this stage, no further germination or prolongation of plumule was marked. In 200 ppm mercury treatment, 76% seeds germinated, but only 60% seedlings established with stunted growth. In the case of 250 ppm of mercury, 20% pigeon pea seeds were germinated but these germinated seeds could not establish altogether (Table.1 and figure 3). No germination was marked beyond 300 ppm of the mercury. Selected healthy seeds were exposed to graded series of concentrations of mercury in sterilized petri plates to find out the lethal concentration and percent survival values for the present set of experiments. Series of pilot tests were conducted and the experiments were repeated at least thrice to determine the lethal concentration values. From the toxicity study, five different concentrations (10, 50, 100, 150, 200 ppm) of the mercury were selected for future experiments. During concentration studies, seeds and seedlings were carefully watched to find out the abnormalities during seed germination and seedling establishment. No significant morphometric change was recorded. Browning of the shoot at the base of the exposed seedlings was marked. The entire root became brown after a few days of exposure (7^{th} day). Control seedlings remained clinically healthy. At very high concentrations of the mercury, the exposed roots became small and less. At higher concentrations of the toxicant, germination was observed but no seedling establishment was marked. Cent percent germination after 168 h of exposure was recorded in the control and at 10, 50, and 100 ppm of mercury concentration. The percentage of seed germination decreased with the increase in mercury concentration. The values obtained in the toxicity testing were significant and indicate the toxic nature of mercury. The values obtained were statistically significant. The effect of mercury on germination was more pronounced at 200 ppm of mercury concentration as only 76% seeds germinated and 60% established. At 250 ppm of mercuric concentration the pigeon pea seeds 20% seeds germinated but these germinated seeds could not establish altogether. No germination was marked beyond 300 ppm of mercuric nitrate (Table-1 and figure 3).

STUDY OF MORPHOLOGICAL PARAMETERS:

A) Shoot length and Root length:

From three replicas 10 seedlings were selected randomly and shoot and root lengths were measured, with the help of a scale. The shoot length of the germinated seedlings showed significant changes in the mercury exposed pigeon pea seeds in petri plate culture. In the control set, the shoot length of the seedling was 7.92 ± 0.29 cm after 168 hrs of germination and the root length was 3.38 ± 0.29 cm in petri plate culture. In case of mercury at 10 ppm the shoot length of the exposed seedling decreased from 7.92 to 7.08 cm showing 10.6% decrease over the control value. In case of 50 ppm of mercury concentration, the shoot length of the exposed seedling further declined from 7.92 to 6.28 cm showing 20.7% decrease when compared to the control value (Table-1). With the increase in mercury concentration, the shoot length decreased significantly to 5.27 cm showing a decrease of -33.45% at 100 ppm mercury concentration (Table-1, Fig. 4 and Fig. 5). When the concentration of mercury increased to 150 ppm, the shoot length decreased significantly from 7.92 to 3.26 cm and recorded a decrease of 58.8%. At 200 ppm mercury concentration, the shoot growth declined significantly and showed 87.2% decrease when compared to control.

The root length of the germinated seedlings showed significant changes in the mercury exposed pigeon pea seeds. In the control set, the root length of the seedling was 3.38 after 168 h of germination. In case of 10 ppm mercury concentration, the root length of the exposed seedling decreased from 3.38 to 2.28 cm showing 15.2% decrease over the control value. In case of 50 ppm of mercury concentration, the root length of the exposed seedling decreased significantly from 3.38 to 2.28 cm showing 32.5% decrease when compared to the control value (Table-1; Fig. 4 and Fig. 5). With the increase in the mercury concentration, the root length further declined to 1.57 cm at 100 ppm concentration (Table-1.Fig. 4 and Fig. 5) showing 53.5% decrease when compared to root length value of control. When the seeds are treated with high concentration of mercury (150 ppm and 200 ppm), the root length decreased significantly from 3.38 to 1.05 and 0.54 showing 68.93% and 84.02% decrease in root length respectively.

B) Shoot weight and Root weight:

Ten seedlings from three replicates were taken and the shoots were separated from the roots. These were washed thoroughly with distilled water, surface dried by means of blotting paper. Then fresh weights of roots and shoots were taken separately by a single pan electronic balance (Dhona make).

After weighing the fresh shoots and roots, they were kept in an oven for 48 hours at a temperature of 80^0C and their dry weights were recorded at 24 hrs. of interval till we got a constant weight of the samples.

The shoot fresh weight of the effluent exposed pigeon pea seedlings decreased from 1.05 to 0.906, 0.878 and 0.778 mg at 10 ppm, 50 ppm, and 100 ppm of mercury concentration, showed 14%, 16% and 26% reduction over the control value. Whereas, at higher concentration of mercury at 150 ppm, the shoot weight decreased significantly from 1.05 to 0.403 mg showed a decrease by 61.6% and at 200 ppm mercury concentration, the fresh weight of shoot decreased from 1.05 to 0.129 mg that showed 87.7% decrease over the control value (Table.1and Fig. 6).

The root fresh weight of the mercury exposed pigeon pea seedlings decreased from 0.248 to 0.1715, 0.1204, 0.0969, 0.0405 and 0.0161 mg at respective concentrations of 10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm of mercury, where a decrease by 20.15%, 43.9%, 54.8%, 81.1% and 92.5% were recorded over the control value (Table-1and Fig. 6).

The shoot dry weight of the mercury exposed pigeon pea seedlings decreased from 0.171 to 0.149 0.005, 0.132, 0.103, 0.065 and 0.025 mg that showed 12.8%, 22.8%, 39.7%, 61.9% and 85% decrease in shoot dry weight at respective concentrations ranging from 10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm when compared to the control value (Table.1and Fig.7).

The root dry weight of the mercury exposed pigeon pea seedlings decreased from 0.0372 to 0.026, 0.0205, 0.0144, 0.0104, 0.0023 mg at concentrations ranging from 10 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm of mercury showed 28.2%, 44.8%, 61.2%, 72.04% and 93.8% indicating drastic decrease when compared to the control value (Table.1and Fig.7).

In the present investigation, the effect of mercury on seed and seed biology during germination and seedling establishment and growth parameters of seedling were studied in mercury treated seedlings. All the parameters of the exposed pigeon pea seedlings were found to be less than the control values. The effect is increasing with increasing concentrations of mercury. Similar observations by previous findings of decrease in germination percentages by higher concentrations of mercury metal reported by in mung bean [25]. The reduction in germination percentages might be attributed to the toxic effect of heavy metals on the activity of enzymes such as amylase, protease and ribonuclease [26] and mobilization of food reserves [26]. The mercury toxicity causes water deficit the seedling that leads to inhibition of root and shoot growth [27]. The decrease in root and shoot length of the treated seedlings at higher concentration was in agreement with the previous findings of [28 and 29]. It was observed from the data that the root was more affected than the shoot. The reduction of growth of shoot and root may be attributed to the change of properties of plasma membranes and cell walls by replacement of cations by mercury metal ions [11]. It has been proved time and again that heavy metals hamper normal functioning of plants and acts as a barrier to metabolic processes in several ways such as the bonding of HMs with sulfhydryl groups of proteins results in the disruption of protein structure [30] and affect the functional groups of various cellular molecules such as pigments or enzymes [31]. Metal toxicity also disrupts the integrity of membranes [32]. All such events lead to the repression of enzyme mediated vital metabolic events such as photosynthesis and respiration.

CONCLUSION:

The results obtained in this investigation show that mercury at higher concentrations show adverse effects on seed germination and seedling growth. Moreover the release of mercury into the immediate environment may enter the cereals, pulses and vegetables we eat and affect the health of human beings. Hence it is an urgent necessity to minimize the use of mercury in industries and mercury containing pesticides and fungicides.

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Growth and Carbon metabolism assessment in finger millet (*Eleusine coracana* L. Gaertn) seedlings undergone PGPR treatment subjected to NaCl stress.

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Abstract
Salinity stress adversely effects the plant growth and productivity. To study the effect of salinity and to investigate the influence of PGPR (Plant Growth Promoting Rhizobacteria) *Acinetobacter calcoaceticus* on finger millet cultivars, we have grown two finger millet cultivars, VR-988 and VR-1076 in three sets of pots. First set of pots are controls (0 NaCl and no PGPR treatment), second set contained NaCl stressed seedlings and the third set consisted of PGPR treated seedlings subjected to NaCl stress. All the three sets of pots included cultivar VR-988 and VR-1076 maintained separately. After 48 hours seed germination was recorded. Root, shoot growth and dry weights were observed after 5th day. Chlorophyll-a, Chlorophyll-b, total Chlorophyll, total carbohydrate, starch content, reducing and non-reducing sugars, total soluble sugars (TSS) and α-amylase activity were studied on 4th, 6th and 8th day for all treatments in VR-988 and VR-1076.

Keywords: α-amylase, Chlorophyll, Plant Growth Promoting Rhizobacteria, Salinity stress.

Introduction
Plants are exposed to various environmental conditions. Plants which develop mechanisms to tolerate the adverse climatic conditions will succeed in the nature. Most of the crop plants are sensitive to the fluctuations in the environment. Majority of the crops show reduced growth and yields under abiotic stresses (Mahajan and Tuteja, 2005). Global population is raising and the food requirements of the growing population are not satisfied because of the limited availability of fertile land for cultivation due to pollution, urbanization and industrialization. This is the challenge facing the globe. One such crop that ensures food security is finger millet (*Eleusine coracana* L. Gaertn), a millet crop widely grown in arid and semi-arid areas of Asia and Africa. Nutrient deficiencies, drought and salinity are the major abiotic stresses which are severely effecting the crop growth and production (Maharajan *et al*., 2018). To attain better yields crop must first withstand and tolerate the adverse conditions like
salinity. Microorganisms growing in saline environments might serve as source to make the plants halotolerant. Scientists are focusing on this area as it is an eco-friendly approach. There were several works in various plants using PGPR, they provided evidence that these halotolerant PGPR promoted growth under saline conditions (Ramadoss *et al*., 2013; Kang *et al*., 2014). Early stages of the life cycle such as seed germination and seedling growth are very much sensitive to stresses like salinity. Hence the seedling stages were selected for most of the research works. If these stages acquire tolerance it might be advantageous for the plant to tolerate in the later developmental stages. The main objective of this work is to study the seedling growth and carbon metabolism of finger millet salt sensitive (VR-988) and salt tolerant (VR-1076) seedlings under controlled, salt stressed and bacterial (PGPR) treated under salt stress condition.

Materials and Methods
We have obtained the seed material from Agriculture Research Station, Vizianagaram, Andhra Pradesh, India. The experiment was conducted in the Physiology laboratory of

Botany department, Andhra University, Visakhapatnam. Salt tolerance assay was conducted for twelve finger millet cultivars. From the 12 cultivars two cultivars VR-988 and VR-1076 were identified as salt sensitive and salt tolerant cultivars respectively based on their performance (seed germination, seedling root length, seedling shoot length and dry weight) under NaCl stress.

Total six bacterial isolates were obtained from the soil samples. Among six isolates MGST-02 showed growth up to 8% NaCl concentration. This isolate was further identified as *Acinetobacter calcoaceticus* based on 16S rRNA sequencing and phylogenetic analysis.

Plant growth promoting assay of selected finger millet cultivars using the halotolerant PGPR *Acinetobacter calcoaceticus*

Healthy, uniform sized seeds of finger millet cultivars VR-988 and VR-1076 (salt sensitive and salt tolerant respectively) were selected then sterilized with hypo chlorite solution and washed with distilled water thrice. For evaluation of germination the seeds were grown in Petri dish containing Whatman No.1 filter paper. To study the growth parameters the seeds were grown in pots of diameter 10 cm. 20 seeds were taken in each pot.

C: control - 5 day old seedlings maintained at room temperature 28 ± 2^0 C and 60-65% relative humidity throughout the experiment were treated as control seedlings.

S: salt stressed -5 day old seedlings grown in pots supplemented with 1500 ppm NaCl solution were considered as salt stressed seedlings.

S+B: bacterial treated under salt stress- 5 day old seedlings treated with *Acinetobacter calcoaceticus* bacterium grown in pots supplemented with 1500 ppm NaCl solution.

Chlorophyll estimation

Total chlorophyll contents (Chlorophyll-a, Chlorophyll-b and total Chlorophyll) were estimated using the method of Arnon (1949). The amount of Chlorophyll-a, Chlorophyll-b and total Chlorophyll were measured as milligrams of Chlorophyll content per gram of plant tissue.

Total Carbohydrate estimation

100 mg of the sample was weighed and taken into a boiling tube. Hydrolysed by keeping it in a boiling water bath for three hours with 5 mL of 2.5 N HCl and cooled to room temperature. It was neutralized with sodium carbonate made up to 100 mL volume and centrifuged. The supernatant was collected and 0.5 and 1 mL aliquots were taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard. '0' served as blank. The volume was made upto 1 mL in all the tubes including the sample tubes by adding distilled water. Then 4 mL of anthrone reagent was added. It was heated for eight minutes in a boiling water bath. Rapidly cooled and read the green to dark green colour at 630 nm.

Starch and Total Soluble Sugars (TSS) estimation

The starch and total soluble sugars were estimated according to the method of Mc Cready *et al*., (1950) as modified by Clegg (1956). Soluble sugars were separated by alcohol extraction and the residue containing starch was brought into solution with perchloric acid.

Estimation of Reducing Sugars

Total reducing sugars were estimated according to the phenol-sulfuric acid method of Dubois *et al*., (1956) as followed by Smyth and Dugger (1980).

Estimation of Non-Reducing Sugars

The reducing sugar content subtracted from the total soluble sugar content was considered as non- reducing sugars.

Estimation of α -amylase activity

Amylase activity was estimated by the method of Filner and Varner (1967) as followed by Kapoor and Sachar (1979).

The readings were taken on 4^{th}, 6^{th} and 8^{th} day.

Statistical analysis

The results obtained in our studies were subjected to statistical analysis on SPSS statistical tool, Standard Errors were calculated in order to signify the results statistically.

Results

Table- 1: showing seed germination and seedling growth of selected finger millet cultivars under various conditions.

Cultivar	Treatment	Percent germination	Root length (cm)	Shoot length (cm)	Dry weight (mg)
VR-988	C	98.5 ± 0.05[a]	3.6 ± 0.33[b]	4.8 ± 0.45 [a]	22.5 ± 0.17 [a]
VR-988	S	56.1 ± 0.28[b]	3.1 ± 0.35[c]	2.4 ± 0.03[b]	13.1 ± 0.09[b]
VR-988	B+S	89.0 ± 0.09 [a]	4.3 ± 0.29 [a]	4.3 ± 0.03 [a]	21.4 ± 0.05 [a]
VR-1076	C	100 ± 0.00[a]	3.9 ± 0.35 [ab]	4.5 ± 0.07 [a]	23.9 ± 0.99[b]
VR-1076	S	65.2 ± 0.43[b]	3.4 ± 0.42[b]	2.9 ± 0.13[b]	16.4 ± 0.05[c]
VR-1076	B+S	93.6 ± 0.84[a]	4.8 ± 0.09 [a]	4.1 ± 0.19 [a]	25.2 ± 0.34 [a]

Note: Different letter indicates the significant difference between different treatments ($p \leq 0.01$). All the results were the average of three replicates. Standard Errors were calculated for the data for significance.

C- Control
S-Salt stressed
B+S- Bacterial treated under salt stress

Table -2: showing the effect of NaCl and the influence of *Acinetobacter calcoaceticus* on chlorophyll content and carbohydrates in finger millet cultivars VR-988 and VR-1076.

Cultivar	Treatment	Day	Chl-a mg/L	Chl-b mg/L	Total Chl mg/L	Total Carbohydrate
VR-988	C	4^{th}	36.12 ±0.40 [a]	45.18±0.11 [a]	81.30±0.31 [a]	4.88±0.05[b]
		6^{th}	36.58±0.18 [a]	48.52±0.31 [a]	85.10±0.11 [a]	5.16±0.01[b]
		8^{th}	37.00±0.21 [a]	50.11±0.33 [a]	87.12±0.26 [a]	5.28±0.03[b]

VR-988	S	4th	22.30±0.33c	21.13±0.19 a	43.44±0.29c	6.87±0.02 a
		6th	23.37±0.29c	23.3 ± 0.17c	46.74±0.83c	6.99±0.05 a
		8th	23.80±0.27c	23.87±0.19c	47.67±0.39c	7.07±0.05 a
VR-988	B+S	4th	33.51±0.46b	38.47±0.05b	71.98±0.28b	4.91±0.01b
		6th	34.12±0.26b	41.54±0.28b	75.66±0.28b	5.39±0.03b
		8th	35.73±0.33b	42.25±0.54b	77.99±0.93b	5.52±0.08b
VR-1076	C	4th	35.58±0.13b	46.6±0.29b	82.28±0.54b	4.70±0.03c
		6th	36.30±0.40b	51.90±0.09b	88.21±0.98b	4.84±0.03c
		8th	37.13±0.11b	53.13±0.28b	90.27±0.84b	4.95±0.02c
VR-1076	S	4th	32.10±0.35c	40.09±0.33c	72.19±0.33c	5.95±0.08 a
		6th	33.38±0.15c	42.69±0.33c	76.07±0.35c	6.03±0.02 a
		8th	34.37±0.26c	45.61±0.85c	79.98±0.34c	6.14±0.01 a
VR-1076	B+S	4th	36.89±0.11 a	51.03±0.08 a	87.93±0.35 a	5.06±0.11b
		6th	38.71±0.19 a	52.11±0.13 a	76.07±0.35c	5.11±0.03b
		8th	38.37±0.19 a	57.06±0.27 a	95.43±0.54 a	5.51±0.03b

Note: Different letter indicates the significant difference between different treatments ($p \leq 0.01$). All the results were the average of three replicates. Standard Errors were calculated for the data for significance.

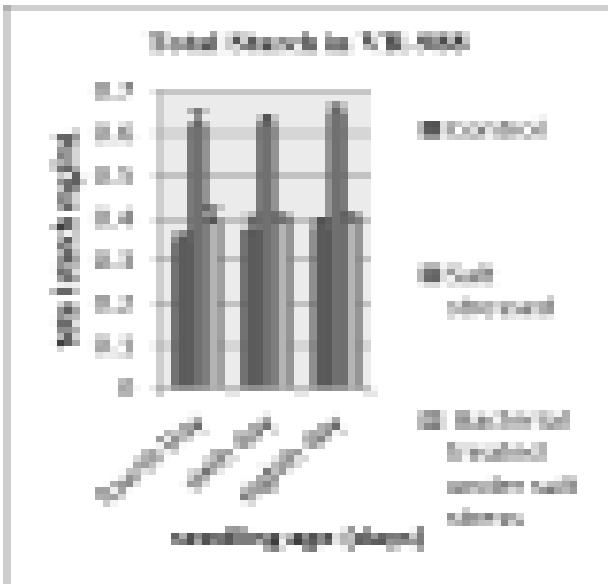

Fig. 1: Total starch content in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

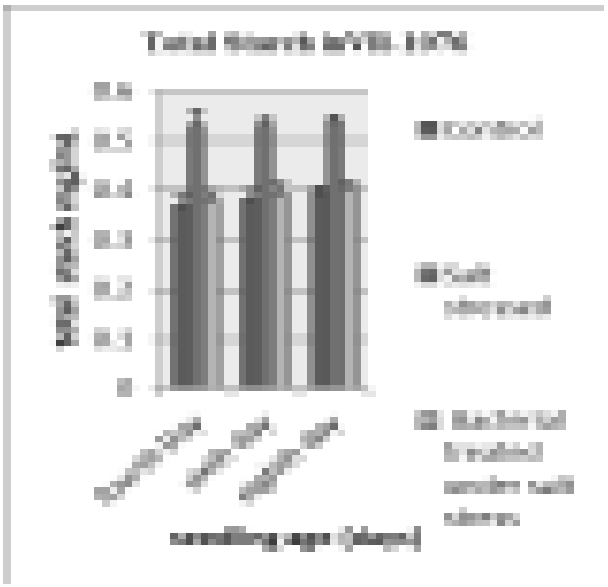

Fig. 2: Total starch content in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

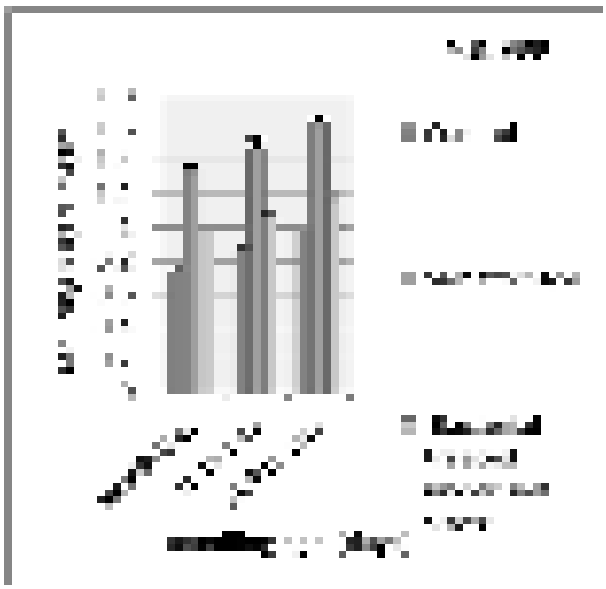

Fig. 3: Reducing sugars content in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

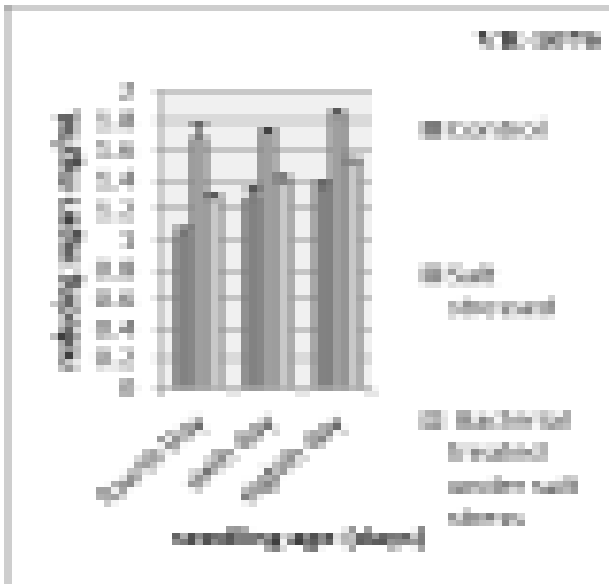

Fig. 4: Reducing sugar content in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

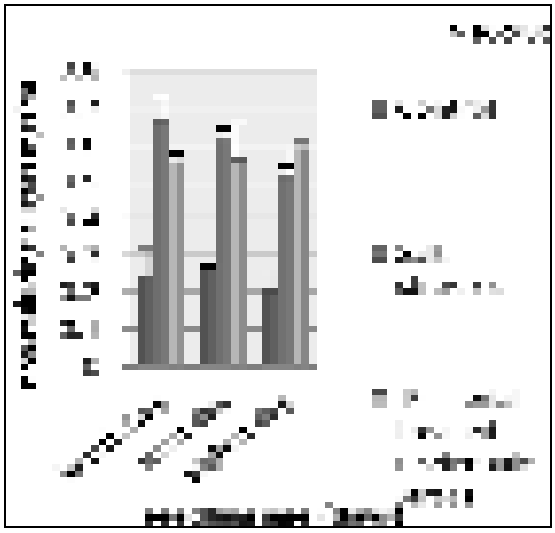

Fig. 5: Non-reducing sugars content in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

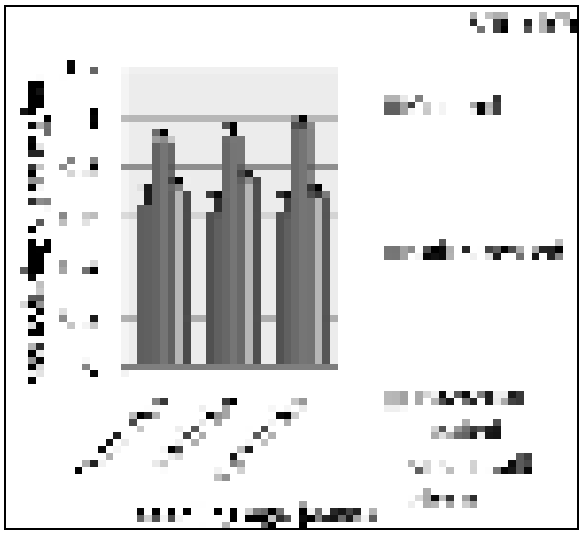

Fig. 6: Non-reducing sugar content in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

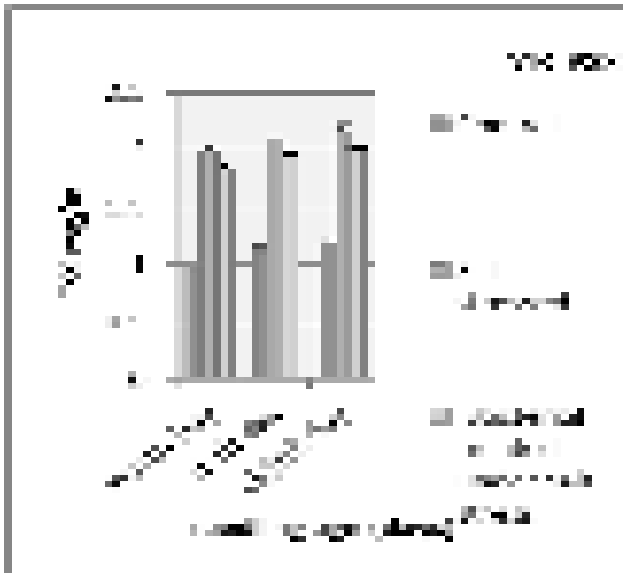

Fig. 7: Total soluble sugars in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

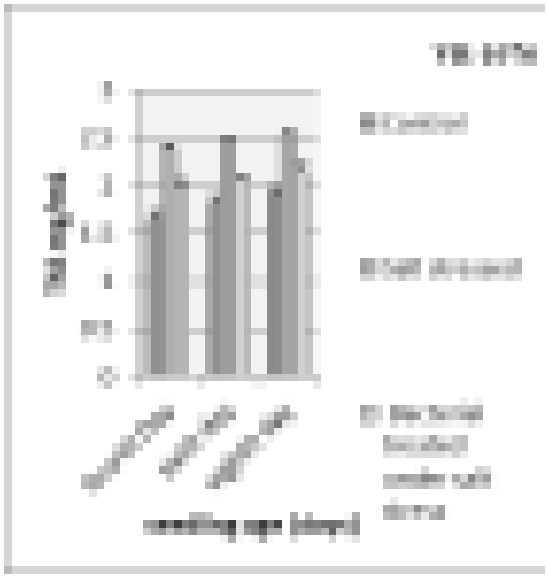

Fig. 8: Total soluble sugars in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

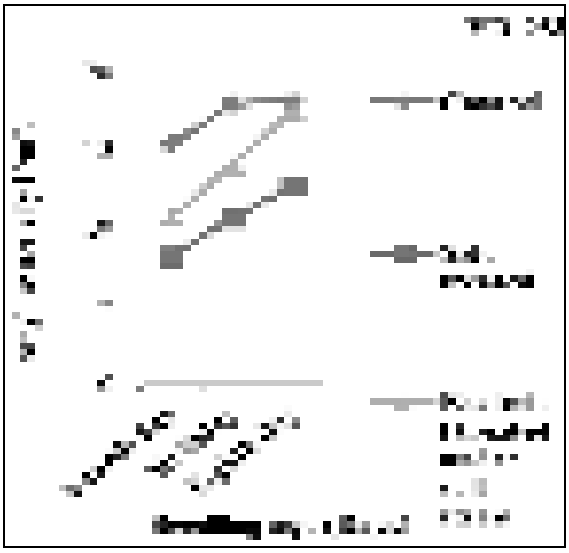

Fig. 9: Amylase activity in VR-988 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

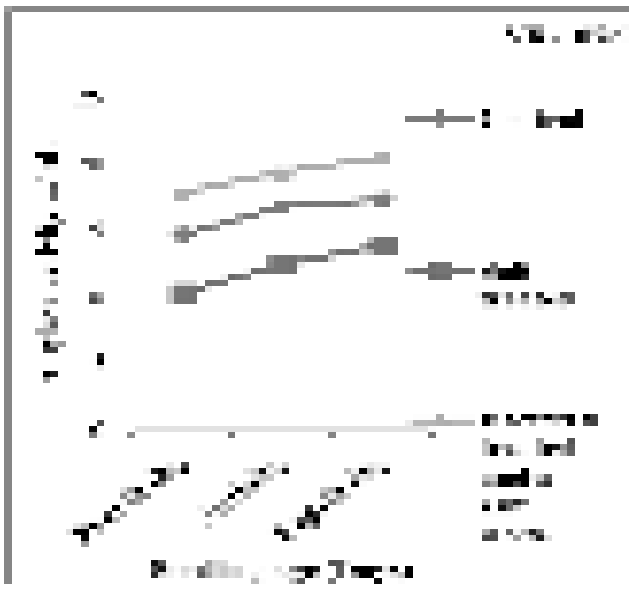

Fig. 10: Amylase activity in VR-1076 seedlings undergone NaCl & PGPR (*Acinetobacter calcoaceticus*) treatment.

Discussion:

Seed germination and seedling growth:

Both the selected finger millet cultivars (VR-988 and VR-1076) showed reduced seed germination, root and shoot growth and dry weight under NaCl stress when compared to their respective control seeds. The seeds undergone bacterial treatment under NaCl stress improved their growth as compared to the seeds grown under NaCl stress. The effect of salt and the influence of the bacterium *Acinetobacter calcoaceticus* was more on salt sensitive finger millet cultivar, VR-988 than salt tolerant cultivar VR-1076.

Our results were in accordance with Kang *et al*., 2014, who worked in cucumber under saline soils using *Acinetobacter calcoaceticus.* Species belonging to the genus *Acinetobacter* were isolated from the rhizosphere of *Pennisetum glaucum* (pearl millet) were studied for their growth promoting activity and were observed that the application of *Acinetobacter* improved the shoot height, root length and dry weight (Rokhbakhsh-zamin *et al*., 2011). Bacteria isolated from natural saline habitats were reported to have the property of enhancing seed germination, seedling growth and dry weight in wheat was studied by Ramadoss *et al*., (2013).

Chlorophyll content:

Irrespective of treatment chlorophyll contents were increased from fourth day to eighth day in VR-988 and VR-1076. Salt stressed seedlings had lower chlorophyll contents than control and bacterial treated seedlings under salt stress. Control seedlings maintained relatively higher chlorophyll contents than salt stressed and seedlings undergone bacterial treatment under salt stress. Chlorophyll contents might be destroyed due to excessive NaCl. Han and Lee, (2005) reported that under NaCl stress lettuce plants inoculated with PGPR showed improved chlorophyll contents. Enhanced chlorophyll content under stress in presence of PGPR was also reported by Nadeem *et al*., (2006).

Carbohydrate, starch and TSS content:

Total carbohydrates, starch, reducing and non-reducing sugars and total soluble sugars (TSS) were greatly enhanced in salt stressed VR-988 and VR-1076. *A. calcoaceticus*
treatment in saline condition resulted in reduction of carbohydrates, starch and sugar contents in both the cultivars but the reduction was more in salt sensitive (VR-988) than salt tolerant (VR-1076) cultivar.

Studies conducted on rice (Amirjani, 2010) revealed that accumulation of carbohydrates was an effective mechanism to tolerate stress. Sugar accumulation under saline conditions provides defence against stress and it shows the extent of salt tolerance of plants (Bohnert and Jensen, 1996). Enhanced sugar accumulation was found during stress in groundnut (Shukla *et al*., 2012). Accumulation of starch after salt treatment was also observed in rice cultivars as reported by Pattanagul and Thitisaksakul (2008). One possible reason to accumulate starch is that the components required for synthesis of starch are simple sugars which are produced by the hydrolysis of sucrose due to the enzymatic activity of alkaline invertase. Under saline condition the enzymatic activity increased in turn leading to the accumulation of starch.

Basic levels of TSS were more in salt tolerant cultivar than salt sensitive cultivar under ideal (control) conditions. Salinity increased the reducing, non-reducing and total soluble sugars (TSS) in both selected cultivars. This demonstrates the role of sugars in providing protection against salinity. *A. calcoaceticus* treatment in saline condition resulted in lowering the sugar contents in VR-988 and VR-1076 than salt stressed seedlings but higher than in control seedlings of both cultivars. Based on this observation it was concluded that *A. calcoaceticus* applied seedlings did not register much stress and accordingly lower sugars were accumulated in bacterial treated seedlings under salt stress in VR-988 and VR-1076. Similar results were found in oat where plants produced high amounts of sugars under stress, salt stressed seedlings inoculated with *Klebsiella* produced fewer sugars than seedlings under stress but have higher levels than controls (Sapre *et al*., 2018).

α -amylase activity

As per the results α-amylase activity reduced in salt stressed seedlings of finger millet cultivars VR-988 and VR-1076 when compared to their control seedlings. Bacterial treated seedlings under salinity stress have showed improved activities of the enzyme.

The reduction in α-amylase activity may be due to NaCl toxicity or due to the less imbibition by the seeds because water as a solvent is necessary to stimulate the enzyme activity. The studies on various plants suggested that salinity has decreased the seed germination by reducing the α-amylase activity in the seeds during germination (Singh *et al*., 2001 in lentil). Amylase activity was observed to be more in salt tolerant cultivars under salt stress when compared to salt sensitive cultivars of rice (Govindaraju and Balakrishnan, 2002).

Conclusion

In our studies conducted in finger millet cultivars VR-988 and VR-1076 we observed that seed germination and seedling growth were reduced under NaCl stress where as the treatment with PGPR *Acinetobacter calcoaceticus* resulted in improved seed germination and seedling growth. Chlorophyll contents and enzymatic activity of α-amylase decreased when subjected to NaCl stress. PGPR treated seedlings under salt stress produced high quantities of chlorophyll when compared to seedlings subjected to NaCl stress without PGPR treatment and also showed improved α-amylase activity as compared to NaCl stressed seedlings. This result was similar to both VR-988 and VR-1076 but the improvement was more in VR-988 which was a salt sensitive cultivar.

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IN -VITRO CYTOTOXICITY OF BIOGENIC BIMETALLIC NANOMATERIALS AGAINST HUMAN BREAST CANCER CELLS

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Abstract

Now- a- days, green synthesis of metallic nanoparticles is an emergent area of research because of their potential therapeutic applications for various diseases. In the present work, we synthesized bimetallic silver cobalt nanomaterials (BMNMs) from silver nitrate ($AgNO_3$) and cobalt nitrate (Co $(NO_3)_2$) precursor solutions using aqueous leaf extract of **Aerva lanata** as bioreducing, stabilizing and capping agent. The synthesized BMNMs were characterized using UV–Vis spectroscopy, FTIR, SEM, EDX, XRD and TEM analyses. The cytotoxic response was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. These biogenic Silver cobalt BMNMs were found to be significantly toxic to MCF-7 cells (human breast cancer cells) via induction of apoptosis.

Keywords: Bimetallic Nanomaterials (BMNMs), Aerva lanata(AL), Cytotoxicity.

1. Introduction

Breast cancer is the most common malignancy in mankind that causes major mortality worldwide. Over the past decenniums, treatments to this notable life-threatening cancer has become more challenging owing to the prevalence of multiple drug resistance, detrimental side effects and the lack of innovative approaches [1]. Nanomedicine is a hopeful and exciting field that could potentially lead to improvements in cancer treatment procedures, offering a modern perspective on tumour identification, prevention and bioremediation. [2]. The successful application of nanoparticles as an anticancer drug is due to their exclusive qualities like large surface area for volume, porosity, solubility, increased bioavailability and different structural properties. Ultimately this can improve the stability and durability of the drugs, moreover it will offer many biomedical perceptions for clinical level applications [3]. Another interesting feature of nanoparticles, it can easily cross the cellular barriers and strongly interact with functional biomolecules [4]. Silver nanoparticles are among the most common and applicable nanostructures, according to their distinctive catalytic, therapeutic activities and stability as well as development of nanodevices and therapeutic preparation for diagnoses and treatment of cancer [5]. The treatment of a variety of cancers with silver NPs has been well documented [6]. Antitumor potentiality (cytotoxicity) of the silver NPs is expressed through oxidative stress as well as inflammation through production of reactive oxygen species that lead to DNA damage and mitochondrial membrane potential disorder, releasing cytochrome c and resulting in mitochondrial related apoptosis and necrosis to cell proliferation and carcinogenesis [7].

Aerva lanata is a medicinal plant that belongs to the family **Amaranthaceae**. It is wealthy source of secondary metabolites that have antibacterial [8], antifungal [9], antioxidant [10], cytotoxic [11], anti-HIV [12], anti tumour[13], anti-diabetic[14,15] and anticancer[16] activities. Moreover, the green synthesized nanoparticles frare found to be more potent antitumour agents than the uncoated nanoparticles as the former are capped with bioactive phytomolecules [17].

2. Experimental

2.1. Preparation of *Aerva lanata* leaf extract: 100g of fresh *Aerva lanata* leaves were taken and cleaned with running tap water to remove dust on surface of leaves followed by deionised water to eliminate other contaminants from leaves and dried up under shade for ten days. The dried leaves were powdered by using home blender. Now 200 mL deionised water was taken in 500 mL beaker to this 10g leaf powder was added. The contents in the beaker heated for 30 minutes at 50^oC with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled leaf broth was filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4^oC. This was taken as leaf extract (figure 1(a)) for the experimental studies.

2.2. Synthesis of Ag-Co bimetallic nanoparticles: Equimolar (25 mM) concentrations of silver nitrate and cobalt nitrate aqueous solutions were prepared separately in 100 ml volumetric flask by dissolving 0.4246 g, 0.7275 g weight of $AgNO_3$ and $Co(NO_3)_2$ in water respectively. Synthesis of Ag-Co BMNMs was done by taking 100mL of $AgNO_3$ solution in a 500 mL beaker, to this 90 mL of leaf extract was added drop wise through burette. 100mL of $Co(NO_3)_2$ solution was added to contents in the beaker by drop wise. During addition process beaker was placed on a magnetic stirrer for continuous agitation. Now this mixture was stirred at 50^oC for 40 minutes at pH 7 on magnetic stirrer. These synthesized BMNMs were separated out by doing centrifugation at 5000 rpm for 30 minutes. The obtained BMNMs were washed with using deionised water two times to remove unwanted constituents. The resultant BMNMs particles were collected (figure: 1(b)) and used for characterization.

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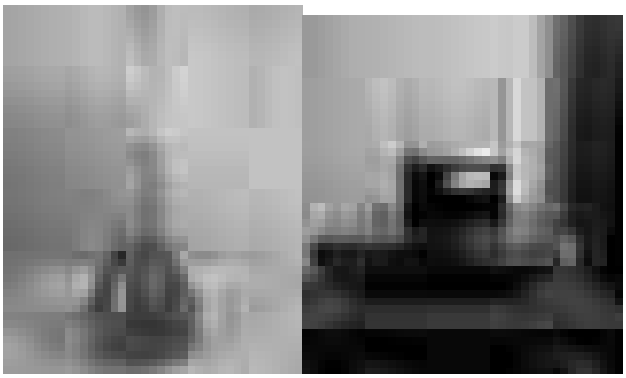


Fig 1(a):Aerva lanata leaf extract Fig 1(b) : Ag-Co BMNMS

2.3 Characterization: Formation of Ag-Co BMNMs was confirmed by UV-Visible absorption spectra using UV-2450 SHIMADZU double beam spectrophotometer, FTIR using Bruker, FESEM, EDX studies are done by using Hitachi S-3700N machine and the morphology of BMNMs was elucidated by HRTEM analysis with FEI Technai machine.

UV-Visible absorption spectrum of Ag-Co BMNMs is revealed in Fig 2(a). The characteristic surface plasmon resonance (SPR) band at around 441 nm is observed in Ag-Co BMNMs which confirms the nano size of the synthesized particles [18]. FTIR spectra of Ag-Co BMNMs (fig 2(b))clearly indicates the existence of the plant extract phytomolecules such as polyphenols , terpenes, amides, carbohydrates, amines etc. on the surface of the Ag-Co BMNMs. The FTIR spectrum of Ag-Co BMNMs exhibits major peak positions at 3215 cm^{-1}, 3411 cm^{-1} and 3507 cm^{-1}which indicate N-H stretching vibrations of amines and O-H stretching of hydroxyl groups of alcohols, phenols. Small peak at 2926 cm^{-1} is due to C-H stretching of alkyl groups. Intense peak at 1640 $cm^{-1}$is due to C=O stretching of amide group [19, 20].

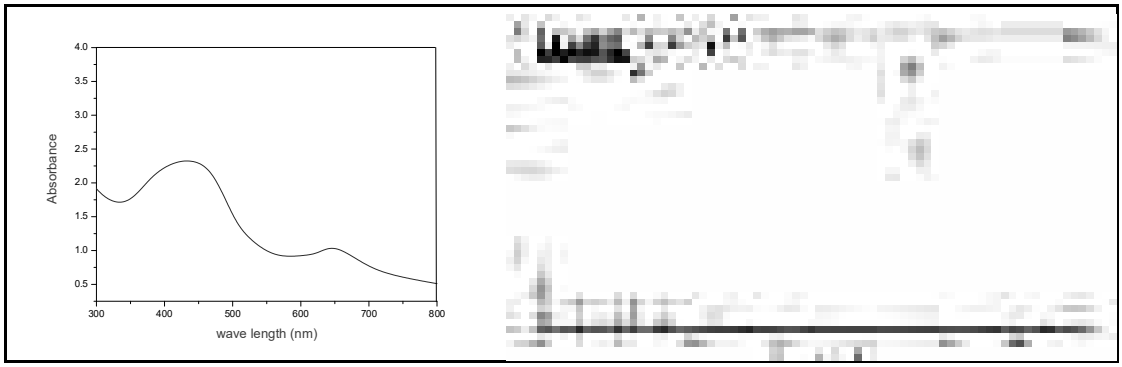


Fig 2(a) : UV-Vis spectrum of Ag-Co BMNMs Fig 2(b) : FTIR spectrum of Ag-Co BMNMs

EDX spectrum (fig 2(c)) indicates the presences of Ag and Co which confirms the formation of Ag-Co bimetallic nanoparticles. This is also supported by the EDX study which gives quantitative data of silver and cobalt compositions in BMNMs**.** As per field emission scanning electron microscopic (FESEM) images of Ag-Co BMNMs (fig 2(d))it is noticed that nanoparticles are in the size range of 30-100 nm in Ag-Co BMNMs. Fig 2(e) shows the HRTEM images for synthesized Ag-Co BMNMs from *Aerva lanata* leaf extract. From these images, it was observed that Ag-Co BMNPs were formed with spherical morphology and crystalline structure below 100 nm in size. Indeed, more explicitly the two metals nanospheres appear to be conjointly placed adjacent to each other giving an overall bilobal structure. This is also in good agreement with FESEM images. The XRD spectrum of green synthesized Ag-Co BMNMs from leaf extract is shown in fig 2(f). The peaks appeared at 2θ values of 32.123^0,37.972^0,44.122^0,64.253^0, 77.216^0 corresponding to the Bragg's reflections of (220), (111), (200), (220) and (311) planes respectively of face centered cubic crystal structure [21].

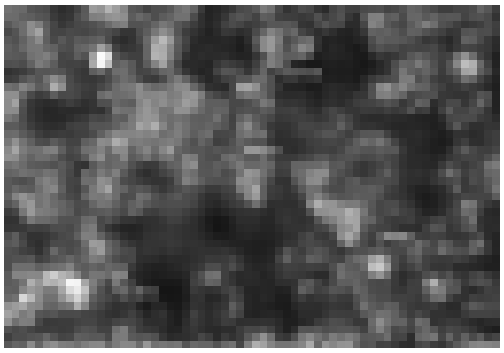


Fig 2(c) : EDX spectrum of Ag-Co BMNMs

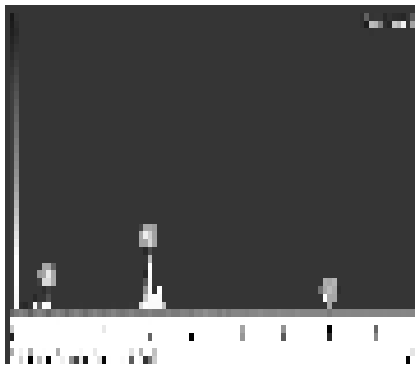


Fig 2(d) : FESEM image of Ag-Co BMNMs

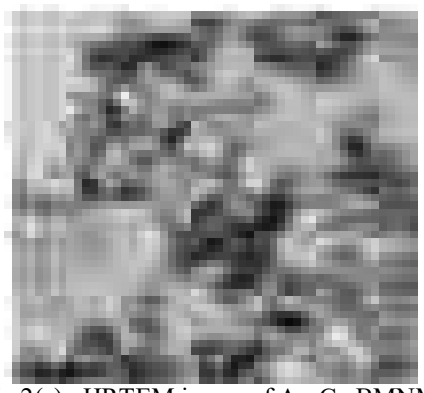


Fig 2(e) : HRTEM image of Ag-Co BMNMs

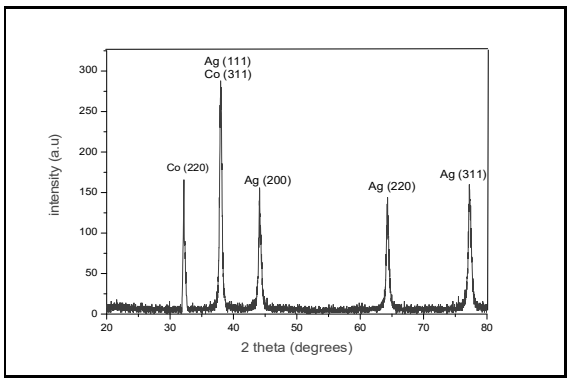


Fig 2(f) : XRD spectrum of Ag-Co BMNMs

2.4 In vitro Cytotoxic Activity by MTT assay

2.4.1 Materials and Methods

Apparatus and chemicals required

- Spectrophotometer
- Incubator
- Inverted microscope
- Centrifuge
- MCF cells (purchaged from NCCS, Pune, India)
- 96 well microplates
- Micropipette
- Doxorubicin
- MTT (1 mg/mL)
- DMSO

MTT assay is a quantitative colorimetric assay for evaluating cellular growth, cell proliferation and cell survival derived from the ability of living cells. The assay was conducted using (3-(4, 5- dimethyl thiazol-2yl) - 2, 5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells resulting a measurable purple product formazan. The amount of formazan formed is directly proportional to the viable cell count and is inversely proportional to the extent of cytotoxic activity. The effect of in vitro cytotoxicity of Ag-Co nanomaterials on Breast cancer cell lines (MCF 7 cell line) is recorded at 24 hours and 48 hours.

2.4.2 Preparation of nano compoundsfor the assay

Five hundred micro litre of stock (100 mg/ mL) nanoparticles were dissolved in 4.5 mL of DMSO for a concentration of 10 mg/ mL. Prior to the assay, the new working suspension was filtered through a 0.45 µm membrane filter. Five gradient concentrations (2 mg, 4 mg, 6 mg, 8 mg and 10 mg) of were used for this analysis. 500 µL of 48 h culture of MCF 7 cell lines at a concentration of 10^5 cells/ mL was applied to each well. Two control wells received only cell suspensions without nanomaterials, also the drug, doxorubicin used as positive control at same concentrations. The plate was placed in a humidified CO_2 incubator for 4 - 6 h at 37°C. Microscopically, the plate was tested for confluent cell monolayer, turbidity and toxicity.

2.4.3 Assay Process: After incubation, the medium from the well was carefully aspirated and then disposed. Each well was washed with Eagle's Minimum Essential Medium (EMEM) without Fetal Calf Serum (FCS). 200 µL of MTT solution (5mg MTT/ ml of PBS, pH 7.2) will be added to each and every well. In a CO_2 incubator with 5 percent CO_2, the plate was incubated for 6-7 h at 37°C. 1 mL of DMSO was applied to each well after incubation, combined with a pipette and left at room temperature for 45 seconds. In the wells, purple formazan was developed [22]. In order to compare full cell viability in cytotoxicity and antitumor activity assessments, cell control and solvent controls were used in each assay. The suspension was moved to a cuvette of the spectrophotometer and the optical density (OD) was calculated as blank at 540 nm using DMSO. With the following formula, cell the %viability was determined.

Cell viability % = Mean OD of wells receiving each plant extract dilution / Mean OD of control wells x 100.

The determination of IC50, the compound concentration needed to inhibit 50 percent cell growth, was calculated by plotting a log graph (extract concentration) vs. percent cell inhibition. A line drawn on the Y axis from the 50% value meets the curve and interpolates to the X axis. The value of the X axis gives the log value (concentration of the compound). The IC50 value is given by the anti-log of that value.

3. Results and Discussion

The synthesized Ag-Co BMNMs were investigated for their cell viability assay and cytotoxic activity against human breast cancer cell line (MCF-7) were assessed by applying standard MTT assay and doxorubicin was used as a standard drug. The compounds were treated with MCF-7 cell line at five different concentrations (2mg, 4mg, 6mg, 8mg and 10mg). The **cytotoxic activities of Ag-Co nano compound and doxorubicin drug against MCF-7 Cell Line at different concentrations are depicted in Table 3.1 and Table 3.2. The** Linear graphs of percent inhibition of Ag-Co nano compound and doxorubicin are shown in Fig 3.1 and fig 3.2. The results clearly demonstrate that the Ag-Co nano compound exhibits a maximum of 35.14 percentage inhibition at 10 mg concentration and a IC50 of 16.55 mg/mL.Figure 3.3 shows the morphological analysis of materials treated with MCF-7 cells.

The results clearly demonstrates that all the synthesized compound Ag-Co (for 24 and 48hrs) have shown moderate to significant cytotoxic activity with values ranging from 2mg to 10mg respectively. So, it is confirmed that all the novel synthesized derivatives exhibit cytotoxic activity and it is also noted that cytotoxic activity increases as the concentration of the BMNMs in the solution increases. Based on these results, compound Ag-Co exhibited remarkable cytotoxic activity comparable to standard drug, it showed 35.14% inhibition at 10mg concentration and the IC50 for Ag-Co was 16.55 mg.

Table 3.1:Cytotoxicity Activity of Ag-Co nanomaterialsagainst MCF-7 Cell Line at Different Concentrations by MTT Assay

Concentration (mg/ml)	OD at 540nm	Percent inhibition	IC 50 (mg/ml)
2	1.21	18.24	
4	1.17	20.95	
6	1.09	26.35	**16.55**
8	1.01	31.76	
10	0.96	35.14	

Fig 3.1: Linear graph for Ag-Co nanomaterials percent inhibition

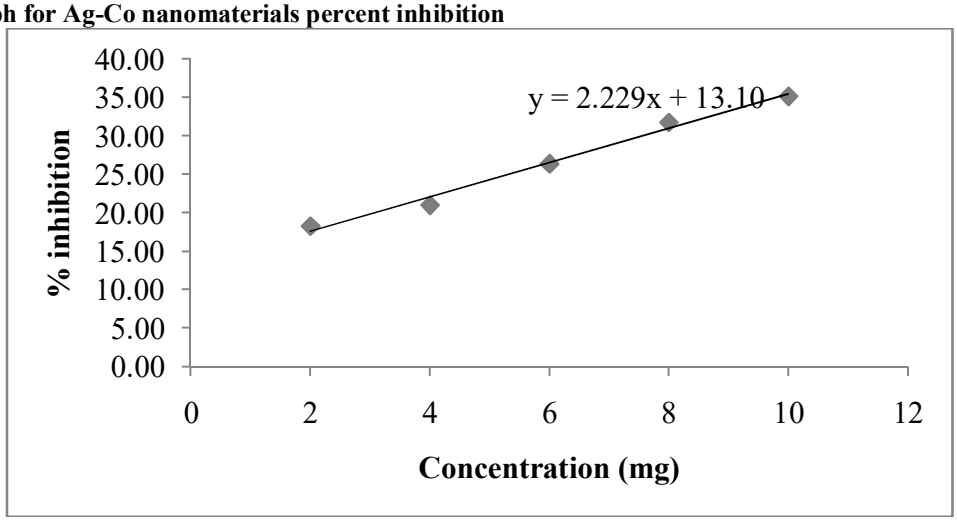
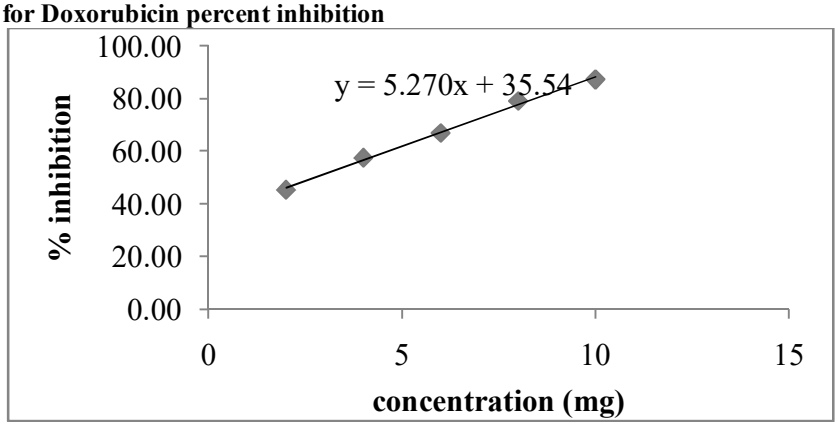


Table 3.2:Cytotoxicity Activity of positive control (Doxorubicin) against MCF-7 Cell Line at Different Concentrations by MTT Assay

Concentration (mg/ml)	OD at 540nm	Percent inhibition	IC 50 (mg/ml)
2	0.81	45.27	2.74
4	0.63	57.43	
6	0.49	66.89	
8	0.31	79.05	
10	0.19	87.16	

Figure 3.2: Linear graph for Doxorubicin percent inhibition



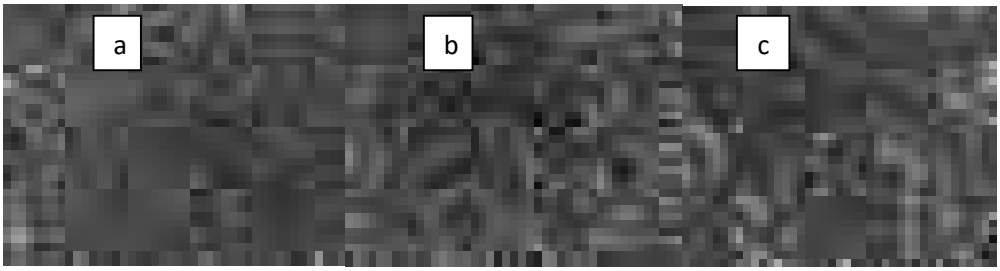


Figure 3.3: Morphological analysis of nano compounds treated MCF-7 cells.
a- control; b- AgCo treated at 10 mg/mL concentration; c- Doxirubicin treated at 10 mg/mL concentration

4. Conclusions

As per the present study Phyto molecules of **Aerva lanata** leaf extract are involved in the bio reduction, formation and stabilization of nanoparticles, the future studies might move towards the optimization of the reaction parameters for generation of high amount of biomolecules to stabilize and cap the formed nanoparticles.

In conclusion, this study implies that green synthesized Ag-Co nano materials may be potent for treatment of MCF7 cells of human breast cancer. A linear correlation was observed between the number of the tumor cells and the dose-dependent cytotoxic effects the synthesized nanomaterials. From the results, it is concluded that these nanomaterials can be used for the development of new preparations for the therapy of tumors. The future research may be directed for the genetic manipulation of plants to increase the metal tolerance and cytotoxicity.

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జాతీయోద్యమ కృషిలో తెలుగు సాహిత్యము, పత్రికలు – బ్రిటీష్ ప్రభుత్వపు నిర్బంధాలు

– సి.హెచ్. తేజస్వి, చరిత్ర ఉపన్యాసకులు, ప్రభుత్వ డిగ్రీకళాశాల, ఆమదాలవలస.

చరిత్ర, సాహిత్యము ఆవిర్భవించిన నాటి నుండి ఒకదానితో ఒకటి అవినాభావ సంబంధాన్ని కలిగి ఉన్నాయి. ఒక ప్రాంతపు భాషా సాహిత్యం పై ఆ ప్రాంతం యొక్క చారిత్రక గతితత్వం, వారసత్వం తిరుగులేని ప్రభావాన్ని చూపుతాయి. అదేవిధంగా ఒకదేశపు లేదా ప్రాంతపు సంస్కృతిని, చరిత్రనూ నిర్మించడంలో అచ్చటి ప్రజల భావజాలాలను రూపొందించడంలో ఆ దేశ భాషల్లోని సాహిత్యం విశిష్టమైన పాత్రను పోషిస్తుంది. సాహిత్యం చరిత్రను దర్పణ మౌనంగా ప్రతిబింబించడమే కాక చరిత్రపు నిర్మాణంలోనూ ఎనలేని సేవలందిస్తుంది.

తెలుగు అతివిశిష్టమైన భాష ఎన్నో మండలికాలతో, ప్రాంతీయ వైవిధ్యాలతో నుడికారాలతో బిన్నత్వంలో ఏకత్వం కలిగిన భాష పద్యం,గేయం, వచన కవిత్వం, కథ, కథానిక నాటకం, నవల వంటిఎన్నో సాహితీ ప్రకియలు తెలుగు భాషను సుసంపన్నం చేశాయి. ఇక సామెతలు, పొడుపుకథలు, జాతీయాలు వంటిభిన్న భాషా రూపాలు తెలుగుకు నిత్య సజీవత్వాన్ని అందించాయి. ఇతర భాషల్లో ఉదయించిన గజళ్ళు, రుబాయీలు, నానీలు, హైకూలు వంటి నవీన పక్రియలను తెలుగు భాష అందిపుచ్చుకుంది. ఈ విధంగా ప్రాచీన, అర్వా చీనత్వాల మేలు కలయికగా తెలుగు సాహిత్యం కొన సాగుతూ వస్తుంది.

చారిత్రక విభాతసంధ్యలతలో తెలుగు నేలపై సామాజిక సంఘర్షణలు, సంక్షోబాలు సంభవించినప్పుడు తెలుగు సాహితీవేత్తలు చైతన్యంతో స్పందించి ఆయా సమస్యలపై విశ్లేషణాత్మక, విమర్శనాత్మక సాహిత్యాన్ని సృజించారు. ఇట్టి సాహిత్యం తెలుగు ప్రజల్లో చైతన్యాన్ని ప్రోదిచేసి అనేక సామాజిక ఉధ్యమాలకు మార్పులకు దారి తీసింది. భారతదేశం వలసపాలకుల ఆక్రమణకు గురైనప్పుడు తెలుగు నాట ఎందరో మేధావులు, రచయితలు జాతీయ చైతన్యంతో కూడిన రచనలు చేసి ప్రజల్లో స్వాతంత్ర్య స్ఫూర్తిని కలిగించారు. తమ రచనలో జాతీయోద్యమం నాటి పరిస్థితులను ప్రతిబింబించారు. వాటిలో నిషేధాలు, శిక్షలనే బహుమానాలుగా పొందిన రచనలు అనేకం ఉన్నాయి.

స్వదేశీ సత్యాగ్రహం, విదేశి వస్తు బహిష్కరణ మొదలైన పోరాట రూపాలు బ్రిటీష్ ప్రభుత్వాన్ని ఎంతగా భయపెట్టాయో బ్రిటీష్ వ్యతిరేక సాహిత్యం కూడా అంతగానే భయపెట్టింది. అటువంటి సాహిత్యానికి వ్యతిరేకంగా ప్రభుత్వం సాగించిన ఆంక్షల పర్వమే దానికి నిదర్శనం. 1898లో బ్రిటీష్ ప్రభుత్వం చేసిన ఒక శాసనంలోని సెక్షన్ 124 యాక్టు 4 ప్రకారం దృశ్య, సాహిత్య కళారూపాలపై ఆంక్షలు విధించబడ్డాయి. దీని ప్రకారం "మౌఖికంగా గాని, రాతపూర్వకంగా గాని, దృశ్య రూపంలో గాని, ఇతర రూపాల ద్వారా గానీ మహా ఘనత వహించిన రాణీగారికి వ్యతిరేకంగా లేక బ్రిటీష్ ఇండియాలో చట్టబద్ధంగా స్థాపించబడిన ప్రభుత్వానికి వ్యతిరేకంగా ప్రజలలో ద్వేష భావాలను రెచ్చ గొట్టేవారు శిక్షింపబడుదురు". వలస పాలనకు వ్యతిరేకంగా వచ్చిన సాహిత్యంపై దేశ ద్రోహ సాహిత్యమని ముద్ర వేసి దాన్ని నిషేధించడానికి వీలుగా ఈ చట్టాన్ని చేశారు.

తెలుగులో జాతీయ వాదరచనలు అనేక సాహితీ ప్రక్రియలలో వెలువడ్డాయి.. 1892 సంవత్సరంలో చిలక మర్తి లక్ష్మీ నరసింహం "అధికార భూతం బులడుగు

లంచములిచ్చి'' అనే దీర్ఘ పద్యం రాశారు. యిందులో బ్రిటీష్ వారి ఆర్థిక దోపిడీ సారాన్ని చక్కగా వివరించారు. 1907వ సంవత్సరం, వందేమాతరం ఉద్యమ సమయంలో బిపిన్ చంద్రపాల్ ఆంధ్రాలో పర్యటించి ప్రసంగించిన సందర్భంలో చిలకమర్తి ఆ ప్రసంగాలకు అనువాదకునిగా వ్యవహరించారు. అప్పుడు బ్రిటీష్ వారి దోపిడీ వివరిస్తూ ''భరత ఖండంబు చక్కని పాడియావు'' అనే నాలుగు పదాల పద్యాన్ని ఆశువుగా చెప్పారు. ఈ పద్యాన్ని ప్రజలు కంఠస్థం చేసి పాడుకోవడమే కాకుండా గోడలపై సైతం రాసి బహుళ ప్రచారం చేశారు.

1922 నాటి సహాయ నిరాకరణోద్యమ సమయంలో గరిమెళ్ళ సత్యనారాయణ ''మాకొద్దీ తెల్లదొరతనము'' అనే 42 చరణాల గేయాన్ని రచించారు. ఈ గేయం ఆంధ్ర దేశం మొత్తాన్నే ఊపివేసింది. ఆనాటి బహిరంగ సభలోనే కాక జైళ్ళల్లో కూడా ఈ గీతం వినిపించేది. ఈ గేయాన్ని రాసినందుకు గరిమెళ్ళకు బ్రిటీష్ ప్రభుత్వం రెండున్నర సంవత్సరాల జైలు శిక్ష విదించింది. ఆయన రచించిన స్వరాజ్య గీతములు గ్రంధ కాపీలను జప్తు చేసి ప్రచురణ కర్త అయిన ఆచంట సత్యనారాయణకు సైతం అరెస్టు వారెంట్లు జారీ చేసింది.

1922 చీరాల - పేరాల ఉద్యమం సందర్భంగా మేడూరి రామమూర్తి రచించిన 'చీరాల -పేరాల గాంధీ', 'దానునాటకము' గ్రంధాలను ప్రభుత్వం స్వాధీనం చేసుకున్నది. ఇదే సంవత్సరంలో విజయనగరం నుండి సోమయాజులసూరి కవి రాసిన 'శ్రీభారతీయ విలాసము' రాజద్రోహకరం అని ఆరోపించి ప్రచురణను అడ్డుకుంది. ముదిగంటి జగన్నాధశాస్త్రి రచించిన 'స్వరాజ్యయుద్ధం', 'దరిద్రనారాయణీయం' కూడా నిషేధించబడ్డాయి. 'స్వప్న ప్రయాణం(1922) ముప్పై ఎనిమిది పేజీల గ్రంధం. దీని రచయిత దీపాల పిచ్చయ్య శాస్త్రి అని తెలుస్తున్నది. ఈ గ్రంధం కూడా బ్రిటీష్ ప్రభుత్వ నిషేధానికి గురయ్యింది. ఈ గ్రంధంలో పిచ్చయ్యశాస్త్రి ''బ్రిటీష్ వారు అనే వింత కోతులు భారతదేశం అనే ఉద్యానవనాన్ని నాశనం చేస్తున్నాయని, వాటిని తరిమి కొట్టాలని'' పద్యాల రూపంలో రచించారు. పల్నాటి పుల్లరి సత్యాగ్రహంలో ఉన్న లక్ష్మీ నారాయణ అరెస్టయి జైలులో ఉన్న సమయంలో 'మాలపల్లి' (1923) నవలను రచించారు. ఇందులో కొన్ని భాగాలను బ్రిటీష్ ప్రభుత్వం నిషేధించింది. ఆ భాగాలు బ్రిటీష్ పాలనా విధానాలను, అవినీతిని, దోపిడీని బట్టబయలు చేసే విధంగా ఉన్నాయి. ఈ నవల 1923లో ఒకసారి, 1936లో మరోసారి నిషేదించబడింది.

తెలుగునాట నాటక సాహిత్యాన్ని కూడా రచయితలు స్వాతంత్ర్య పోరాట ప్రచారానికి ఉపయోగించుకున్నారు. నాటకం సమాహార కళా రూపంగా ప్రదర్శనకు అనుకూలమైన సాహితీ ప్రక్రియ అయినందు వలన ప్రజల హృదయాలలోనికి విశేషంగా చొచ్చుకునిపోయింది. సహాయ నిరాకరణోద్యమం సమయంలో దామరాజు పుండరీకాక్షుడు 'గాంధీమహౌదయము' అనే నాటకాన్ని రచించారు. ఈ నాటకం అచ్చువ్వకుండా స్టేజీపై ప్రదర్శించబడకుండా బ్రిటీష్ ప్రభుత్వం అడ్డుకుంది. ఇదే కాక పుండరీకాక్షుడు రచించిన 'గాంధీ విజయం', 'పాంచాల పరాభవం', 'పంజాబుదురంతము' నాటికలను నిషేదించింది. పుండరీకాక్షుడు రచించిన గ్రంధాలన్నీ పోగుచేసి తగులబెట్టించింది. ఈయన రచించిన 'కలియుగ ప్రహ్లాద' హరి కధను కూడా ప్రభుత్వం నిషేధించింది.ఈ నాటకాలు ఒక పక్క నిషేధించబడుతూ ఉంటే మరో పక్క మారుపేరుతో ప్రదర్శించబడుతూ ఉండటం విశేషం. ఈ నాటక ప్రదర్శనలు ప్రజలపై విశేష ప్రభావాన్ని చూపాయి. కొండా వెంకటప్పయ్య మాటల్లో చెప్పాలంటే ''1920-21 లగాయతు ఆంధ్ర దేశంలో జరిగిన రాజకీయ ప్రచారంలో సగం వంతు పుండరీకాక్షుడు ప్రచురించిన నాటకములు మున్నగు రచనల వల్లనే జరిగింది''. తిలకమరణాంతరం భారత జాతీయ కాంగ్రెసు రాజకీయాల్లో గాంధీయుగం ఆరంభమైన విషయాన్ని 'గాంధీ మహౌదయము' నాటకం ధృవపరుస్తుంది.

తెలుగులో వచ్చిన అనువాద సాహిత్యంపై కూడా బ్రిటీష్ ప్రభుత్వం నిషేధాజ్ఞల కొరడా ఝుళిపించింది. గోర్కీ రచించిన 'ది మదర్' గ్రంధాన్ని క్రొవ్విడి లింగరాజు అమ్మ పేరుతో తెలుగులోనికి అనువదించారు. 1933లో బయటకు వచ్చిన ఈ పుస్తకాన్ని 1935 లో ప్రభుత్వం నిషేధించింది.

రచయితల భావ ప్రకటనాస్వేచ్ఛను గౌరవించే విషయంలో హైదరాబాదు సంస్థానంలోని నిజాం ప్రభుత్వం కూడా బ్రిటీష్ ప్రభుత్వం బాటలోనే నడిచింది. సూల్తాన్ బజార్లోని అణాగ్రంధమాల వారు అతి తక్కువ ధరకు పుస్తకాలు విక్రయించేవారు. వీరు ప్రచురించిన ''నుభాష్ చంద్రబోస్'' ''అల్లూరి సీతారామరాజు'' ''ఎమ్.ఎన్.రాయ్'' ,''రైతు'' గ్రంధాలను నిజాం ప్రభుత్వం ఫర్మానాల ద్వారా నిషేదించింది. రచయితలకు జరిమానాలు, జైలు శిక్షలు విధించింది. 'అగిధ్రార' 'గ్రంధ రచయిత దాశరధి', 'ఓ నిజాముపిశాచమా' కానూడు నిన్ను బోలిన రాజుమాకెన్నడేని అని నిజాంనిరంకుశ త్వాన్ని నిరసించాడు. దాశరధీకృష్ణమాచార్యులు, కాళోజీ నారాయణరావు, పొట్లపల్లి రామారావు మొదలైన ఎందరో రచయితలను నిజాం ప్రభుత్వం జైళ్ళలో నిర్బందించింది. వట్టికోట ఆళ్వారు స్వామి 'ప్రజల మనిషి' నవలలో సాహిత్యంపై నిజాం ప్రభుత్వం విధించిన నిషేధాలను ప్రస్తావించారు.

భారత స్వాతంత్ర్యోద్యమంలో పత్రికలు చేసిన సేవ అనుపమానమైనది. ఇవి బ్రిటీష్ ప్రభుత్వ దోపిడీ స్వభావాన్ని ప్రజలకు తెలియచేసేలా వార్తల్ని ప్రచురించి, ప్రచారం కల్పించేవి. అయితే ఇట్టి పత్రికలు ఎదుర్కొంటున్న ఆంక్షలు, నిషేధాలు అన్నీ ఇన్నీ కావు. అసలు భారత దేశంలో పత్రికల రాజకీయ చరిత్రయే నిషేధాలతో మొదలయిందని చెప్పవచ్చును. బ్రిటిష్ పౌరుడైన జేమ్స్ అగష్టన్ హికీ 27-01-1780 తేదీన బెంగాల్ గెజిట్ అండ్ కలకత్తా జనరల్ అడ్వేర్టైజర్ పేరుతో నాలుగు పేజీల హిందీ వార్తా పత్రికను ప్రచురించారు. ఈ పత్రికలో హికి బ్రిటిష్ విధానాలనుదుయ్య బట్టారు. ఇది నవంబరు 1780 నుండి అనేక ఆంక్షలు, నిషేధాలను ఎదుర్కొన్నది. జేమ్స్ హికీ జైలుపాలై చివరకు జైలులోనే మరణించాడు.

19వ శతాబ్దం ద్వితీయార్ధం నుండి తెలుగునాట అనేక పత్రికలు వెలవడినవాటిలో సుజన రంజని (1862 - 67), తత్వబోధిని (1864 - 70), ఆంధ్రభాషా సంజీవని (1871), పురుషార్ధప్రదాయని (1872 - 78) సఖల విద్యాభివర్ధిని (1873 - 79), సుధీరంజని (1873 - 80), లోకరంజని (1874), విద్యజ్ఞనమనోహరిణి (1874), వివేకవర్ధిని (1874 - 85), జనవినోదిని (1875 - 85), భారతి (1875), సతీ హితబోధిని (1883 - 87), అముద్రితగ్రంధ చింతామణి (1885 - 1904), నీతి దర్పణం (1885 - 87), హిందూ జన సంస్కారిణి (1885-95), స్వదేశి (1875), ఆంధ్రప్రకాశిక (1888 - 1928), చిత్తూరు వర్తమాని (1890), సత్యసంవర్ధిని (1897 - 97), తెలుగు జనానా (1893 - 1907), కల్పవల్లి (1894), దేశాభిమాని (1896), బాలిక (1896 - 97), శారద (1896), మంజువాణి (1898 - 1904), సరస్వతి (1898 - 1912), రత్నాకరం (1899 - 1902), వాగ్వల్లి (1899 - 1903), కళావతి (1900 - 1907), కృష్ణాపత్రిక (1902), సువర్ణలేఖ (1905 - 17), భారతమాత (1906 నుండి), నవయుగ (1907 - 08) వంటి పత్రికలున్నాయి. వీటిలో ఎక్కువ భాగం సాహిత్య పత్రికలు. కొన్ని సంఘసంస్కరణ కోసం ప్రచురించ బడినవి. అయితే 20వ శతాబ్దారంభం నుండి పత్రికల్లో బ్రిటీష్ వ్యతిరేకరాజకీయ చైతన్యం వెల్లివిరిసింది. ఇది ఆంగ్లేయులను భీతావహుల్ని చేసింది.

1970లో శ్రీ పాదకృష్ణమూర్తి 'వందేమాతరం' అను వారపత్రికను స్థాపించినారు. ఇది స్వదేశి ఉద్యమ ప్రచారం కోసం స్థాపించబడినది. గాడిచర్ల హరిసర్వోత్తమ రావు వందేమాతరం ఉద్యమకాలంలో నడిపిన 'స్వరాజ్య' పత్రికలో బ్రిటీష్ ప్రభుత్వాన్ని తీవ్రంగా విమర్శించి నారు.1980లో తిరునల్వేలి జిల్లాలో పోలీసు కాల్పులలో ఇద్దరు పౌరులు చనిపోయిన సందర్భంలో హరిసర్వోత్తమ

రావు 'స్వరాజ్య' పత్రికలో రాసిన సంపాదకీయ వ్యాసంల బ్రిటీష్ ప్రభుత్వాన్ని "క్రూరవ్యాఘ్రమా!" అని సంబోధించి తీవ్రంగా విమర్శించినారు. ఈ సంపాదకీయం రాసినం దుకు 1908లో బ్రిటీష్ ప్రభుత్వం గాడిచర్ల పత్రిక ముద్రా పకులైన బోడి నారాయణరావు పైన రాజద్రోహనేరం మోపింది. కృష్ణా జిల్లా సెషన్స్ కోర్టు హరి సర్వోత్తమరావుకు 6 నెలలు, బోడి నారాయణరావుకు 9 నెలలు కఠిన కారాగార శిక్ష విధించినది. ఈ శిక్ష చాలా తక్కువని భావించి బ్రిటీష్ ప్రభుత్వం మద్రాసు హైకోర్టుకు అప్పీలు చేసినది. హైకోర్టు గాడిచర్లకు విధించబడిన 6 నెలల శిక్షను 3 సంవత్సరాల శిక్షగా మార్చి తీర్పునిచ్చింది. ఉదార బుద్ధితో తక్కువ శిక్ష విధించాడని సెషన్స్ జడ్జ్ కెర్వాన్స్ పదవిని సబ్ కలెక్టరు స్థాయికి తగ్గించి నర సారావుపేటకు బదిలీ చేసినది. దరిమిలా హరిసర్వోత్తమ రావు 1200 రోజులు అత్యంత దుర్భరమైన కారాగార శిక్ష అనుభవించినారు. వందేమాతరం ఉద్యమంలో జైలు శిక్ష పొందిన తొలి తెలుగు రాజకీయ ఖైదీలు నారాయణ రావు, హరిసర్వోత్తమరావులు.

1917లో చిలకమర్తి లక్ష్మీ నరసింహం నడిపిన 'దేశమాత' పత్రికలో బ్రిటీష్ వారిని విమర్శిస్తూ ఒక వ్యాసం ప్రచురితమైనది.దానికి గాను బ్రిటీష్ ప్రభుత్వం పత్రిక ముద్రనశాలపై వేయి రూపాయల ధరావతు విధించినది. హోంరూల్ ఉద్యమకాలంలో గాడిచర్ల హరిసర్వోత్తమరావు తన ఆధ్వర్యంలోని 'నేషనలిస్ట్' పత్రికలో హోంరూల్ ఉద్యమాన్ని ప్రచారం చేసినారు. ఎంత మంచి ప్రభుత్వాని కైన సరే తన ప్రజలు స్వయంపాలనకు అర్హులు కారని చెప్పే హక్కులేదని చాటారు. దీనితో ఆగ్రహించి ప్రభుత్వం '1911 ముద్రణాలయ చట్టం' క్రింద నేషనలిస్ట్ పత్రికకు చెల్లించిన దఖావతు సొమ్మును జప్తుచేసింది. హోంరూల్ ఉద్యమ సమయంలో అనీబిసెంట్ నిర్వహించిన 'న్యూఇండియా' పై మొదటి రెండువేలు, అనంతరం పదివేల భారీ జరిమానాలను విధించినది.పత్రిక ప్రచురణ నిలపకూడదన్న ఉద్దేశ్యంతో అనిబీసెంట్ పది వేల రూపా యలు ధరావతు చెల్లించి పత్రిక ప్రచురణను కొన సాగించినది.

శాసనోల్లంఘన సమయంలో సైతం బ్రిటీష్ ప్రభుత్వం ప్రజలపైన, జాతీయవాద పత్రికలపైన తీవ్ర నిర్భంధం అమలు చేసినది. ఏలూరులో 'సత్యాగ్రాహి' పత్రికను, ముద్రణాలయాన్ని స్వాధీనం చేసుకున్నది. పత్రికా సంపాద కుడు ఆత్మకూరి గోవిందాచార్యులను అరెస్టు చేసినది. రాజమండ్రి నుంచి మద్దూరు అన్నపూర్ణయ్య నడిపిన 'కాంగ్రెస్' పత్రికపై దాడిచేసి మూసివేయించింది.మంగళా పాండే వీరగాధ అయిన 'చిచ్చురపిడుగు' ప్రచురించి నందుకు సంపాదకుడు అన్నపూర్ణయ్యకు రెండు సంవత్స రాల కఠిన కారాగార శిక్ష విధించింది. అన్న పూర్ణయ్య అరెస్టు తరువాత క్రొవ్విడి లింగరాజు రహస్యంగా పత్రికను వెలువరించగా ప్రభుత్వం ఆయనను కూడా అరెస్టు చేసింది. తరువాత చందుభట్ల హనుమంతరావు, రామచంద్రుని వెంకటప్ప మొదలుకొని 8 మంది సంపా దకులు ఒకరి వెంట మరొకరు అరెస్టు చేయబడ్డారు. ఇది భారత పత్రికారంగ చరిత్రలోనే ఒక అపూర్వ విసయం. మద్దూరి అన్నపూర్ణయ్య జాతీయ ఉద్యమాల్లో మొత్తం 14 సంవత్సరాలు జైలు శిక్షలు అనుభవించినారు. ఆయన భార్య రమణమ్మ కూడా జైలు శిక్షకు గుర య్యారు.

పత్రికా స్వాతంత్ర్యాన్ని హరించడంలో కూడా హైద రాబాదు సంస్థానపు నిజాం ప్రభుత్వం బ్రిటీష్ అడుగు జాడల్లో నడిచింది. పత్రికా స్వాతంత్రంపై ఉక్కుపాదం మోపింది. హైదరాబాదు సంస్థానంలో 1912లో శ్రీనివాస శర్మ ప్రారంభించిన సరోజినీ విలాస్ మొదలుకొని ఆంధ్రమాత (1917), తెలుగుపత్రిక (1920), నీలగిరి (1920), దేశబంధు (1920), ఆదిహిందూ, దక్కనుకేసరి (1934), 1936 (1936), తెలుగుతల్లి (1936), శోభ (1937) మొదలైన పత్రికలు వచ్చాయి. 1938 నుండి 1945 వరకు నిజాం ప్రభుత్వం కొత్త పత్రికల ప్రచురణను అనుమతించలేదు. 1945 తరువాత, ది ఆంధ్రకేసరి,

సందేశం పత్రికలు వచ్చాయి. తెలంగాణ పత్రిక జాతీయ వాదాన్ని బలపరిచింది. అబ్దుల్లతీఫ్ 1931 నుండి ప్రచురించిన 'న్యూఎరా' పత్రిక నిజాం ప్రభుత్వం చేస్తున్న దుబారా ఖర్చులపై వ్యాసాలు ప్రచురించింది. అయితే వీటిలో ఏ పత్రిక ఐదు సంవత్సరాలకు మించి కొనసాగలేక పోయింది. సురవరం ప్రతాపరెడ్డి 1925లో స్థాపించిన 'గోల్కొండ' పత్రిక మాత్రం విశాలాంధ్ర స్థాపన జరిగే వరకూ కొనసాగి జాతీయవాదానికి దన్నుగా నిలిచింది. బెజవాడ నుండి వెలువడే 'ప్రజాశక్తి' పత్రిక ఆంధ్ర మహాసభ యొక్క కార్యక్రమాలను వివరంగా ప్రచురిస్తూ నిజాం ప్రభుత్వ ఆగ్రహానికి గురైంది. దీనిపై నిజాం ప్రభుత్వం 1947లో నిషేధాజ్ఞ జారీ చేసింది. నిజాం దురం తాలపై వరుస కథనాలను ప్రచురించిన 'ఇమ్రోజ్' పత్రిక సంపాదకు డుషోయబుల్లాఖాన్ 1948లో రజాకార్ల చేతిలో హత్యకు గురయ్యాడు.

ఎన్నో నిర్భందాలను, ఆంక్షలను, నిషేధాలను ఎదు ర్కొన్నప్పటికి భారత స్వాతంత్ర్యోద్యమకాలంలో తెలుగు సాహిత్యమూ, పత్రికలూ వెనుకంజ వేయక నిర్భీతిగా తమ కర్తవ్యాన్ని నిర్వర్తించినవి. ఇది స్ఫూర్తిదాయకమైన చరిత్ర.

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Phytochemical Screening Of Leaf Extract Of Aerva Lanata Collected From Agricultural Lands Of India

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Abstract - **Medicinal plants possess biologically active components called phytochemicals which are administered for curing of various human ailments and also take part in significant role in healing. Phytochemicals are of two categories i.e., primary and secondary constituents. Primary constituents include proteins, chlorophyll, sugar and amino acids. Secondary constituents contain alkaloids and terpenoids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities because of these phytochemicals. The aqueous extract of leaf samples of the medicinal plant Aerva lanata are collected from agricultural lands of S.Kota. This extract is used for the phytochemical analysis to find out the phytochemical constituents in the plants with an objective to check the presence or absence of the phytochemical constituents in the selected plant. The results of the phytochemical analysis of the plant leaf extract shows that the terpenoids, tannins, reducing sugar, flavonoids and alkaloids are found to be present in afore mentioned medicinal plant. The phytochemical analysis of the plants is very much essential commercially and has huge attention in pharmaceutical industries for the fabrication of the novel drugs for treating various diseases. It is supposed that the key phytochemical properties identified by this study will be very helpful in the treatment of various diseases.**

keywords - **Phytochemicals, Aerva lanata, alkaloids, flavonoids, terpenoids, FTIR, tannins.**

1.1 INTRODUCTION

Phytochemistry is the part of chemistry that deals with the chemical processes involved in plant life. Phytochemicals are naturally occurring and biologically active chemical substances present in plants. Proteins, chlorophyll and regular sugars are the primary constituents and alkaloids, terpenoids, phyto sterols, flavonoids, glycosides, tannins and phenolic compounds are being the secondary constituents [1]. Phytochemicals guard plant cells from pollution, drought, stress and pathogenic attack [2]. Phytochemicals are synthesized in almost all parts of the plant like leaves, root, bark, stem, root, fruits, flower, seeds etc. [3, 4]. Phytochemicals are responsible for the colour and organoleptic properties of plant. Recent research shows that phytochemicals play a vital role in protecting humans against diseases. To extricate these compounds from plants phytochemical screening is inevitable. Phytochemical screening deals with the extraction, screening, and identification of the bioactive substances found in plants [5, 6].

Aerva lanata is a woody perennial shrub belonging to the family Amaranthaceae which is commonly found in the lands and fields of India (**Figure 1.1**). It is a good source of phytochemicals terpenoids, flavonoids, alkaloids, phenolic compounds, glycosides, gums, tannins, terpenes, carbohydrates and aminoacids. Thus, it plays inherent role to cure human diseases [7]. It is used as antiurolithiatic and diuretic drug in indian Ayurveda with the name of Pashanabeda (which means stone dissolving) for urinary disorders [8]. Studies on *Aerva lanata* proved that betulin and quercetin are the two compounds isolated from the plant have inhibitory property on enzyme activity which is responsible for kidney stone formation [9, 10]. There are other constituents such as ferulic acid, syringic acid, narcissin and feruloyltyramine (**Figure 1.2**) which are isolated from methanoic extract of *Aerva lanata* which are responsible for its antibacterial, antioxidant, anti asthmatic and anthelmintic activities [11]. This plant is also used traditionally for arresting hemorrhage during pregnancy, as an anti inflammatory, to treat nasal bleeding, scorpion sting . The amount of phytochemicals varies from species to species and plant to plant, based on the age and different ecological and climatic factors [12]. This chapter reports the preparation of *Aerva lanata* leaf extract and its phytochemical screening.

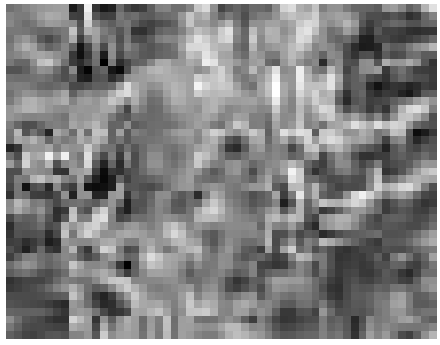

Figure 1.1: *Aerva lanata* plant

Figure 1.2: Chemical constituents identified in *Aerva lanata* plant

Aerva lanata is a good source of phytochemicals terpenoids, flavonoids, alkaloids [13], phenolic compounds, glycosides [14], gums, tannins, steroids, carbohydrates [15] and essential oil. Thus, it plays inherent role to cure human diseases [6]. Literature shows that like other plants *Aerva lanata* is wealthy sources of secondary metabolites and have antibacterial [16], antifungal [17], antioxidant [18], cytotoxic [19], anti-HIV [20], anti tumour [21], anti diabetic [22, 23] and anticancer [24] activities. This chapter reports the phytochemical screening of *Aerva lanata*.

1.2 MATERIALS AND METHODS

1.2.1 Chemicals required

The chemicals required for the phytochemical screening of the leaf extract are Mayers reagent (potassium mercuric iodide), Hager's reagent, Molisch's reagent, Benedict's reagent, Fehling's reagent, Schiffs reagent sodium nitroprusside, NaOH, ferric Chloride, benzene, H_2SO_4, chloroform, lead acetate, gelatin, HNO_3, acetic anhydride, ferric chloride, Ninhydrin reagent, copper acetate, sodium bicarbonate, hydrochloric acid, litmus papers, 2,4-DNP, Tollens reagent, iodine solution and deionized water.

1.2.2 Collection of *Aerva lanata* leaves

Fresh leaves of *Aerva lanata* plant are collected from agricultural fields located at S. Kota mandal in Vizianagaram district, Andhra Pradesh, India (**Figure 1.3**). 100 g of leaves are weighed and thoroughly cleaned with running tap water to eliminate debris on surface of leaves followed by deionized water to remove other contaminants from leaves and dried up under shade for nine days i.e., until the weight of the dried leaves remains constant. These leaves are sliced into tiny pieces and made homogenized powder by using home blender. The obtained powder is stored in an air tight container for further usage.

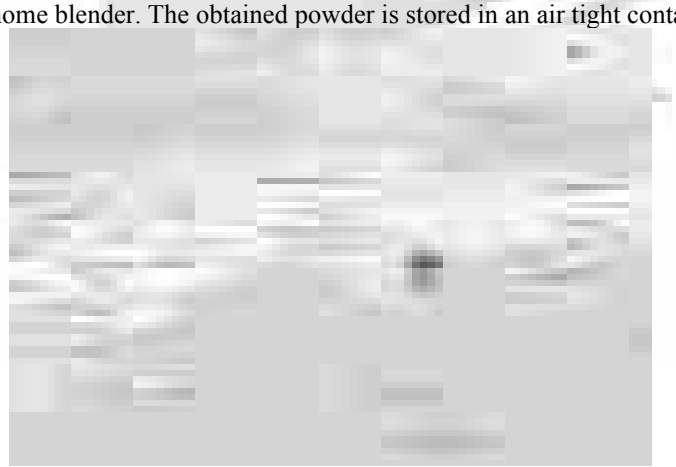

Figure 1.3: Map showing plant collection site in India

1.2.3 Preparation of leaf Extract

200 mL deionized water is taken in 500 mL beaker to this 10 g stored powder weighed and added. The contents in the beaker boiled for 30 minutes with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled leaf broth is filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4^{o}C. This is taken as leaf extract throughout the experiment. **Figure 1.4** shows image of *Aerva lanata* leaf extract prepared.

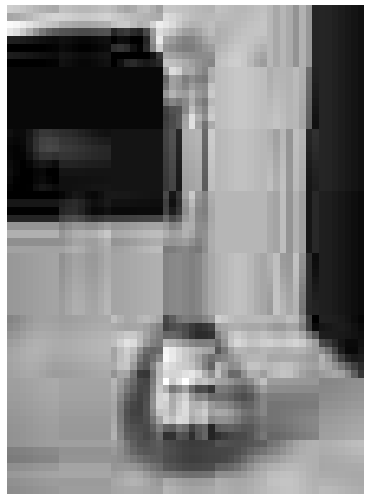

Figure 1.4: Image of *Aerva lanata* leaf extract

1.3 PHYTOCHEMICAL SCREENING TESTS

Aqueous extract of *Aerva lanata* is screened to various phytochemical tests. Standard methods are used for phytochemical screening [25].

1.3.1 Test for Alkaloids

a) Mayers Test

To 5 mL of 1% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of the filtrate is treated with two drops of Mayer's reagent. Formation of yellow precipitate indicates the presence of Alkaloids.

b) Hager's Test

To 5 mL of 1% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with 2 drops of Hager's reagent. Formation of yellow precipitate shows the presence of alkaloids.

c) Wagner's Test

To 5mL of 1% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with two drops of Wagener's reagent. Formation of brown colour precipitate indicates the presence of alkaloids in leaf extract.

1.3.2 Test for Carbohydrates

a) Molisch's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Molisch's reagent (alcoholic solution of α-naphthol solution) is added followed by the addition of concentrated sulphuric acid along the walls of the test tube. Formation of violet ring indicates the presence of carbohydrates.

b)Benedict's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Benedict's reagent is added and heated gently for two minutes. Formation of red precipitate indicates the presence of carbohydrates (reducing sugars).

c) Fehling's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 1mL of each Fehling solution A and B is added and boiled in a water bath for 2 min. Formation of brown precipitate indicates the presence of carbohydrates (reducing sugars).

1.3.3 Test for Glycosides

a) Legal's Test

5 mL of extract is treated with 4mL of pyridine contained 2 mL of sodium nitroprusside solution. This is neutralized with 10% NaOH. Appearance of pink colour shows the existence of glycosides.

b) Modified Borntrager's Test

5 mL of extract is treated with 2 mL of $FeCl_3$ solution and immersed in boiling water for about five minutes. The mixture is cooled and extracted with equal volumes of benzene. The benzene layer is separated and treated with ammonia solution. Formation of rose-pink colour indicates the presence of anthranol glycosides.

c) Keller-kilani test

Another test to confirm the presence of glycoside is carried out by mixing the crude extract (2 mL) with glacial acetic acid (2 mL) containing 1-2 drops of 2% $FeCl_3$ solution. The mixture is then transferred into another test tube already containing 2 mL concentrated H_2SO_4. Appearance of brown ring at the interphase confirms the presence of cardiac glycosides.

1.3.4 Test for Saponins

a) Foam Test

To 5 mL of crude extract 20 mL of distilled water is added and this solution shaken vigorously in a 100 mL conical flask for 15min. Persistent foaming on shaking confirms the presence of saponins.

1.3.5Test for Steroids and Phytosterols

a) Libermann-Burchard's Test

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 is added. Formation of reddish brown colour indicates the presence of steroid ring.

b) Salkowski's Test

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of conc. H_2SO_4, shaken and allowed standing. Appearance of golden yellow colour indicates the presence of steroids.

1.3.6Test for Phenolic compounds

a) Ferric Chloride Test

The extract is dissolved in 5mL of distilled water and 2-4 drops of 5% $FeCl_3$solution is added. Formation of deep green colour specifies the presence of phenolic compounds.

b) Lead Acetate Test

2mL of 5% lead acetate solution is added to the extract solution. Formation of yellow precipitate indicates presence of phenolic compounds.

1.3.7Test for Tannins

a) Gelatin Test

To 1% gelatin solution containing 10% NaCl 5mL diluted algal extract is added. The formation of white precipitate indicates the presence of tannins.

1.3.8 Test for Flavonoids

a) Alkaline Reagent Test

5mL of extract is treated with few drops of sodium hydroxide solution. Formation of an intense yellow colour, which becomes colourless on addition of dilute acid connotes the presence of flavonoids.

1.3.9 Test for proteins

The extract is treated with few drops of Conc. HNO_3. Formation of yellow colour suggests the presence of proteins.

1.3.10 Test for Amino acids

a) Ninhydrin Test

5mL of extract is diluted by the addition of 15mL of distilled water. To the extract, 0.25% w/v Ninhydrin reagent is added and boiled for a few minutes. Formation of blue colour denotes the presence of amino acids.

1.3.11Test for Diterpenes

a) Copper acetate Test

Extract is dissolved in water and treated with 2-4 drops of copper acetate solution. Formation of emerald green colour confirms the presence of diterpenes.

b) Crude extract (2 mL) is dissolved in chloroform (2 mL) and then evaporated to dryness. Concentrated H_2SO_4 (2 mL) is added to the resulting solid and heated for 2 minutes. The appearance of greyish colouration indicates the presence of terpenoids.

1.4 Detection of functional groups present in the *Aerva lanata* leaf extract by FTIR analysis

The FTIR spectrum is used to identify the functional groups of the active components based on its peak values in the region of IR radiation [26].

The strong intense peaks observed between 3200 cm^{-1} to 3950^{-1} may be due to N-H, O-H stretching of 1^0 amines and polyhydroxy groups present in the extract (**Figure 1.5**). The strong absorption at 1643 cm^{-1}indicates the presence of C=O group of amides. This result gives the evidence about the high protein content of the extract. The small peak at 2892 cm^{-1} may be due to C-H symmetrical stretching of methylene groups. The peak position at 1519 cm^{-1} may be due to the C=C stretch of aromatic ring. The peak at 598 cm^{-1} is denoting the presence of C-Cl group.

Fig. 1.5: FTIR spectrum of *Aerva lanata* leaf extract

3.5 CONCLUSIONS

The phytochemical screening and FTIR spectroscopic analysis of *Aerva lanata* leaf extract confirm the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds, tannins, flavonoids, proteins, amino acids and terpenes. These are the plant secondary metabolites present in the leaf extract. The important phytochemical properties recognized by this study will be very useful in the development of new drugs for the treatment of various diseases of mankind.

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HEALTH & WELLNESS – A Simple Way to Enhance the Quality of Life

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ABSTRACT

The combined phrase health and wellness can basically be relatedto as a P**ursuit of** overall health by adopting certain convenient physical activities.Wellness is simply the state of good mental, physical and emotional health. An example of wellness is eating correctly and exercising every day. The term is used frequently as a tool for promoting a better lifestyle.

In today's life the majority of our waking hours are often spent working and taking care of our responsibilities. If those hours are lacking energy, enthusiasm, excitement and engagement, then it's time to reevaluate how it's affecting your life. Today's world is complicated, hectic and challenging. Despite the fact that technology is supposed to make things easier, it's done the exact opposite for far too many people. Being accessible at all hours of the day and night have blurred the lines between work and home, and information overload makes us feel like there is constantly more to get done. To-do lists are overloaded, and it is just not humanly possible to take care of it all.Most of one's time gets lost in attending the demands either at professional front or personal front. This makes life more of mental work and less of physical. Health and wellness is a journey defined by its ups and downs – this path requires patience, curiosity, open-mindedness, and even conviction. What and when we eat, our sleep patterns, the amount of mobility or exercise, our work habits and mental attitude all play a part in energy and performance and we have the power to confront and make small adjustments to-change-every-part.

Keywords: Health & Wellness, Life style management, Health Management, Quality of life

INTRODUCTION

This paper aims at understanding the importance of Wellness, threats of sedentary way of life, its identification, differentdimensions of Wellness and finally, how one can find different means to be Healthy and Happy by adopting simple tips.

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