# GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING FLOWER EXTRACT OF PHYLA NODIFLORA

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**Abstract**

Present work the progress of green chemistry in the synthesis of nanoparticles with the use of plants has engrossed a great attention. Zinc oxide nanoparticles were received potential interest due to their vast applications in the food industry. For such purpose, the development of novel and biological techniqus is in considerable demand for raising these materials to an industrial level. This letter portrays a novel method for the biosynthesis of ZnO nanoparticles using a Phyla nodiflora for the first time. The morphology structure and stability of the synthesized ZnO nanoparticles were studied using UV-spectro photometer and Fourier Transform Infrared (FTIR) spectroscopy. The results were depicted that the synthesized nanoparticles are moderately stable, roughly spherical with maximum particles in size range within 9-10 nm in diameter.

## Introduction

Many plants were synthesized substances that are useful to the maintenance of health in humans and other animals. These includes of aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Most of the secondary metabolites, of which at least 12,000 have been isolated a number estimated to be less than 10% of the total. Many of the herbs and species used by humans to season food yield useful medicinal compounds.

Natural compounds were extracted from plants, particularly higher plants, have been suggested as alternative sources for antibiotics. The chemical features of the compounds constituent differ considerably among different species. This approach is alluring, in part, because they constitute a potential source of bioactive compounds that have been professed by the general public as comparatively safe and often act at multiple and novel target sites, thereby reducing the potntial for resistance

Ayurveda an ancient system of Indian medicine has recommended in number of drugs from indigenous plant/animal sources or the treatment of several diseases or disorders. Use of plant products, as medicine is inherent in Ayurveda, the ancient Indian system of health care. In industrialized countries, herbal medicine is become increase in popular, However the expanded use of herbal medicine has lead to concerns relating to assurance of safety, quality and efficacy. The use of herbal medicine for the treatment of some important diseases and infections is as old as mankind

The medicinal plants constitute one of the important raw materials for drugs for treating various ailments of human being, although there has been significant development in the field of synthetic drug chemistry and antibiotics. During the last two decaded considerable changes have taken place in the medicinal system all over the world. Because of general awareness of toxicity, harmful effects, which associate with the long use of synthetic drugs and antibiotics, the western society, prefer drugs from natural sources.

In this present work the Phytochemical constituents *Abrus precatorius L*(Kundumani)leaves, and identification of the phytoconstituent by using UV-VIS Techniques, Separation of Phenolic compounds by TLC. To identify the biologically active compound by GC-MS technique.

## MATERIALS AND METHODS

Homogenate was prepared by weighing 20grams of fresh *Flower* of *Phyla nodiflora* collected from Pattukkoti. Washed thoroughly (thrice) in distilled water and homogenized using a mortar and pestle. The homogenate was then filtered using a sterile gauze cloth. This homogenate extract prepared was then transferred to a sterile container and used for the study.

### Preparation of Zno Solution

For the synthesis nanoparticle 50 ml of *Phyla nodiflora flower* extract was taken and boiled to 60-80 degree Celsius using a stirrer heater. 5 grams of Zinc Oxide was added to the solution as the temperatures reached 60 degree Celsius. This mixture is then boiled until it reduced to a deep yellow colored paste. This pastes were collected in

a ceramic crucible and heated in an air heated furnace at 400 degree Celsius for 2 hours. A light white colored powder was obtained and this was carefully collected and packed for characterization purposes. The material was mashed in a mortar-pestle so as to get a finer nature for characterization.

Optimization of Various Parameters for Nanoparticles Synthesis

UV-VIS spectra analysis

The bioreduction of Zn ions in solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and 800 nm ranges.

FTIR analysis

For FTIR measurements, the Zn nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the Zinc nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a Shimadzu IR-IR Affinity1 model in the diffuse reflectance mode operating at a resolution of 4 cm−1.

ANTIMICROBIAL STUDIES

Test Organisms

The pure cultures of bacteria maintained in the microbiology Laboratory were used for the microbiological work. The test organisms were maintained on Nutrient agar medium.The test organism were used for work are, *Staphylococcus aureus, Escherichia coli, P.aurenginosa , Bacillus.*

Preparation of inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of microorganism from the stock cultures to test tubes of Nutrient broth, and incubated for 24 hrs at 37°C. The cultures were diluted with fresh Nutrient broth.

Preparation of Media

The medium was prepared by dissolving the different ingredients in water and autoclaved at 121°C for 15 minutes. This was used for preliminary antibacterial studies.

Microorganisms and culture media

Bacterial cultures such as, *Pseudomonos* , *Staphylococcus* , *candida tropicalis* , *candida albicans* were obtained from St. Joseph College, Trichy. Bacterial strains were maintained on nutrient agar broth, (Himedia) at 4°c.

Nutrient Broth Medium

Bacterial cultures were sub cultured in liquid, medium (Nutrient broth) at 32°C for 24 - 48 hours and used for experiments. The nutrient broth medium consisted of following composition.Peptone -59, Beef extract - 3g, Sodium chloride -5g, Yeast extract -1.5g, Distilled water -1000 ml. After adding all the ingredients into the distilled water, it was boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ibs for 15 minutes.

Inoculums preparation

Bacterial cultures were sub cultured in liquid medium (nutrient broth) at 37c for 8h and further used for the test. These suspensions were prepared immediately and the test was carried out.

Preparation of culture media

Nutrient agar medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes.

Ingredients ; grams/liter

Peptone - 5 gm, Beef extract - 3gm, Agar - 15 gm, Sodium chloride - 5 gm, Yeast extract -1.5gm, Distilled water - 1000ml, PH - 7.0

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib.psi pressures (121 c) for 15 minutes.

Agar Well Diffusion Method

Agar well method is also known as hole plate diffusion method or cup diffusion method.

Principle

It is an important method for studying the inhibitory effect of any compound (plant extract or antibiotics) on the growth and multiplication of a particular bacteria or fungus. Here well or cups are made using a sterilized cork borer on the seeded nutrient agar or potato dextrose agar in a Petri dish to which the test compound is added. The treated Petri dishes are incubated at 37°C for 24 hrs for bacteria and 48 hr for fungus. The inhibition zone formed around each well indicates the antimicrobial activity.

| S.No | Plant leaf extract+ZnNo3 | Color change | | pH change | | Color intensity | Time | Result |
|---|---|---|---|---|---|---|---|---|
| | Scientific name | Before | After | Before | After | | | |
| 1 | *Phyla nodiflora* | Light Yellow | Brown | 4.0 | 4.60 | +++ | 20min | Positive |

Table 1: Indication of Color Change in Synthesis Zno nano Particle (SNPs)

Color intensity: +++ = very dark colour

Procedure

Nutrient agar or potato dextrose agar was used as the culture medium for this assay. The molten nutrient agar or potato dextrose agar was dispensed in pre-sterilized Petri dishes (25 ml) and allows cooling. These agar plates were homogenously incubated with the test bacterium previously suspended in distilled water. The plates were allowed to solidify. After solidification holes/wells of 6 mm diameter were punched into the agar with the. Help of flamed cork borer. Three wells prepared for each plate. One hole was filled with 100% concentration of plant extract another one well was filled with 50% concentration. Final one hole was filled with solvent as a control. The Petri dishes were incubated at 37°C for 24 hrs for bacteria. After

incubation period the diameter of the inhibition zone formed around each hole (well/cup) was measured and the values were recorded.

A well was prepared for each experiment by filling up with only solvent as negative control. The value obtained here is deducted in the experimental values for analysis.

## RESULT AND DISCUSSION

### Zno NPs Synthesis:

The synthesis and application of nanomaterial is in the limelight in modern nanotechnology. Plants including herbs, lower plants, higher plants, weeds, roots, etc. contain an array of secondary metabolites such as phenolic compounds, terpenoids, essential oils, flavonoids, and more natural products which helps in the reduction of metal ion and formation of nanoparticles(Haverkamp and Marshall 2009). The present investigation demonstrate the formation of Zinc nano particles by the reduction of aqueous Zinc metal ions by *flower* extracts prepared from *Phyla nodiflora* .

Zinc Oxide nanoparticles were synthesized successfully by the green synthesis method using Neem (Azadirachta indica) leaf extracts (Hot and Cold methods). During exposure to leaf extracts, reduction of zinc ions into zinc nanoparticles was observed as a result of the colour change from pale white to brown colour which occurred due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which helps in the formation of the Surface Plasmon Resonance absorption band, which is due to the united vibration of the electrons of metal nanoparticles in resonance with light wave (Kanthimathi et al., 2013)

### Detection and Characterization of Phyto Zinc Nanoparticles

### Visual Observation:

After treatment of *Phyla nodiflora* extract with AgNO3, the colour change of the reaction mixture was visually observed. The time taken for the reaction mixture to change the colour was noted accurately.

The reduction of Zinc ions into Zinc particles during exposure to the plant extract is followed by colour change from colorless or pale yellow to yellowish brown. It is well known that Zinc nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in Zinc nano particles.

*UV- Vis- Spectroscopy*

Visible wavelengths cover a range from approximately 400 to 800 nm. Optical properties of the as-prepared ZnO nanostructure sample was revealed by UV–Vis spectrum and photoluminescence spectroscopy at room temperature, as shown in Figure 1. It can be seen from the Figure .1 that there was intensive absorption in the ultraviolet band of about 300-1100 nm. The absorption wavelength at about 208 and 215 nm of ZnO suggested the excitonic character at room temperature

Fig 1:UV-VIS SPECTROSCOPY

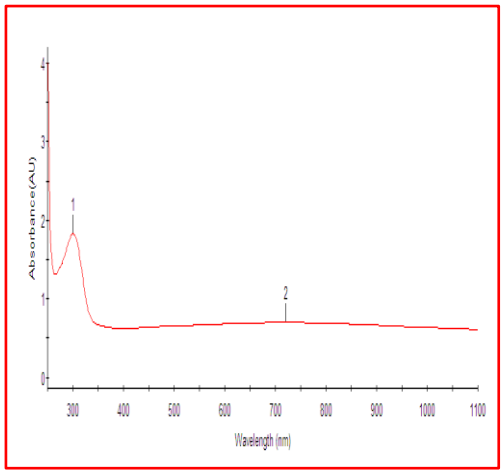
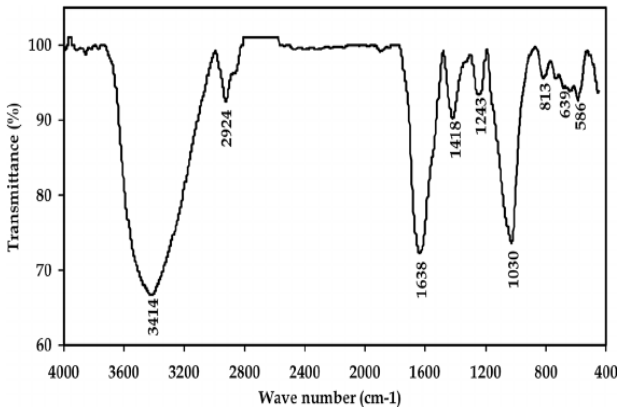



Fig 2: FT-IR analysis




FT-IR analysis:

Two milligram of Zno nanoparticales were prepared by mixing with 200 mg of spectroscopic grade KBr. FTIR spectra were recorded using a Nicolet 520P spectrometer with detector at 4000-400 cm-1.

The results of the FTIR spectrum of hot and cold methods of neem (Azadirachta indica) extracts of Zinc Oxide nanoparticles are depicted in Figure 1and 2. The band at 437-445 $cm^{-1}$ and 509-511$cm^{-1}$ is attributed to ZnO nanoparticles. The broad peak at 3402-3419$cm^{-1}$ correspond to O-H band and C=O indicating the compound to be aliphatic carboxylic acid. The band at 1554-1558$cm^{-1}$ is attributed to the presence of aromatic ring. The band at 1028-1033$cm^{-1}$ correspond to saturated primary alcohol. The band at 2927-2931$cm^{-1}$ is due to doublet absorption of C-H stretching vibration of an aromatic aldehyde. These bands are indicative of terpenoid group of compounds present in aqueous neem

(Azadirachta indica) extract ( Jha and prasad ,2010 and Senthilkumar and Sivakumar, 2014).From FTIR analysis, it can be inferred that alcohols, terpenoids ketones, aldehydes and carboxylic acid were surrounded bysynthesized nanoparticles. Phenolic compounds flavonoids, lignans, coumarins, tannins, quercetin, alkaloids, cynogenic glycosides present in the leaves formed a strong capping on the nanoparticles(Awwd et al., 2013). The prominent doublet absorption at 2927. The FT-IR studies clearly indicates thereduction and capping agents ie. biomolecules present in the neem (Azadirachta indica) leaf extract.

| s.no | Frequency range | Bond | Type and group |
|---|---|---|---|
| 1 | 3414 | O–Hstretch, H–bonded | alcohols, phenols |
| 2 | 2974 | C–H stretch | alkanes |
| 3 | 1638 | –C(triple bond), C– stretch | alkynes |
| 4 | 1418 | N–H bend | primary amines |
| 5 | 1243 | C–H bend | alkanes |
| 6 | 1030 | C–C stretch (in–ring) | aromatics |
| 7 | 813 | C–H wag (–CH2X) | alkyl halides |
| 8 | 639 | C–Cl stretch | alkyl halides |
| 9 | 586 | C–Cl stretch | alkyl halides |

Table 3: FT IR RESULT FOR FLOWER EXTRACT OF *Phyla nodiflora*

ANTIMICROBIAL ACTIVITY

The *flower* extract of *Phyla nodiflora* ZnoNPs showed highest percentage of bacterial inhibition to *Staphylococcus* than compared to *Pseudomonas* . The zone of inhibition was found to be 16mm against *Staphylococcus.* The zone of inhibition was found to be 15mm against *Pseudomonas.* The reason have been that the smaller size of the particles which leads to increased membrane permeability and cell destruction. Similar results were found in *Boswellia ovalifoliolata* .

The antimicrobial effect of green synthesized ZnoNPs is attributed that the micro-organisms having of peptidoglycan, which is a complex structure and often contains teichoic acids or lipoteichoic acids and these are having strong negative charge. This charge may contribute to the sequestration of free Zinc ions. The finding (Sereemaspun *et al*.,2008)suggested species that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane. SNPs have an ability to interfere with metabolic pathways and bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptide substrates critical for cell viability and division( Shrivastava *et al*.,2007). The SNPs synthesized from stem of *Shorea tumbuggaia* toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications.

Zno is highly antimicrobial and this effect is de- pendent on superficial contact with enzymatic systems of the respiratory chain by alter DNA synthesis .Size reduction i.e. the nano-scale particle syntheses involve the increase of surface area for contact, which is an im- portant tool for the effects of Zinc and reasons to con- sider Zinc as superior is its broad antimicrobial activity (Spacciapoli *et al*.,2001)

Ever growing antibiotic resistant strains of bacteria constantly forces the scientific community to search for and to develop novel antibiotics and many new antibio- tics have been introduced in the last decade and unfortu- nately none of them was successful in combatting the multi-drug resistant strains (Conlon *et al*.,2003).

As the nanoparticles have delivered and demonstrated effective antimicrobial activities, the development of novel preparations in this field finds attractive alternative to antibiotics. To authen- ticate, nanoparticles have been examined for their ability to suppress microbial infections in skin ( Paddle-Ledinek *et al.*,2006) and burn wounds ( Ulkur *et al*., 2001), and even in preventing bacterial coloniza- tion and the results are promising(Paddle-Ledinek *et al.*,2006).

Studies reveal that the antimicrobial activity of the Zinc particles is due to their positive charge that qualifies them in reacting with the negatively charged proteins on the cell membranes and thus contributing to their antimicrobial activities .Many reports have suggested the efficacy of Zinc nanoparticles of their antimicrobial activities and to mention some as follows. Kim *et al*.,

have obtained positive results against *E. coli and S. aureus* where as a more profound effect was seen against *E. coli* and comparably a milder effect against S.aureus. (Hamouda *et al*.,2000)

The removal of free Zinc ions, due to the teichoic acid or liptoeichoic acid present in peptidoglycan, may be control the growth of pathogen. A recent study stated that the AgNPs also control the bacterial growth by inhibiting the oxidation based biological process by entering of metallic nano sized particles through the microsomal membrane.

Table:4 Antimicrobial activity of *Phyla nodiflora* ,Zinc nitrate and Zinc nano particle isolated from *Phyla nodiflora*

| S.N0 | Pathogens | Zone Of Inhibition In mm | | |
|---|---|---|---|---|
| | | Zinc Oxide | *Phyla nodiflora* | Zinc NPs |
| 1 | Pseudomonos | 10 | 7 | 15 |
| 2 | Staphylococcus | 12 | 8 | 16 |
| 3 | *Candida tropicalis* | 09 | 6 | 13 |

Antifungal Activity:

The antifungal activity against C*andida tropicalis* and *Candida albicans* (Table.4 )were studied. The maximum toxicity was observed in ZnNPs treated cells than zing nitrateand *flower* extract. With the fungal treatment, we observed the highest activity against *Candida tropicalis* Since it is observed the treated fungal cells with ZnNPs expressed significant damage like pit in their cell walls and pores in their plasma membrane.

In the case of fungal analysis, Zinc nano particle shows a promising growth inhibition. While increasing the concentration of the Zinc nano particles exhibit significant growth inhibition towards Candida albicans .The higher inhibition zone was observed for the higher

concentration. From this result it was observed that, nano particles shows better growth inhibition against the tested microorganisms.

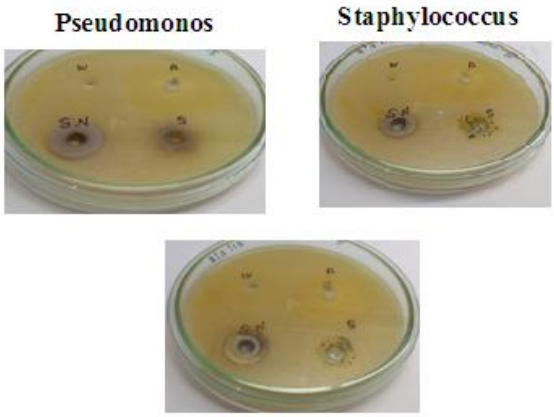
Fig 3 :Antibaterial effect of Silver

The mechanism of the antimicrobial action of Zinc ions were closely related to their interaction with thiol groups, although other target sites remain a possibility. Zinc was also proposed to act by binding to key functional groups of enzymes ( Nie *et al*., 1997). Zinc ions were caused the release of K+ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes, is an important target site for Zinc ions. In addition to their effects on bacterial enzymes, Zinc ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules. They were inhibited cell division and damaged the cell envelope and contents of bacteria. Bacterial cells were increased in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibited structural abnormalities. Finally, Zinc ions have interact with nucleic acids, they are interact preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of their lethal action is unclear .It was shown in the present study that the antimicrobial activity of the Zinc nanoparticles synthesized from *Phyla nodiflora* had sensitivity against the microbial strains Staphylococcus aureus, Pseudomonas aeuruginosa *Candida tropicalis* and Candida albicans. This was also evidenced by the work of (Wang *et al*.,2007)which used Pleurotus caju Zinc nanoparticles. Microbes are unlikely to develop resistance against Zinc, as they do against conventional and narrrow target antibiotics because the metal attacks a broad range of targets in the organisms which means that they would have to develop host mutations simultaneously to protect themselves.

*In-vitro* studies areperformed with microbicidal activity of the methanol extract of *Origanum majorana L* was tested against seven fungi (*Fusarium solani, Candida albicans, Aspergillus niger, A. parasiticus, Rhizopus oryzae, Rhizoctonia otyzae-sativae* and *Alternaria brassicicola*) and six bacteria (*Bacillus subtilis, B. megaterium, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*). The methanol extract of *O. majorana* can be used as an effective herbal protective species against different pathogenic

bacteria and fungi. High toxicity against the growth of *Aspergillus niger* was diagnosed (Leeja*et al*.,1995).

## Nano particles



Candida albicans

CONCLUSION

Nanotechnology finds extensive applications in nanomedicine, an emerging new field. Nanoparticles can be synthesised by chemical and physical methods but these methods are quite expensive and toxic . Use of biological organisms, plant extracts could be an alternative method for production of nanoparticles.The phytosynthesis of Zinc nanoparticles was demonstrated by visual inspection and by performing some spectral techniques (UV-VIS absorption, FTIR spectroscopy and SEM analysis).FTIR results proved that bioactive compounds responsible for Zing bioreduction could be proteins and flavonoids presumed to act as reducing and capping agents for the Zinc nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.These environmentally benign ZnNPs were further confirmed by using UV-Vis spectroscopy .The present study included the bio-reduction of Zinc ions through medicinal plants extracts and testing for their antimicrobial activity.The antimicrobial assay showed that Zinc nanoparticles presented good antibacterial performance against clinical pathogens.The results indicated that ZnNPs have good antimicrobial activity against different microorganisms. It is confirmed that ZnNPs of *Phyla nodiflora* are capable of rendering

antimicrobial efficacy and hence has a great potential in the preparation of drugs used against bacterial and fungal diseases

To conclude we have used unreported, inexpensive, nontoxic, ecofriendly and abundantly available *Phyla nodiflora* for the rapid synthesis of ZnO NPs in the range of 9-10nm. FT-IR studies of aqueous Citrus aurantifolia extract reveals the presence of phyto constituents like alcohol, aldehyde and amine which were the surface active molecules stabilized the nanoparticles and this phytochemicals have interacted with the zinc surface in the stabilization of zinc oxide nanoparticles. The green synthesis approach shows that the environmentally begined and renewable Citrus aurantifolia extract can be used as an effective stabilizing as well as reducing agent for the synthesis of zinc oxide nanoparticles

## BIBLIOGRAPHY


1. Abha verma1, prakash joshi2 & arvind arya (2013) synthesis of plant-mediated Zinc nanoparticles using plant Extract of *sonchus asper* International Journal of Nanotechnologyand Application (IJNA)Vol. 3, Issue 4, Oct 2013, 11-18
2. Ramesh P. et al. / Asian Journal of Phytomedicine and Clinical Research. 2(4), 2014, 189 - 195.
3. Acker C I, Brandão R, Rosário A R, Nogueir C W. (2009)Antioxidant effect of alkynylselenoalcohol compounds on liver and brain of rats in vitro Environmental Toxicology and Pharmacology 2009; 28: 280–287 Res.11, 1453–1463
4. Biswas, A., Aktas, O.C., Schumann, U., Saeed, U., Zaporjtchenko, V. and Faupel, F. (2004). Tunable multiple plasmon resonance wavelengths response from multicomponent polymer-metal nanocoposite systems. Applied Physical Letter, 84: 2655-2657.
5. De N, James NA (2002). Antibacterial spectrum of extracts of Ocimum gratissium L. (Basil) and Xylopia aetiopica A. Rich. (Dunah). Niger. J. Basic Appl. Sci. 11:165-175.
6. Debabrat Baishya*, Nakul Sharma and Rituparna Bora(2012) Green Synthesis of Zinc Nanoparticle using Bryophyllum pinnatum (Lam.) and monitoring their antibacterial activities .

7. Dewanjee S, Kundu M, Maiti A, Majumdar R, Majumdar A and Mandal SC, (2007). In vitro evaluation of antimicrobial activity of crude extract from plants Diospyros peregrina, Coccinia grandis and Swietenia macrophylla. Trop. J. Pharm. Res., 6: 773-78

8. E. Ulkur, O. Oncul, H. Karagoz, E. Yeniz and B. Celikoz, (2005) "Comparison of Zinc-Coated Dressing (Acticoat), Chlo- rhexidine Acetate 0.5% (Bacti-Grass), and Fusidic Acid 2% (Fucidin) for Topical Antibacterial Effect in Methicil- lin-Resistant Staphylococci-Contaminated, Full-Skin Thi- ckness Rat Burn Wounds," Burns, Vol. 31, No. 7, 2005, pp. 874-877. doi:10.1016/j.burns.2005.05.002

9. Farnsworth AC (1982). The role of ethnopharmacology drug development from plants. John Wiley and Sons, England (Ciba found. Symp. 154). pp. 2-10.
10. Farooqui MA, Chauhan PS, Krishnamoorthy P, Shai J. (2010) Extraction of Zinc nano-particles from the leaf extracts of *Clerodendrum inerme*. *Di* Kaushik Thakkar N, Snehit Mhatre S, Rasesh Parikh Y, Biological synthesis of metallic nanoparticles. Nanomedicine: Nanotechnology, *Biology and Medicine*. 2010; 2, 257-262. *gest Journal of Nanomaterials and Biostructures*. 2010; 5: 43-49.
11. Govindraju, K., Kiruthiga, V. and Singaravelu, G. (2008). Evaluation of biosynthesized Zinc nanoparticles against fungal pathogens of mulberry Morus indica. Journal of Biopesticides, 1: 101-104.
12. Govindraju, K., Kiruthiga, V., Ganesh Kumar, V. and Singaravelu, G. (2009). Extracellular synthesis of Zinc nanoparticles by a marine alga, Sargassum wightii Grevilli and their antibacterial effects. Journal of Nanoscience and Nanotechnology, 9: 5497-5501

13. I. Dragieva, S. Stoeva, P. Stoimenov, E. Pavlikianov and K. Klabunde, (1999)"Complex Formation in Solutions for Che- mical Synthesis of Nanoscaled Particles Prepared by Borohydride Reduction Process," Nanostructured Mate- rials, Vol. 12, No. 1, 1999, pp. 267-270. doi:10.1016/S0965-9773(99)00114-2

14. Indrayan AK, Sharma A, Gideria BS, Gupta CP (2002). Antimicrobial activity of dye from Caesalpina sappan (Patang/Braziluvoral). Indian J. Microbiol. 42: 359-360.

15. J. C. Huralikuppi, A. B. Christopher, P. M. Stephen 1991.Anti-diabetic effect of *Nelumbo nucifera* (Gaertn): Part I preliminary studies in rabbits. Journal of Biopesticides, 1: 101-104

16. J. E. Paddle-Ledinek, Z. Nasa and H. J. Clelanz(2006) "Effect of Different Wound Dressings on Cell Viability and Pro-liferation," Plastic and Reconstructive Surgery, Vol. 117, No. 7, 2006, pp. 110S-118S.

17. J. M. Conlon, J. Kolodziejek and N. Nowotny, (2004) "Antim-icrobial Peptides from Ranid Frogs: Taxonomic and Phy-logenetic Markers and a Potential Source of New Thera-peutic Agents," Biochimica et Biophysica Acta, Vol. 1696, No. 1, 2004, pp. 1-14.
18. Jain D, Daima HK, Kachhwaha S, Kothari SL. (2009)Synthesis of plant-mediated Zinc Nanoparticle using papaya fruit extract and evaluation of their anti-microbial activities. *Digest journal of nanomaterials and biostructures*. 2009; 4:557

19. Leeja and Ameh 1275 Cheesbrough M (1995). Biochemical Tests to Identify Bacteria. In: Laboratory Practice in Tropical Countries Cambridge Edition. pp. 63-70.
20. M. E. Barbinta Patrascu, A. Cojocariu, L. Tugulea, N. M. Badea, I. Lacatusu, A. Meghea, J (2011).Optoelectron Adv Mater 13(9), 1165 (2011).
21. M. Sendova-Vassileva, M. Sendova, R. Ruther, J. C. Pivin, J Optoelectron Adv Mater 9(1), 186 (2007).
22. Nameirakpam Nirjanta Devi1, Dheeban Shankar. P, Sutha.S (1074-1079.)Biomimetic Synthesis nanoparticle-hydrogel composites. Journal of Advanced Materials, 16: 1074-1079.
23. Nguyen Thi Mai Tho; Tran Nguyen Minh An; Mai Dinh Tri; Thupakula Venkata Madhukar Sreekanth; (2013)Jae-Soon Lee; Patnamsetty Chidanandha Nagajyothi; Kap Duk Lee(2013)Green Synthesis of Zinc Nanoparticles Using Nelumbo nucifera Seed Extract and its Antibacterial Activity. Journal of Biopesticides, 1: 101-104

24. Nie, S. and Emory, S.R. (1997). Probing single molecules and single nanoparticles by surface enhanced Raman Scattering. Science, 275: 1102-1106.