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ORIGINAL RESEARCH ARTICLE

Biosynthesis and Antimicrobial Activity of Silver Nanoparticles from *Murraya koenigii, Ocimum teniflorum*, Chitin and Chitosan

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ABSTRACT

Green synthesis of silver nanoparticles and the study of their antimicrobial properties are of fundamental importance in the advancement of recent research. In this paper we describe the synthesis of silver nanoparticles using plant extract of *Murraya koenigii* (Green Curry Leaves), *Ocimum teniflorum* extract and polysaccharides-Chitin and Chitosan. Synthesized silver nanoparticles were confirmed by sampling the reaction mixture and the absorption maximum was scanned by UV–Visible spectra, at the wavelength of 300–600 nm. The antibacterial activity against different pathogen (*Escherichia coli*, *Staphylococcus aureus*) and control along with Growth kinetics of *Bacillus subtilis* and *Klebsiella pneumonia* were reported. The zone of inhibition is observed both in gram positive and gram negative bacterial strains. They appeared to have satisfactory inhibitions against the four mentioned microorganisms.

Key words- *Murraya koenigii* extract, *Ocimum teniflorum* extract polysaccharides, Chitin, Chitosan, AgNPs, antibacterial

INTRODUCTION

In recent years, Nanotechnology has attracted many researchers from various fields like biotechnology, physics, chemistry, material sciences, engineering, and medicine. Nanoparticles are synthesized by physical and chemical methods; these are suffering from drawbacks like expensive reagent, hazardous reaction condition, longer time, tedious process to isolate nanoparticles. Hence, there is scope to develop new methods for the synthesis of nanoparticles which should be required inexpensive reagent, less drastic reaction condition and eco-friendly [1-4].

The Curry tree (*Murraya koenigii*) is a tropical to sub-tropical tree in the family Rutaceae, which is native to India and Sri Lanka. It is a small tree, growing 4–6 m (13–20 feet) tall, with a trunk up to 40 cm diameter. The leaves are fresh and pleasant and enhance the taste of the dish in which they are used. The leaves of *Murraya koenigii* are also used as an herb in Ayurvedic medicine. They are believed to possess anti-diabetic properties [5]. Curry leaf has recently been found to be a potent antioxidant due to high concentrations of carbazoles, a water soluble heterocyclic compound which has been reported by Rai *et al*. may be responsible for the reduction and stabilization of metal ions [6-10].

Ocimum teniflorum (local name Tulasi) is a traditional medicinal plant of India has a source of bio-reduction and stabilizers. The constituent of Tulsi are alkaloids, glycosides, tannins, saponins and aromatic compounds. It is used in the treatment of headaches, coughs, diarrhea, constipation, worts, worms and kidney malfunctions. Recent interest on *Ocimum* has resulted from its inhibitory activity against HIV-1 reverse transcriptase and platelet aggregation induced by collagen and ADP22 (adenosine-5-diphosphate). *Ocimum teniflorum* leaf extracts have been used in the synthesis of silver nanoparticles and gold nanoparticles [11].

Chitin/chitosan is the collective name for a family of de-N-acetylated chitin with different degree of deacetylation. In general, when the number of N-acetylglucosamine units exceeds 50%, the

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biopolymer is termed chitin, whereas the term "chitosan" is used to describe the polymer when the N-acetylglucosamine content is less than 50%. Chitin/chitosan has been studied as a natural cationic biopolymer because of its excellent biocompatibility, biodegradability, nontoxicity, antimicrobial capability, and stimulation of wound healing [12,13]. The aim of this work was to formulate and evaluate the antibacterial activity of simple and cost-effective silver nanocomposites using *M. koenigii* extract, *O.teniflorum* extract Chitin and chitosan.

MATERIALS AND METHODS

Broth extraction for *Murraya koenigii*

The curry leaf extract was prepared with 10g of fresh curry leaves, which were thoroughly rinsed with deionized water and cut into small pieces. The chopped leaves were boiled in 75mL of deionized water for 3 minutes. The leaf broth was then cooled and filtered yielding 50mL of broth.

Synthesis of silver nanoparticles

5mL curry leaf broth was added to 100mL 10^{-3} M silver nitrate and allowed to react at ambient conditions. The observed color change of reaction mixture from transparent yellow to dark brown indicates the formation of silver nanoparticles. Further the reduction of the Ag ions was monitored over time by UV-visible spectral analysis. The suspension of silver nanoparticles was allowed to settle and the excess liquid was removed. The particles were then rinsed to remove any organic residue and re-suspended in 95% ethanol (Fisher scientific) for further characterization.

Preparation of *Ocimum teniflorum* plant leaf extract, silver ion complex and green synthesis silver nanoparticles

The plant leaves of *Ocimum teniflorum* were washed thrice with tap water and distilled water and kept in the room temperature for air dry. After drying the known amount of leaf samples were chopped into fine and small pieces. The chopped 25 gram of leaves added with 100 ml of distilled water and boiled up to 100℃ for 30 minutes. After the desired reaction period the desired samples were filtered through Whatmann filter paper to get the leaf extract. Leaf extracts were stored at -20℃ for further study.

For the preparation of 1mM silver nitrate, 0.0421gm of $AgNO_3$ was added to 100 ml of double distilled water. The solution was mixed thoroughly and stored in colored bottle in order to prevent auto oxidation of silver. For the synthesis of plant mediated silver nanoparticles, the leaf extract and 1mM silver nitrate solution were taken in 1:4 ratio respectively and kept on a water bath at 60℃ for 30 minutes until the color change was observed. This indicates the preliminary confirmation for the formation of plant mediated silver nanoparticles.

Preparation, characterization of Chitin Bionanocomposites

Preparation of AgNPs

Briefly, 0.50 g of silver-containing glass powder was dispersed in 50 mL of an aqueous solution of 0.25, 1, or 4.0 wt% glucose in a 100 mL glass vial. The mixture was at 121° C and 200 kPa for 20 min. The mixture was then gradually cooled to room temperature and centrifuged at 3000 rpm for 10 min. The supernatant containing the Ag NP suspension was removed and stored in the dark at 4° C.

Preparation of Ag NP/ Chitin Composites

In this study, 10 mg of chitin (<5% DAc) was added to 1 mL of each Ag NPs suspension (about 60 μg/mL). The mixture was mixed well (at pH 7.0) on a shaker for 30 min. The insoluble Ag NP/chitin composites were centrifuged at 6000 rpm for 10 min. The centrifuged composites were washed twice with distilled water by centrifugation at 6000 rpm for 10 min. The washed composites were dried up at 70C on a blockheater for 2 h.

Preparation, characterization of Chitosan Bionanocomposites

Preparation of silver-chitosan nanocomposites

A solution of chitosan (1 - 3 mg/ml) in acetic acid solution (1 - 2 %) was first prepared. Due to the poor solubility of chitosan, the mixture was vortexed to achieve complete dissolution, and then kept overnight at room temperature. The solution was filtered through a 0.22 µm millipore syringe filter to remove any impurity before use. Silver-chitosan nanocomposites were obtained by chemical reduction of the silver salt to yield the corresponding zero valent silver nanoparticles with $NaBH_4$. To ensure complete reduction, the concentration of $NaBH_4$ was 10 times that of the silver salt. The silver nanoparticles were separated by centrifugation at 15000 rpm and dried at 60 ºC for 24 h on a Petri dish, yielding a thin layer.

UV-VIS spectra analysis

The silver nanoparticles show the Plasmon resonance at 320 to 500 nm in the UV-Visible

spectrum. The UV-Visible spectrum of synthesized silver nanoparticles was analysed by spectrophotometer (UV-Visible Perkin ElmerLambda)

Antimicrobial activity

The microorganisms used for the study were *Escherichia coli* and *Staphylococcus aureus.* Mueller Hinton agar (HI media) was used for the performance of the antimicrobial assay. Erythromycin (10µg) was used as controls for the bacteria's. Wells were made (6mm diameter) by using an autoclave sterilized metallic borer. Well isolated fresh colonies of the microorganisms were used to prepare inoculums suspension equivalent to 0.5 Standard McFarland Turbidity (which is 1.5×10^8 Colony Forming Units per ml); microbes were inoculated and incubated at 37ºC for 24 hours. After 24 hours the media were examined for inhibition zones and results were recorded in millimeter.

Growth kinetics

For this assay 1ml (10^4 cells/ml) of freshly grown *Bacillus subtilis* and *Klebsiella pneumonia* were inoculated to the each flask containing 50ml of prepared nutrient broth and the culture was incubated with silver nanoparticles with the concentration 40 µg/ml for 24hrs in orbital shaking incubator for 24hrs at 37ºc with 120rpm. To know the growth kinetics the OD values were taken at 600nm for each and every 2hours of interval time along the control.

RESULTS AND DISCUSSION

Nano-science is the study of phenomena and manipulation of materials at atomic molecular and macromolecular scales. Addition of $AgNO_3$ to the leaf broth of *Murraya koenigii* resulted in the formation of orange color at 30 minutes which changed to dark brown after incubation at 90 minutes indicating completion of reduction. The colour of the reaction medium in the case of *Ocimum teniflorum* changed rapidly from colorless to brown in the 1:4 ratios. That brown colour indicated that surface plasmon vibrations, typical of silver nanoparticles. Further, Chitin (<5% DAc) was added as stabilizer to the Ag NP suspensions to remove the generated caramel and to prevent agglomeration and precipitation of the AgNPs. The composites so formed were twice with water to remove the caramel. The composites were brown coloured. Similarly, addition of $NaBH_4$ leads to reduction of $AgNO_3$ whereby chitosan is added as stabilizer for synthesis of AgNP's. The AgNP's so produced are dark brown in colour. Similar results are reported by earlier worker [14-17].

UV-VIS spectra analysis

The formation of silver nanoparticles was confirmed through measurement of UV-Visible spectrum of the reaction mixture. The UV-Visible spectrophotometric analysis of silver nanoparticles using *M.koeingii* leaf extract showed peak at 330 nm. Laura *et al* reported absorbance peak at 435 nm for *M.koeingii* leaf extract nanoparticle[14]. The maximum peak was observed at 390 nm in the case of *O.teniflorum* leaf extract. Vikas et al reported maximum peak at 440 nm for the nanoparticle synthesized using *M.indica* leaf extract [15]. The UV-Spectra showed maximum peak at 370 nm for Chitin nanoparticle and at 380 nm for Chitosan Nanoparticle. Similar results were reported by Vihn *et al* whereby the UV spectra showed maximum peak at 390.5 nm for Chitin Nanoparticle whereas Honary *et al* reported absorbance bands between 400-420 nm for Chitosan Nanoparticle (**Fig 3. 4, 5 & 6**) [14,15,5,16].

Antimicrobial activity

The antimicrobial activity of the synthesized nanoparticles and standard was analysed using disk diffusion method. Synthesized nanoparticles showed antibacterial activity comparable to the standard used against both bacteria (*E.coli and S.aureus*). The zone of inhibition was found to be highest against Chitin AgNP's in case of *E.coli* and *Murayya koenigii* AgNP's in case of *S.aureus* and the antimicrobial activity was found to comparable to that of the commercial antibiotic. The zone of inhibition was observed and was found to be highest against Chitosan AgNP's in case of *E.coli* and in *O.teniflorum* AgNP's in case of *S.aureus* depicting antimicrobial strengths similar to that of the commercial antibiotic. Sushmita reported that *S.aureus* was inhibited at the low concentration of Ag nanoparticles, whereas the growth-inhibitory effects on *E.coli* were mild in the case of *M.koeingii* AgNPs [18]. Vikas et al indicated that *M.indica* silver nanoparticles undergo an interaction with bacterial cell and displayed the strong action against *Staphylococcus aureus, Escherichia coli, Pseudomonas fragi, Bacillus subtilis, Streptococcus agalactiae* and *Proteus vulgaris* [15] (**Table 1 & 2**).Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems [18].

Growth kinetics

Silver nanoparticles at concentration 40 µg/ml were added to the flask containing 50 ml of nutrient broth inoculated with *Bacillus subtilis* and *Klebsiella pneumonia*. To study the growth kinetics the OD values were taken at 600nm for each and every 2hours of interval time along the control. And the curve was plotted and shown in. It has been observed from the Growth kinetics plot that the optical absorption in growth medium induced with the silver nanoparticles showed consistent decline as compared to that of the control indicating substantial amount of antimicrobial activity displayed by the nanoparticles (**Figure 7, 8, 9 & 10**) Bhanu Prakash *et al* showed that the absorbance in the growth media seeded with *Vinca roseus* silver nanoparticles was less as compared to that of the control when tested against *B.subtilis* and *P.aeruginosa*.[19]

Klabunde and co-workers demonstrated that reactive metal oxide nanoparticles show excellent bactericidal effects. Studies conducted by researchers in the recent past revealed that metal oxide nanoparticle formulations possessed significant antibacterial activity. Recently it was shown that highly concentrated and nonhazardous nanosized silver particles can easily be prepared in a cost-effective manner and tested as a new type of bactericidal nanomaterial. These silver nanoparticles may be used in effluent treatment process for reducing the microbial load. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic nanomaterials [20,21].

Table1: Antimicrobial activity of AgNPs of *Murraya koenigii* , Chitin and Chitosan by disk diffusion method

Zone of Inhibition (in mm)				
Bacteria	***Murraya koenigii* AgNP's**	**Chitin AgNP's**	**Chitosan AgNP's**	**Standard (Erythromycin)**
E.coli	14	20	16	22
S.aureus	28	26	26	30

Table2: Antimicrobial activity of AgNPs of *O.teniflorum,* Chitin and Chitosan by disk diffusion method

Zone of Inhibition (in mm)				
Bacteria	***O.teniflorum* AgNP's**	**Chitin AgNP's**	**Chitosan AgNP's**	**Standard (Erythromycin)**
E.coli	16	14	20	12
S.aureus	31	30	28	32

Figure1: Photograph of *Murraya koenigii* nanoparticles, Chitin (CH) and Chitosan (CS) Bionanocomposites

Figure2: Photograph of *O.teniflorum* nanoparticles, Chitin (CH) and Chitosan (CS) bionanocomposites

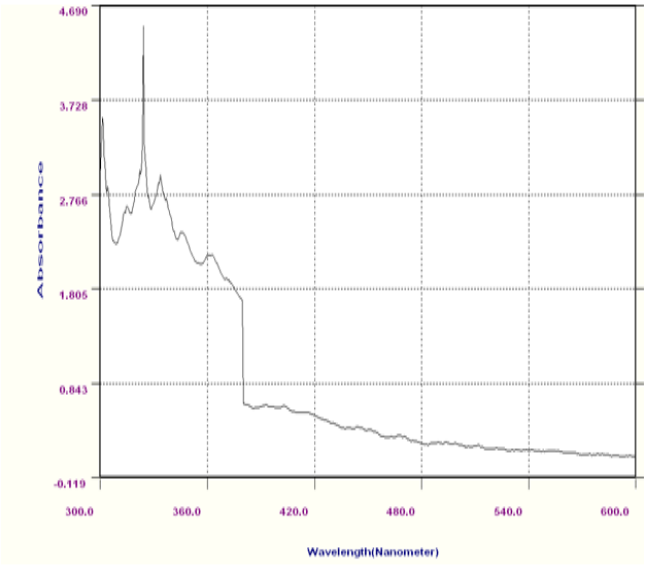

Fig 3: UV- visible spectra of *M.koeingii* leaf Nanoparticle as a function of time

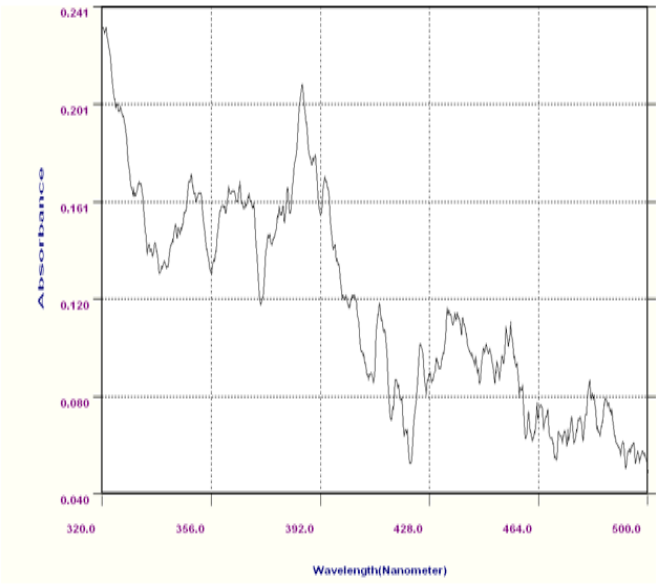

Fig 4: UV- visible spectra of *O.teniflorum* leaf Nanoparticle as a function of time

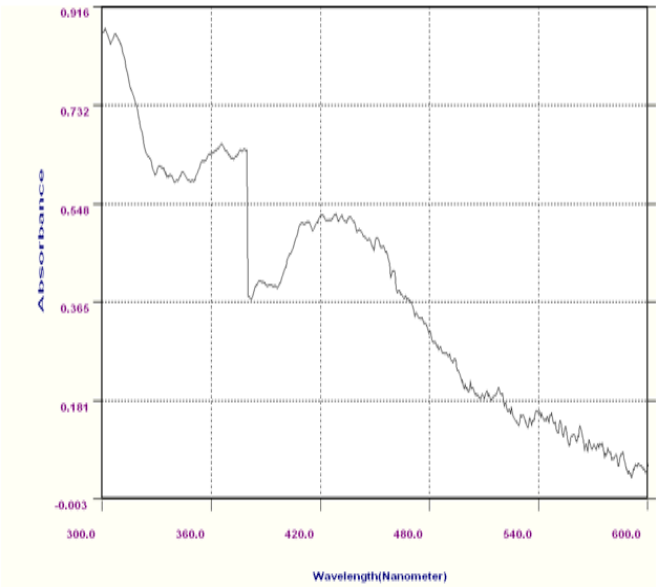

Fig 5: UV- visible spectra of Chitin Nanoparticle as a function of time

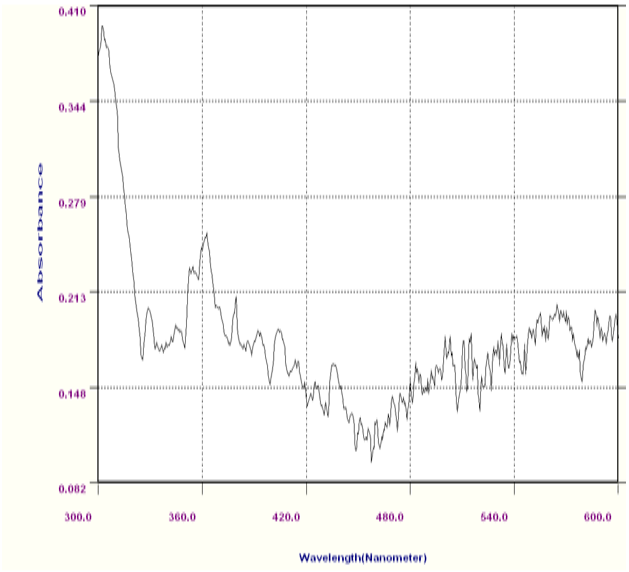

Fig 6: UV- visible spectra of Chitosan Nanoparticle as a function of time

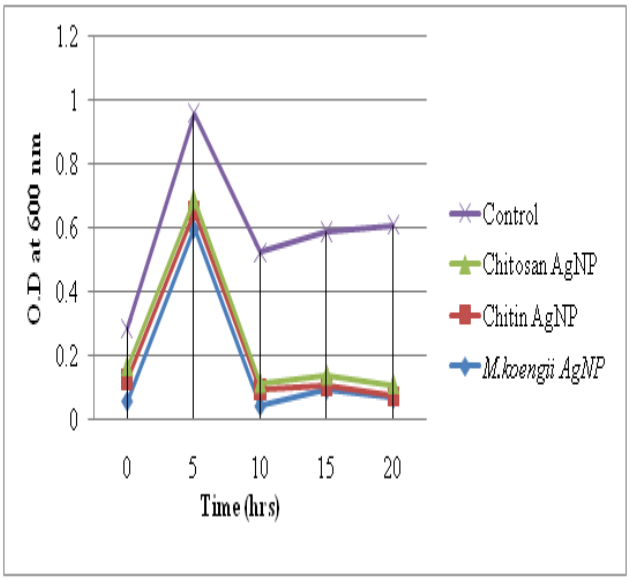

Figure 7:.The effect of green synthesized silver nanoparticles on the growth of a) *B. subtilis*

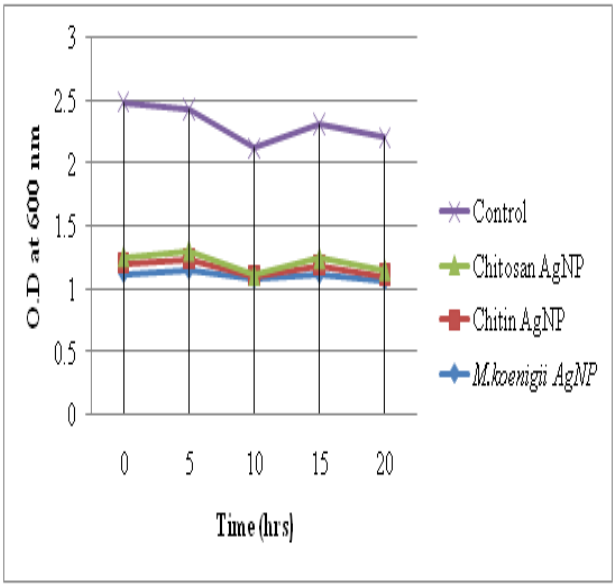

Figure 8: The effect of green synthesized silver nanoparticles on the growth of b) *K.pneumonia*

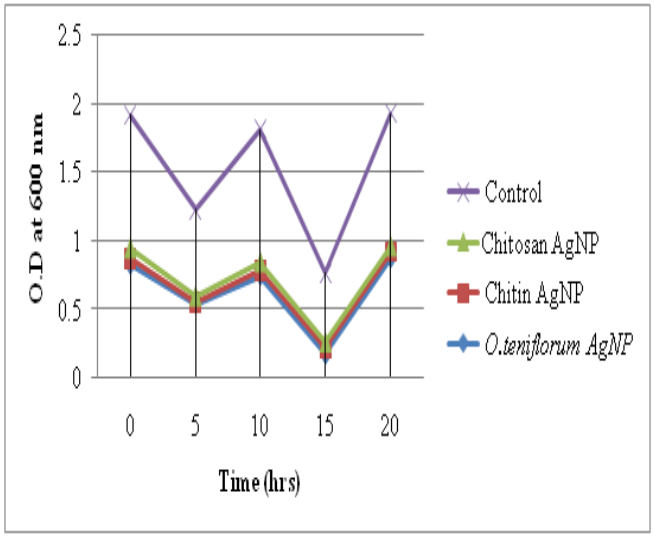

Figure 9: The effect of green synthesized silver nanoparticles on the growth of a) *B. subtilis*

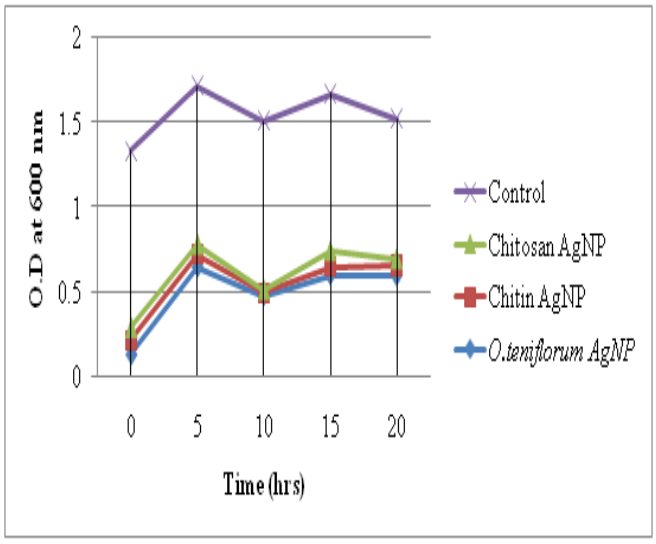

Figure 10: The effect of green synthesized silver nanoparticles on the growth of b) *K.pneumonia*

REFERENCE

1. Lanje A. S., Sharma S. J., Pode R. B., Ningthoujam R. S., Synthesis and optical characterization of copper oxide nanoparticles Advances in Applied Science Research (2010) 1 (2), 36-40.
2. Yang G., Chai S., Xiong X., S. Zhang, YU L., Zhang P. Preparation and tribological properties of surface modified Cu nanoparticles Trans. Nonferrous Met. Soc. China (2012) 22(2), 366-372.
3. Borkow G.,Molecular mechanisms of enhanced wound healing by copper oxide –impregnated dressings Wound Repair Regen (2010) 18(2), 266-275.
4. Borkow G., Zatcoff R. C., Gabbay J., Reducing the risk of skin pathologies in diabetics by using impregnated socks Med. Hypotheses (2009) 73(6),883-886.
5. Arulselvan P, Subramanian SP., Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic beta-cells in experimental diabetes in rats, 2007,155–64.
6. Kumar, V, Yadav, S. K. J. Chem. Technol. Biot. 2009, 84: 151.
7. Adebajo, A. C.; Olayiwola, G.; Verspohl, J. E.; Iwalewa, E. O.; Omisore, N. O. A.; Bergenthal, D. et al, Pharm. Biol. 2004; 42(8): 610.
8. Tachibana, Y.; Kakusaki, H.; Lajis, N. H.; Nakatani, N. J. Agr. Food Chem. 2003; 51: 6461.
9. Tachibana, Y.; Kikuzaki, H.; Lajis, N. H.; Nakatani, N. J. Agr. Food Chem. 2001; 49, 5589
10. Rai, M.; Yadav, A.; Cade, A. Crit. Rev. Biotechnol. 2008; 28: 277
11. Mallikarjuna K., Narasimha G., Dillip G. R., Praveen B. , Shreedhar B., Sree Lakshmi C., Reddy B. V. S.,Prasad Raju B. D., Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization Digest Journal of Nanomaterials and Biostructures (2011) 6(1), 181 - 186.
12. C. Shi, Y. Zhu, X. Ran, M. Wang, Y. Su, and T. Cheng, “Therapeutic potential of chitosan and its derivatives in regenerative medicine,” Journal of Surgical Research, 2006 133; 185– 192.
13. J. Dutta, S. Tripathi, and P. K. Dutta, “Progress in antimicrobial activities of Chitin, Chitosan and its oligosaccharides: a systematic study needs f or food application,” Food Science and Technology International, 2012; 18:20–31.
14. Laura Christensen, Singaravelu Vivekanandhan, Manjusri Misra, Amar Kumar Mohanty, Biosynthesis of silver nanoparticles using *Murayya koenigii* (curry leaf):An investigation on the broth concentration in reduction mechanism and particle size Adv. Mat. Lett. 2011; 2(6): 429-434
15. Vikas Sarsar, Krishan K. Selwal and Manjit K. Selwal Green synthesis of silver nanoparticles using leaf extract of *Mangifera indica* and evaluation of their antimicrobial activity J. Microbiol. Biotech. Res., 2013; 3 (5): 27-32

16. Vinh Quang Nguyen, Satoko Kishimoto,Yasushi Miyahira,Masayuki Ishihara, Hidemi Hattori, and Takemi Matsui, et al , Preparation of Size-Controlled Silver Nanoparticles and Chitin-Based Composites and Their Antimicrobial Activities. Journal of Nanomaterials ,2013; 3:1-7
17. S Honary, K Ghajar, P Khazaeli and P Shalchian, Preparation, Characterization and Antibacterial Properties of Silver-Chitosan Nanocomposites Using Different Molecular Weight Grades of Chitosan, Tropical Journal of Pharmaceutical Research February 2011; 10 (1): 69-74
18. Sushmita Deb Synthesis Of Silver Nano Particles Using *Murraya Koenigii* (Green Curry Leaves), *Zea Mays* (Baby Corn) And Its Antimicrobial Activity Against Pathogens International Journal of PharmTech Research 2014; 1: 91-96
19. M. Bhanu Prakash and Subhankar Paul, Green synthesis of silver nanoparticles using *Vinca roseus* leaf extract and evaluation of their antimicrobial activities, International Journal of Applied biology and Pharmaceutical Technology, 2012; 3(4): 105-111.
20. P.K. Stoimenov, R.L. Klinger, G.L. Marchin, K.J. Klabunde Langmuir, 2002; 18: 66-79.
21. Sondi, D.V. Goia, E. Matijevic J. Colloid Interface Sci., 2003; 260: 75.