Received: 21-02-2014
Accepted: 20-03-2014

ISSN: 2321-4902
Volume 1 Issue 6

Online Available at www.chemijournal.com

International Journal of Chemical Studies

Green synthesis and antimicrobial activity of silver nanoparticles from *Calotropis gigantea, Catharanthus roseus,* Chitin and Chitosan

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Silver nanoparticles (AgNPs) due to biocompatibility, antibacterial action and its various applications in the field of electronics, optical, magnetic have attracted considerable attention. In this study crystalline silver nanoparticles were successfully produced from silver nitrate using *Calotropis gigantea* extract, *Catharanthus roseus* extract and polysaccharides-Chitin and Chitosan. The AgNPs were characterized using UV-visible spectroscopy. The antibacterial activity against different pathogens (*Escherichia coli*, *Staphylococcus aureus*) and growth kinetics for *Bacillus subtilis, Klebsiella pneumonia* were reported. The zone of inhibition is observed both in gram positive and gram negative bacterial strains. They were found to have considerable inhibitory action against the four mentioned microorganisms.

Keyword: *Calotropis gigantea* extract, *Catharanthus roseus* extract, polysaccharides, Chitin, Chitosan, AgNPs, antibacterial.

1. Introduction

Bionanotechnology has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for synthesis of nanomaterials. Nanoparticles (AgNPs) are clusters of atoms in the size range of 1–100 nm. "Nano" is a Greek word synonymous to dwarf meaning extremely small. The use of nanoparticles is gaining impetus in the present century as they possess defined chemical, optical and mechanical properties. The metallic nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains [1].

The plant *Calotropis gigantea* belonging to the family Asclepiadaceae, also called as Alarka, Shwetarka, Mandara, Vasuka; is distributed throughout India, the dry waste land. *Calotropis gigantea* is a species of *Calotropis* native to Cambodia, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka, India and China. The different parts of the plant are used in Indian traditional medicine for the treatment of painful muscular spasm, dysentery, fever, rheumatism, asthma and as an expectorant and purgative [2, 3, 4, 5].

Catharanthus roseus (*C. roseus*) (L.) G. Don. (Apocynaceae) is one of the important medicinal plants, due to the presence of the indispensable anti-cancer drugs, i.e., vincristine and vinblastine.

The roots of this plant are the main source of the anti-hypertension alkaloid ajmalicine. It is also a popular ornamental plant. There are commonly two varieties of this plant based on the flower colour viz., pink flowered rosea and white flowered alba. It contains more than 70 alkaloids mostly of the indole type. It has medicinal importance owing to the presence of alkaloids like ajmalicine, serpentine and reserpine, which are well known for their hypotensive and antispasmodic properties. *C. roseus* exhibited high in vitro antiplasmodial activity, which may be due to the presence of compounds such as alkaloids, terpenoids, flavonoids and esquiterpenes that were previously isolated from the plant. It also possesses known antibacterial, antifungal, antibiotic, antioxidant, wound healing and antiviral activities [6, 7, 8, 9, 10, 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 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 *Calotropis gigantea* extract, *Catharanthus roseus* extract, Chitin and chitosan for possible use as dressings.

2. Materials and Methods

2.1 Preparation of Plant extract

Freshly collected *Calotropis gigantea* leaf were washed with double distilled water and finely cut the leaf. A known amount of leaf was added to 100 ml of deionized water and boiled for 15 min in a water bath. The mixer was then filtered to obtain the aqueous extract of definite concentrations.

2.2 Preparation of silver nanoparticles

Silver nanoparticles were synthesized by reducing the freshly prepared 1 mM silver nitrate ($AgNO_3$) and stored under dark conditions with aqueous extract of the plant. The reaction mixture was prepared in a ratio of 9: 1 (V/V) comprised of freshly prepared silver nitrate solution and plant extract respectively. The initial color of the solution was observed. The solution was stored at room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours the reaction mixture was centrifuged and pellets were collected followed by washing with deionized water and dried in a water glass.

2.3 Preparation of *Catharanthus roseus* plant leaf extract

10 g of *Catharanthus roseus* plant leaves were taken and cut down into small pieces, then they were surface cleaned by running tap water and followed by distilled water and boiled in 100 ml of distilled water at 60 °C for 20 mins. After that the aqueous leaf extract was filtered through the Whatman.No.1 filter paper, followed by Millipore filter (0.22 µm) and the filtrate was stored at 4 °C for further experiments.

2.4 Silver Nanoparticle synthesis

For preparation of a silver nanoparticle synthesis 2 ml of aqueous leaf extract was added to the Erlenmeyer flask containing 98 ml of $AgNO_3$ (1 mM).The mixture was kept in incubation period for 20 minutes at room temperature.

2.5 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.

2.6 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 70º C on a blockheater for 2 h.

2.7 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.

2.8 UV-VIS spectra analysis
The silver nanoparticles show the Plasmon resonance at 320 to 500 nm for in the UV-Visible spectrum. The UV-Visible spectrum of synthesized silver nanoparticles was analysed by spectrophotometer (UV-Visible Perkin ElmerLambda).

2.9 Antimicrobial activity
The microorganisms used for the study were *Escherichia coli*, *Staphylococcus aureus.* Mueller Hinton agar (HI media) was used for the performance of the antimicrobial assay. Erythromycin (10 µg) was used as controls for the bacteria's. Wells were made (6 mm 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 37 ºC for 24 hours. After 24 hours the media were examined for inhibition zones and results were recorded in millimeter.

2.10 Growth kinetics
For this assay 1 ml (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 24 hrs in orbital shaking incubator for 24 hrs at 37 ºC with 120 rpm. To know the growth kinetics the OD values were taken at 600 nm for each and every 2hours of interval time along the control.

3. Results and Discussion
3.1 Formation of Silver Nanoparticles
Several approaches have been employed to obtain a better synthesis of silver nanoparticles such as chemical and biological methods [13, 23]. Addition of Silver nitrate to the *C. gigantea* leaf extracts in the ratio of 9:1 resulted in the change of the reaction medium from colourless to brown (Fig. 1). That brown colour indicated that surface plasmon vibrations, typical of silver nanoparticles [1]. The control $AgNO_3$ solution (without extract) showed no colour change. As the *C. roseus* aqueous leaf extract was added to the silver nitrate solution and incubated the mixture color was changed rapidly from the transparent color to brown yellowish color due to the formation of AgNP's. The appearance of yellowish brown color was due to the excitation of surface plasmon vibrations of silver nanoparticles. Chitin (<5% DAc) was added as stabilizer. The generated AgNP/ Chitin composites were washed with distilled water twice to remove caramel. The color of the composites was brown. Next Chitosan was used as stabilizer with $NaBH_4$ as reducing agent. The AgNO3/ Chitosan suspension was colourless which turned to dark

brown on the addition of $NaBH_4$. Similar results are reported by earlier worker [14,15,5,16].

Fig 1. Photograph of *C. gigantea* nanoparticles, Chitin (CH) and Chitosan (CS) Bio-nanocomposites

Fig 2: Photograph of *C .roseus* nanoparticles, Chitin (CH) and Chitosan (CS) Bio-nanocomposites

3.2 UV-VIS Spectra Analysis

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles. The green synthesis of silver nanoparticles using *C. gigantea* leaf extract, *C. roseus* leaf extract, Chitin and Chitosan was successfully carried out. The change in the colour of the solution exhibited the reduction of the silver nitrate in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles [16]. 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 *C. gigantea* leaf extract showed peak at 320 nm. Sivakumar *et al*. reported absorbance peak at 420 nm after 24 hours incubation for *C. gigantea* leaf extract nanoparticle. The maximum peak was observed at 300 nm in the case of *C. roseus* leaf extract nanoparticle. Ponarulselvam *et al*. reported maximum peak at 410 nm for the nanoparticle synthesized using *C. roseus* leaf extract. The UV-Spectra showed maximum peak at 330 nm for Chitin nanoparticle and at 310 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 [14, 15, 5, 16].

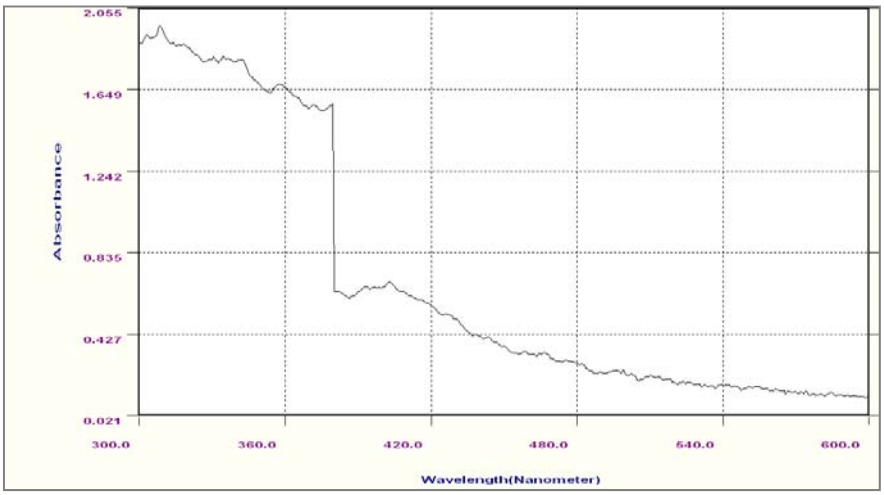

Fig 3: UV- visible spectra of *C. gigantea* leaf extract nanoparticle as a function of time

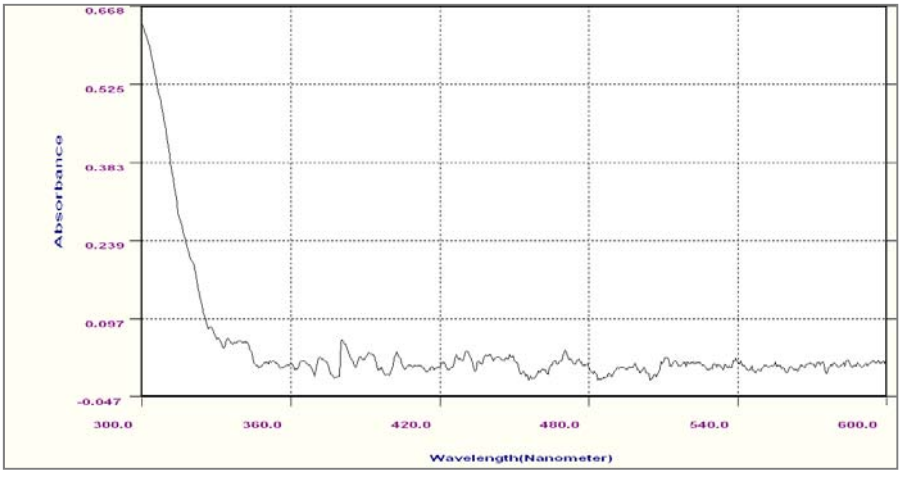

Fig 4: UV- visible spectra of *C. roseus* leaf extract nanoparticle as a function of time

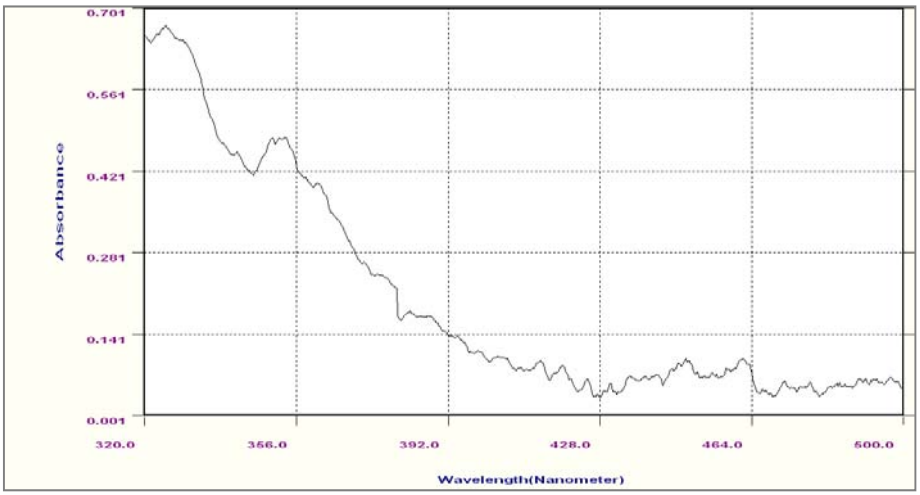

Fig 5: UV- visible spectra of Chitin nanoparticle as a function of time

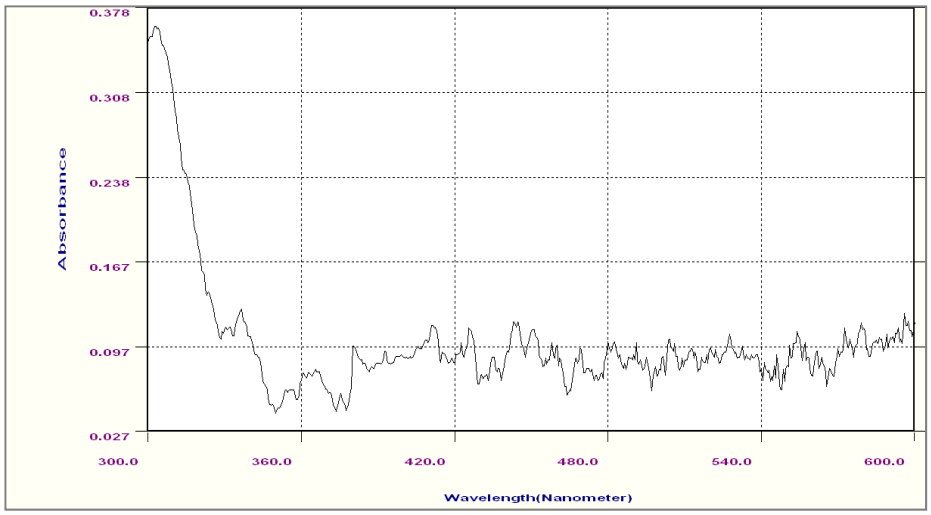

Fig 6: UV- visible spectra of Chitosan nanoparticle as a function of time

3.3 Antimicrobial activity

The antimicrobial activity of the synthesized nanoparticles and the standard was analyzed using disk diffusion method. Synthesized nanoparticles showed antibacterial activity comparable to the standard used against the two bacteria (*Escherichia coli*, *Staphylococcus aureus*). The zone of inhibition was observed and was found to be highest against Chitin AgNP's in case of *E. coli* and in Chitosan AgNP's in case of *S. aureus* with each of them having antimicrobial strengths similar to that of the commercial antibiotic

Table 1: Antimicrobial activity of AgNPs of *C. gigantea*, Chitin and Chitosan by disk diffusion method

Zone of Inhibition (in mm)				
Bacteria	***C. gigantea* AgNP's**	**Chitin AgNP's**	**Chitosan AgNP's**	**Standard (Erythromycin)**
E. coli	21	22	12	22
S. aureus	27	32	36	33

The zone of inhibition of *C. roseus* against Chitin and Chitosan nanoparticles was tested and was found to be highest against Chitin AgNP's in both cases indicating comparable antimicrobial activity to that of the commercial antibiotic used. Manjul *et al*. reported that the highest zone of inhibition for *C. procera* nanoparticles was observed in the case of *K. Pneumonia* and *S. typhi* and the efficacy were found to be less against both *E. coli* and *S. aureus* [17]. Bhanu Prakash *et al.* reported maximum inhibition against Gram positive as against Gram negative organisms in case of *Vinca roseus* nanoparticle. [18] (Figure 7, 8, and Table 1, 2)

Table 2: Antimicrobial activity of AgNPs of *C.roseus*, Chitin and Chitosan by disk diffusion method

Zone of Inhibition (in mm)				
Bacteria	***C. roseus* AgNP's**	**Chitin AgNP's**	**Chitosan AgNP's**	**Standard (Erythromycin)**
E. coli	16	18	15	21
S. aureus	25	30	18	34

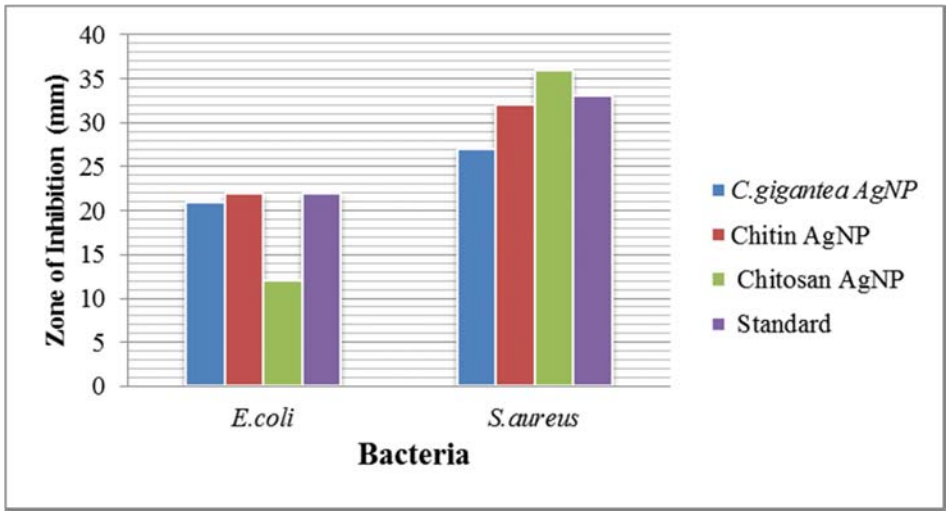

Fig 7: Antibacterial activity of AgNPs from *C. gigantea*, Chitin and Chitosan by disk diffusion method

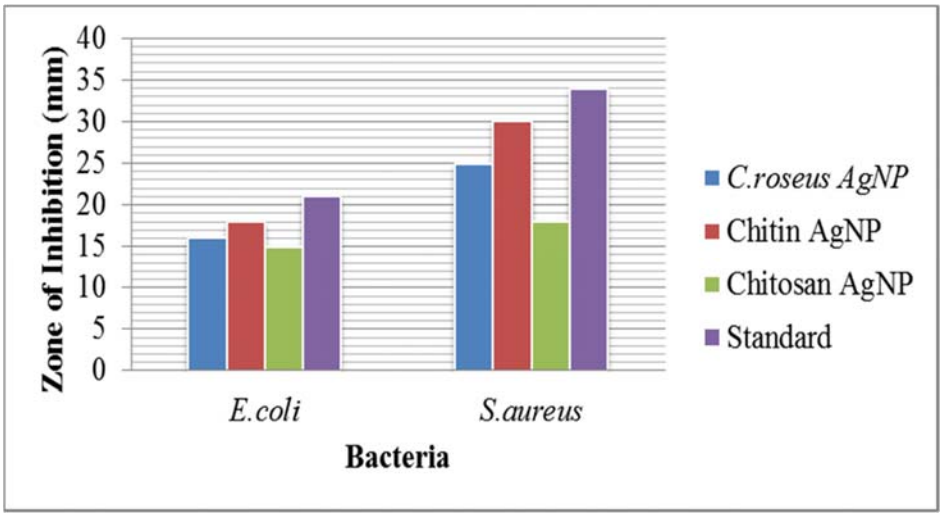

Fig 8: Antibacterial activity of AgNPs from *C. roseus*, Chitin and Chitosan by disk diffusion method

3.4 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 (Figure 9, 10, 11, 12). Bhanu Prakash et al showed that the absorbance in the growth media seeded with *Vinca roseus* silver nanoparticles was less than that compared to the control when tested against *B. subtilis* and *P. aeruginosa* [18].

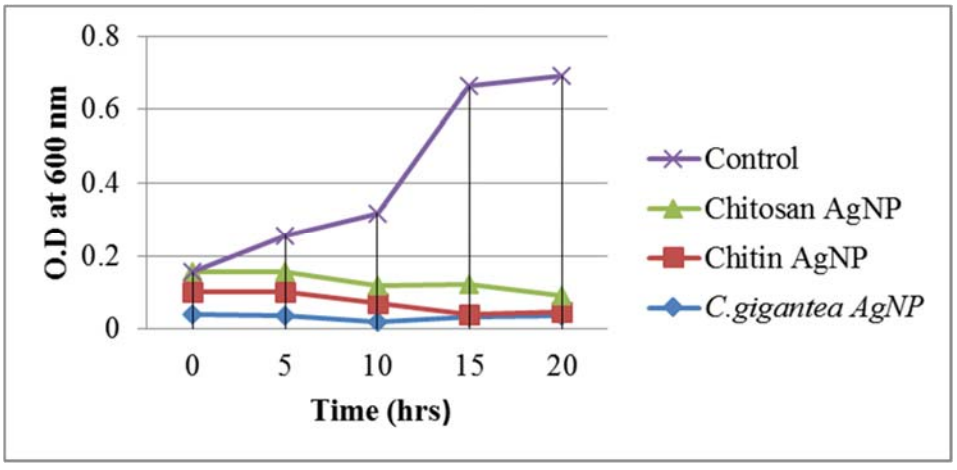

Fig 9: The effect of green synthesized silver nanoparticles on the growth of a) *B. subtilis*

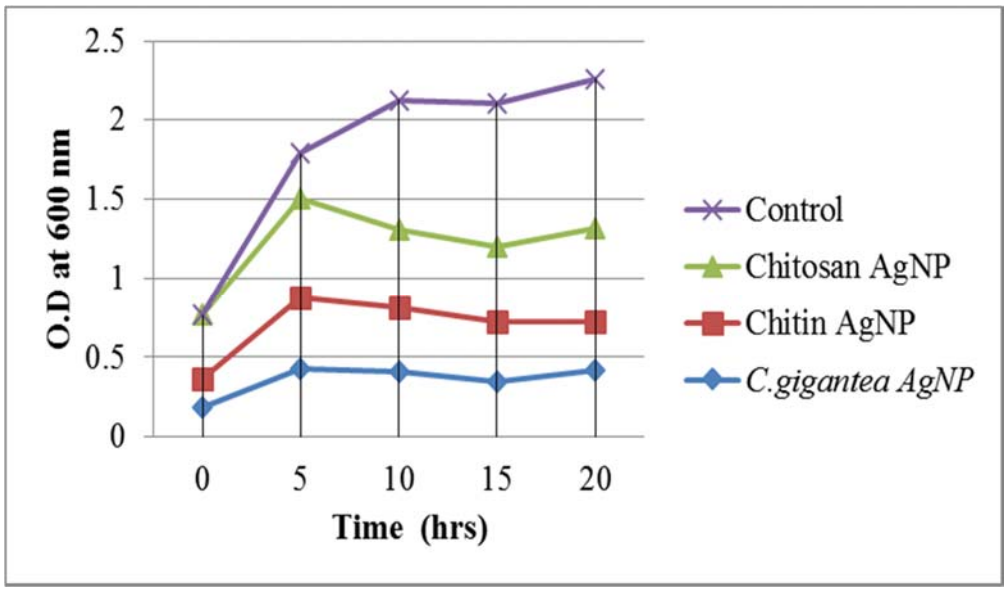

Fig 10: The effect of green synthesized silver nanoparticles on the growth of b) *K. pneumonia*

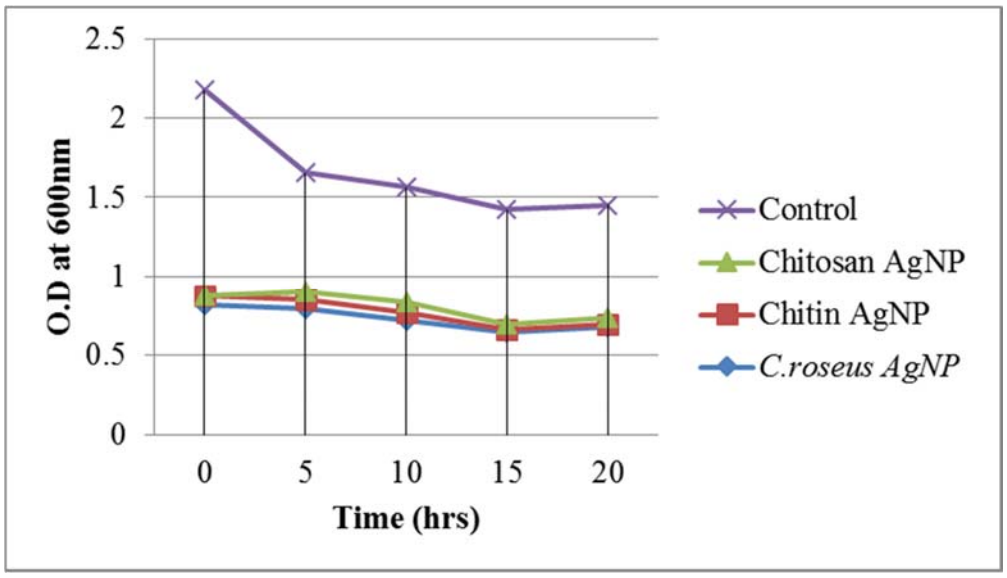

Fig 11: The effect of green synthesized silver nanoparticles on the growth of a) *B. subtilis*

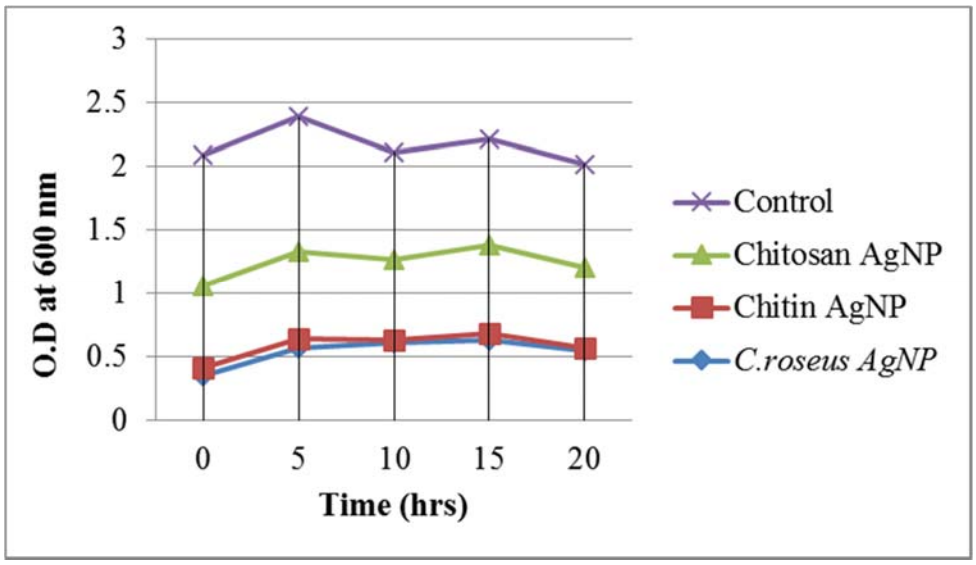

Fig 12: The effect of green synthesized silver nanoparticles on the growth of b) *K. pneumonia*

It has been observed from the Growth kinetics plot that the optical absorption in growth medium induced with the silver nanoparticles showed a steep decline as compared to that of the control indicating a substantial amount of antimicrobial activity displayed by the nanoparticles.

Several factors influence the formation of silver nanoparticles such as plant source, organic compound in plant extract. Organic compounds like alkaloids, polyphenols, and proteins and even some pigments are present in plant extracts. The phenolic acids are reported to be powerful antioxidants. It has been reported that they possess hydroxyl and carbonyl groups which are able to bind to metals [19]. Chitin with<5% DAc was added as a stabilizer to Ag NP suspensions to remove the generated caramel and to prevent agglomeration and precipitation of the AgNPs. The AgNPs adsorbed onto chitin powder were substantially stabilized compared to those in the absence of chitin [16]. Wei *et al*. using FTIR also showed possible interaction between silver salts and chitosan molecules, which may account for the reduction of Ag ions and stabilization of silver-chitosan nanoparticles. Thus the attachment of silver to the nitrogen atoms in chitosan reduced the vibration intensity of the N-H bond due to increased molecule weight after silver binding. The inhibitory effect of silver on microorganisms tested is effected via two possible mechanisms First, is the electrostatic attraction between the negatively charged cell membrane of the microorganisms and the positively charged Ag, and second, is the formation of 'pits' in the cell wall of bacteria related to Ag concentration [20].

4. References

1. Gong P, Li H, He X, Wang K, Hu J, Tan W. Preparation and antibacterial activity of Fe O @Ag nanoparticles. Nanotechnol 2007; 18:604-11.
2. Alagumuthu G, Kirubha R. Green synthesis of silver nanoparticles using *Cissus quadrangularis* plant extract and their antibacterial Activity University. International Journal of Nanomaterials and Biostructures 2012; 2:30-33.
3. Naheed A, Sharma S. Green Synthesis of Silver Nanoparticles Using Extracts of Ananas comosus. Green and Sustainable Chemistry 2012; 2:141-147.
4. Singh RP, Shukla VK, Yadav RS, Sharma PK, Singh PK, Pandey AC. Biological approach of zinc oxide nanoparticles formation and its characterization. Advanced Materials Letters 2011; 2:313-317
5. Honary S, Ghajar K, Khazaeli P, Shalchian P. Preparation, Characterization and Antibacterial

Properties of Silver-Chitosan Nanocomposites Using Different Molecular Weight Grades of Chitosan. Tropical Journal of Pharmaceutical Research 2011; 10(1): 69-74.
6. Collu G, Unver N, Peltenburg-looman AGM, Van der Heijden R, Verpoorte R, Memelink J. Geraniol 10-hydroxylase1, a cytochrome p 450 enzyme involved in terpindole alkaloid biosynthesis. FEBS Lett 2001; 508:215-220.
7. Hirose F, Ashihara H. Metabolic regulation in plant cell culture fine control of purine nucleotide biosynthesis in intact cells of *Catharanthus roseus*. J Plant Physiol 1984; 116:417-423.
8. Jaleel CA, Gopi R, Panneer SVR. Alterations in nonenzymatic antioxidant components of *Catharanthus roseus* exposed to paclobutrazol, gibberellic acid and Pseudomonas fluorescens. Plant Omics 2009; 2:30-40.
9. Jaleel CA, Panneerselvam R. Variations in the antioxidative and indole alkaloid status in different parts of two varieties of *Catharanthus roseus*, an important folk herb. Chin J Pharm Toxicol 2007; 21(6):487-494.
10. Jayanthi M, Sowbala N, Rajalakshmi G, Kanagavalli U, Sivakumar V. Study of antihyerglycemic effect of *Catharanthus roseus* in alloxan induced diabetic rats. Int J Pharm Pharm Sci 2010; 2 (4):114-116.
11. Nayak BS, Pinto PLM. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. BMC Complement Altern Med 2006; 6:41.
12. Shi C, Zhu Y, Ran X, Wang M, Su Y, Cheng T. Therapeutic potential of chitosan and its derivatives in regenerative medicine. Journal of Surgical Research 2006; 133:185–192.
13. Dutta J, Tripathi S, Dutta PK. 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. Sivakumar J, Premkumar C, Santhanam P, Saraswathi N. Biosynthesis of Silver Nanoparticles Using *Calotropis gigantean* leaf. African Journal of Basic & Applied Sciences 2011; 3(6):265-270.
15. Ponarulselvam S, Panneerselvam C, Murugan K, Aarthi N, Kalimuthu K, Thangamani S. Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. Asian Pacific Journal of Tropical Biomedicine 2012; 574-580.
16. Vinh QN, Satoko K, Yasushi M, Masayuki I, Hidemi H, Takemi M, Yasutaka M, Masanori F, Shingo N, Yasuhiro K, Takeshi O. Preparation of Size-Controlled Silver Nanoparticles and Chitin-Based Composites and Their Antimicrobial Activities. Journal of Nanomaterials 3:1-7.
17. Gondwal M, Pant GJN. Biological Evaluation and Green Synthesis of Silver Nanoparticles using aqueous extract of *Calotropis procera.* Int J Pharm Bio Sci 2013; 4(4):635–643.
18. Bhanu MP, Paul S. 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.
19. Harborne JB. Biochemistry of Phenolic Compounds, Academic Press: London, 1966.
20. Wei D, Qian W. Facile synthesis of Ag and Au nanoparticles utilizing chitosan as a mediator agent. Colloid Surface B 2008; 62:136-142.