

**ANTIBACTERIAL POTENCY OF NANOSILVER SYNTHESIZED FROM
THE FRUIT FILTRATE OF *CITRUS SINENSIS*****D.SUBHASHINI***

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ABSTRACT

Nanotechnology is being paid vast attention in this century for its intrinsic characters at the nanoscale level. This is due to the particles possessing high surface area to volume ratio, which can be modified or manipulated to achieve defined and improved surface properties. Among the nanometer particles so far reported, silver is versatile since it has a wide range of biomedical applications. In the current study, silver nanoparticles were synthesized from silver nitrate solution by using the aqueous fruit filtrate of *Citrus sinensis*. There was a change of colour from colourless solution to dark brown, which indicated the reduction of silver ions due to surface plasmon resonance. The particles were absorbed in the UV range and the corresponding peak displayed at 417nm. The characterization study was carried out by using SEM and the percentage of silver nanoparticles was identified by using EDAX. The antimicrobial activity of the particles was checked against *E.coli* (ATCC 25922) and *P.aeruginosa* (ATCC 27853).

KEYWORDS: *Citrus sinensis*, Phyto synthesis, Silver nanoparticles, Antimicrobial activity.

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INTRODUCTION

In the recent era, Nanoscale materials have attracted scientists to create a revolution in the field of nanotechnology. There are two methods by which one can produce a nanomaterial – Top down approach and bottom up approach. Basically, synthesis of nanoparticles can be achieved by physical and chemical methods, which are top down approaches. But, the procedure requires higher temperature, pressure, expensive raw materials or may result in the generation of harmful byproducts. In order to avoid the prevailing disadvantages, biological synthesis was preferred as it is simple to perform and eco-friendly. It's from time immemorial; silver had been utilized for treating various diseases. It possesses anti-bacterial activity¹ and anti-inflammatory activity^{2,3,4}. Biological origin of nanoparticle is generally considered as a bottom up approach. Many researchers have reported the synthesis of silver nanoparticles from various biological sources, which includes N-cholyl amino acids⁵, leaf extracts of *Acalypha indica*⁶, *Lantana camara*⁷, *Iresine herbstii*⁸, *Pulicaria glutinosa*⁹, *Sphaeranthus amaranthoides*¹⁰, fungal extracts of *Fusarium oxysporum*¹¹, *Fusarium solani*¹², *Candida albicans*¹³, peel extract of *Citrus sinensis*¹⁴, fruits of papaya¹⁵, *Solanum torvum*¹⁶, seed extract of *Terminalia chebula*¹⁷ and so on. *Citrus sinensis* is no more exceptional fruit to consider for the nano synthesis. It has tremendous medicinal properties, which were used right from the ancient times to treat and cure various diseases. The methanolic extract of the fresh peels of the fruit were revealed to contain tannins, cardiac glycosides, reducing sugar, saponins and flavonoids¹⁸. A specific flavanoid, 5, 8-dihydroxy-6, 7, 4'-trimethoxyflavone has been isolated from the ethyl acetate extract of its roots¹⁹. The dried peel extract of the fruit was revealed to possess antibacterial activity²⁰ against diarrhoeal pathogens such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. flexneri*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, and *V. cholera* and was proved to contain hesperidin, an anti-ulcer agent²¹. Their seeds possess anti-glycative activity²². Exploiting the use of fruits of *C. sinensis* towards the synthesis of silver nanoparticles has not been reported yet. Hence, in the present study, the fruit filtrate of

C. sinensis was attempted for the synthesis of silver nanoparticles.

MATERIALS AND METHODS

The healthy, disease free fruits of *Citrus sinensis* were purchased from a local market in Chennai, India. The bacterial strains of *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were obtained. Silver nitrate was procured from Himedia Laboratories Privated Limited, India. All the chemicals used for the investigation were of analytical grades.

(i) Preparation of Fruit filtrate

2g of *C. sinensis* fruits was weighed (excluding their peel and seeds) and they were macerated in a mortar and pestle without adding water. Later, it was filtered in four layers of cheese cloth and passed through whatmann no.1 filter paper. The golden orange coloured filtrate thus obtained was stored in an air tight container at room temperature.

(ii) Synthesis of Silver Nanoparticles⁶

The silver nanoparticles were synthesized by biological mean following the described procedure⁶ with slight modification. 1mM Silver Nitrate Solution (AgNO₃) was prepared by gently agitating at 120rpm for 5 minutes and was stored at room temperature in a screw capped brown bottle. 9ml of Silver nitrate solution was taken in a conical flask and the fruit filtrate was added in drops till a change in colour was observed. The synthesized solution was thrice washed with sterile distilled water and was centrifuged at 10000 rpm for 10 mins to remove the impurities. The pellet, thus obtained was lyophilized and stored at 4⁰C for characterization and antibacterial studies.

(iii) Characterization of silver nanoparticles synthesized from *C. sinensis* (CsAgNPs)

The synthesis of silver nanoparticles (AgNPs) was confirmed by recording UV spectra in Spectrascan UV-2700, Thermo Scientific instrument. The morphology of the CsAgNPs was characterized by SEM and the elemental

composition of the sample was identified by EDAX (Hitachi S-4500 SEM instrument).

(iv) Antibacterial activity of CsAgNPs⁸

The biologically synthesized silver nanoparticles, CsAgNPs were tested for its antibacterial efficacy against two bacterial strains: *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) by adopting agar well diffusion method⁸ with less modification. Briefly, Mueller Hinton agar plates were prepared and 100 μ l of the as said strains were inoculated onto the medium in different petriplates. To the 6mm diameter of punched wells, standard antibiotic disc (erythromycin) was placed at centre of the inoculated plate and the surrounding wells were inoculated with different concentration of silver nanoparticles (10 μ l, 20 μ l, 30 μ l, 40 μ l and 50 μ l). The plates were then incubated at 32^oC for 24 hours. The Zone of Inhibition (ZOI) was recorded.

RESULTS AND DISCUSSION

1. Phytosynthesis of Silver Nanoparticles

The simple mixing of the fruit filtrate of *Citrus sinensis* with 1mM silver nitrate solution at room temperature resulted in remarkable colour changed from colourless to light yellow, light brown and finally to dark brown indicating the formation of silver nanoparticles (Fig 1 insert). Similar studies demonstrated such colour change from colourless to dark brown^{10,25} by some workers.

2. Characterization of CsAgNPs

Figure 1 shows the UV-Vis spectra recorded for the CsAgNPs. The Surface Plasmon Resonance (SPR) wavelength, λ_{max} occurred at 417 nm. Similar SPR peak at 420nm, 425nm and 438nm were reported for silver nanoparticles synthesized from the leaf extract of *Acalypha indica*⁶, seed extract of *Psoralea corylifolia*²⁸ and thalli of *Anthoceros*²⁹ respectively. This spectral characteristics of synthesized CsAgNPs interprets the NPs were polydispersed.

UV-Vis spectra of CsAgNPs

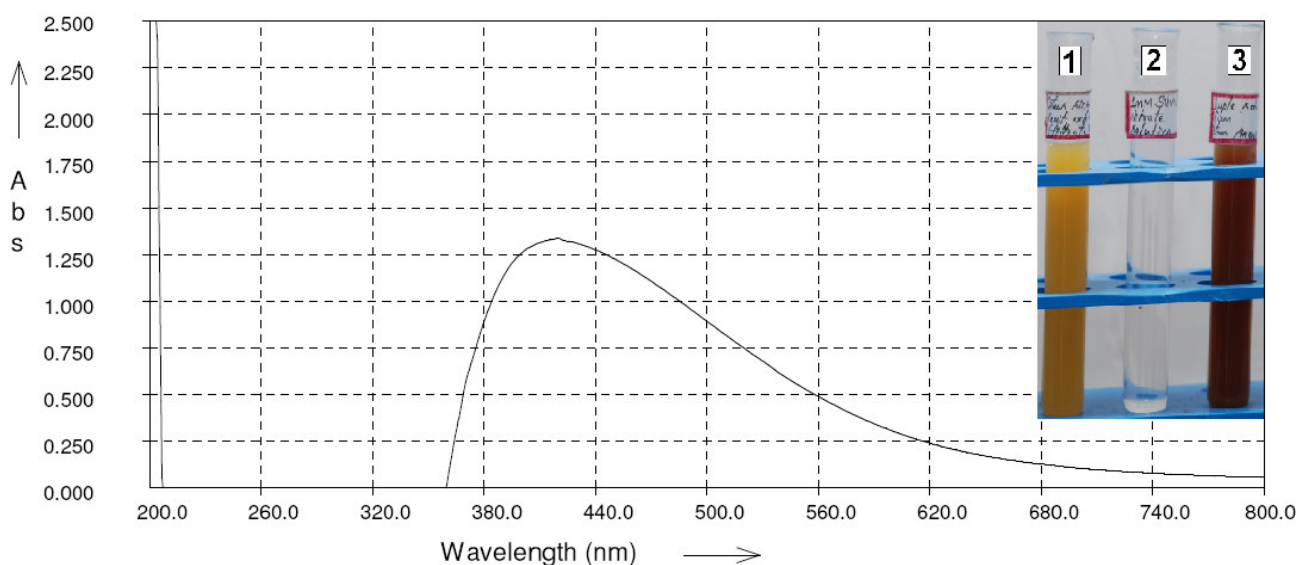


Figure 1

Absorption spectra obtained a characteristic peak at 417nm for the CsAgNPs synthesized. Insert shows the test tubes containing: 1. Fruit filtrate of *C. sinensis*, 2. 1mM Silver nitrate solution, 3. CsAgNPs synthesized.

The morphology of the biosynthesized CsAgNps was determined by employing Hitachi S-4500 SEM instrument. Fig 2 depicts the particles were bit oval in shape

with the size ranged from 50nm to 73nm. This size of polydispersed CsAgNps is in proximity to the reported size of AgNPs from 44nm to 64nm⁸. The phytochemicals^{26,27}

such as alkaloids, flavonoids, phenols, saponins, tannins, proteins, steroids, sugars etc., present in the pulp of *C. sinensis* might be responsible for the reduction of Ag^+ to Ag^0 . The component analysis of the sample was identified by using EDAX. There was a

sharp, characteristic peak for the silver and the EDAX spectrum shows a strong signal for Ag (95.73%). The presence of other peaks of C, O, Cl, obtained in the EDAX may possibly refer to the presence of phytochemicals in the fruit filtrate (Fig 3).

CsAgNPs observed under Hitachi S-4500 SEM

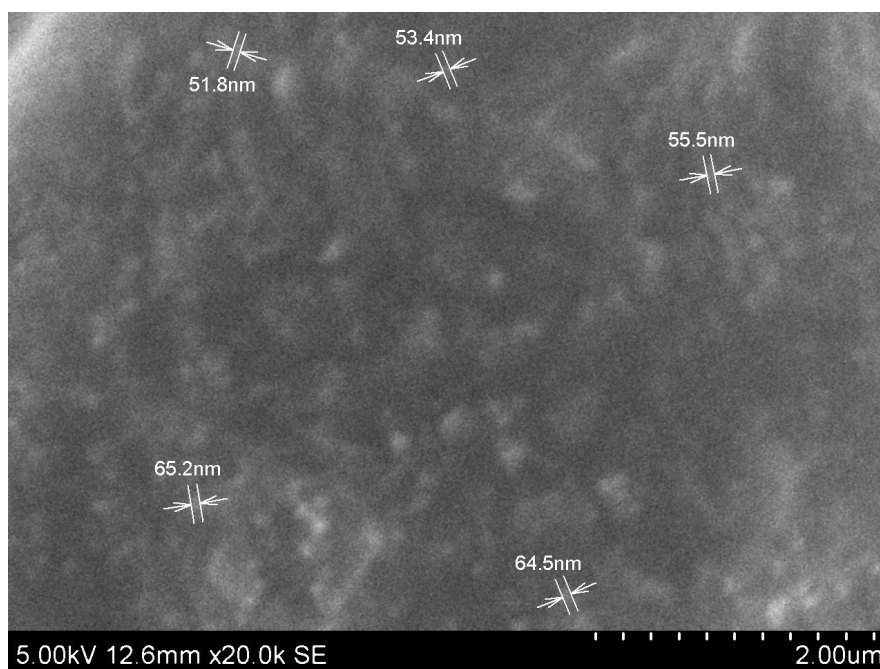


Figure 2
SEM image of CsAgNPs synthesized using the fruit filtrate of *C. sinensis*

EDAX spectra for CsAgNPs

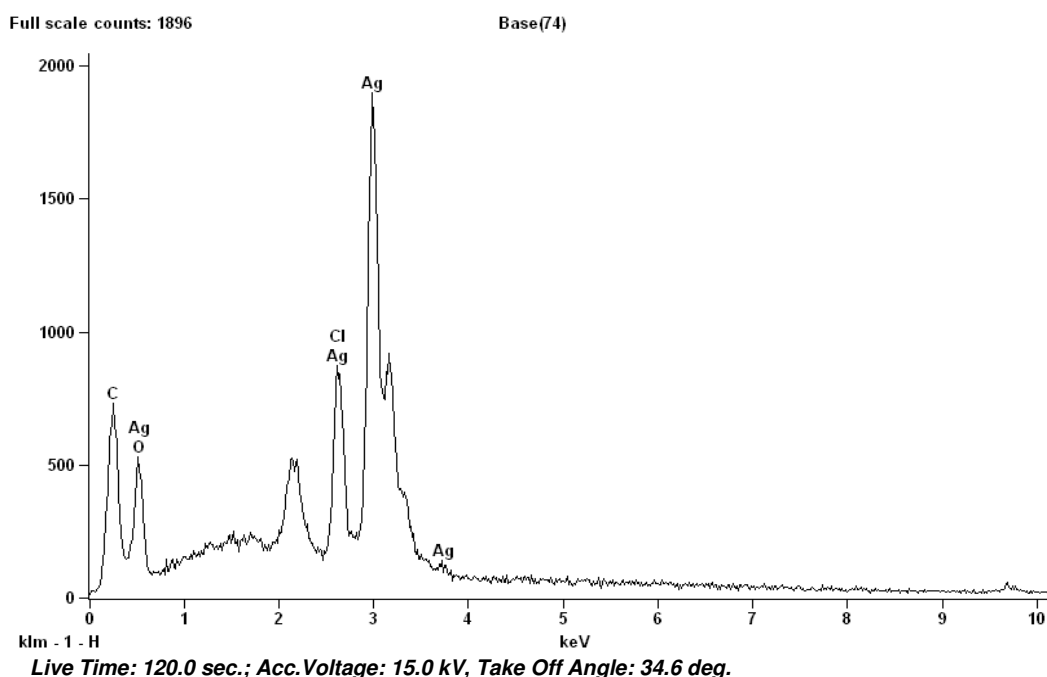


Figure 3
EDAX showing the characteristic peak of CsAgNPs

3. Antibacterial efficacy of CsAgNPs

After 24 hours of incubation, Silver nanoparticles exhibited significant antibacterial activity against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), compared to the standard antibiotic, erythromycin. The control, erythromycin disc (15 µg) had formed Zone of Inhibition of 17mm diameter against *E.coli* and

11mm diameter against *Pseudomonas aeruginosa*, whereas the CsAgNPs at a volume of 10µl showed a ZOI of 11.5mm against *E.coli* and 18mm against *P. aeruginosa* respectively. When compared to the control, nanoparticles were showing distinct zone of inhibition against *P. aeruginosa* (Fig 4).

Antibacterial efficacy of CsAgNPs against *E. coli* and *P. aeruginosa*

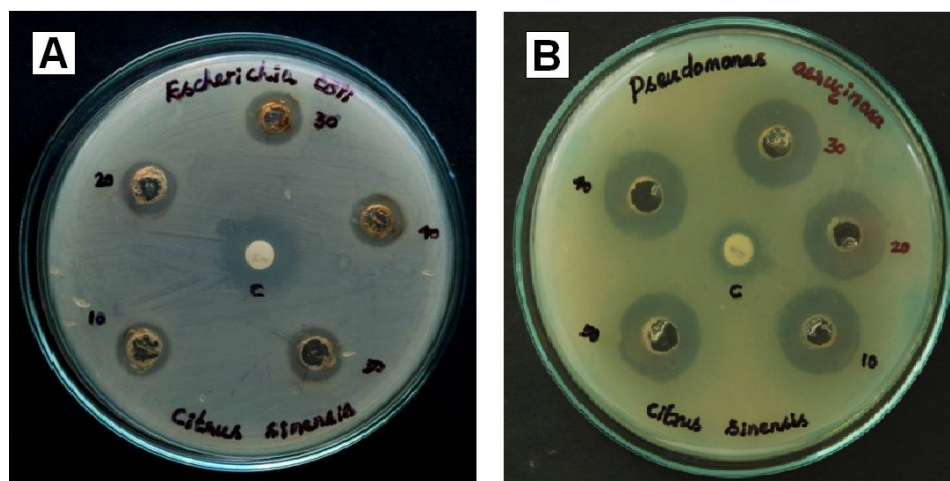


Figure 4

Distinct, clear, Zone of Inhibition formed by CsAgNPs against A) *E. coli* (ATCC 25922) B) *Pseudomonas aeruginosa* (ATCC 27853)

Volume of silver nanoparticles loaded (µl)	Zone of Inhibition against <i>E. coli</i> (ATCC 25922) in mm	Zone of Inhibition against <i>P. aeruginosa</i> (ATCC 27853) in mm
Erythromycin disc (Positive control)	17	11
10	11.5	18
20	12	18.5
30	13	19
40	13.5	19
50	13.5	19.5

Table 1

Zone of Inhibition (mm) formed by CsAgNPs against *E.coli* and *P. aeruginosa*

As shown in the Table 1, the ZOI formed by CsAgNPs was reported to be 13.5mm and 19.5 mm against *E.coli* and *P. aeruginosa* at a concentration of 50µl which is more efficient than the standard antibiotic, erythromycin. This is in concurrence with the reported result of antibacterial activity of silver nanoparticles synthesized from the leaves of *Spinacia oleracea* and *Lactuca sativa*²⁵. However, the present study shows the similar value of 11.5mm against *E. coli*, which is nearer to the reported data against the same strain. But,

against *P. aeruginosa* strain, there is a significant difference between the reported and the obtained values. The reported values were 11mm and 12 mm, whereas the current investigation formed a minimum zone of inhibition of 18mm. Thus, the result suggests that CsAgNPs possesses efficient antibacterial activity and strongly supports the usage of silver nanoparticles against *P. aeruginosa* (ATCC 27853).

CONCLUSION

The phytochemicals present in the edible fruit has numerable activities, which decreases the preventive as well as chronic degenerative diseases²³. The fibres in the fruit had been reported to reduce the intensity of obesity, arthritis, cancer and coronary heart disease²⁴. Those phyto and fibre constituents may possess synergetic effects over the synthesis process and may be responsible for the reduction of Ag⁺ to Ag⁰ in the solution. They might have also acted as stabilizing and capping agents. Further, FTIR should be performed to identify the functional groups involved in the synthesis. The study should be extended to identify the compound responsible for the reduction of silver in the fruit filtrate of *C. sinensis*. The present investigation concludes that the fruit filtrate of

C. sinensis can also be used to synthesize the silver nanoparticles. The method is simple, eco-friendly and feasible to synthesize. The particles possesses promising antibacterial activity against *P.aeruginosa*.

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