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Research Article

BIOSYNTHESIS OF SILVER NANOPARTICLES USING *CITRUS LIMON* (LINN.) BURM. F. PEEL EXTRACT AND ITS ANTIBACTERIAL PROPERTY AGAINST SELECTED URINARY TRACT PATHOGENS

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ABSTRACT

The study is focused to biosynthesize silver nanoparticles using lemon peel extract and to assess the antibacterial activity of AgNPs against the selected urinary tract pathogens. 4 g of finely cut *Citrus limon* peel was added to 40 ml double distilled water, boiled for 2 minutes and filtered. 3 ml of the extract was added to 40 ml of 1 mM $AgNO_3$ solution and change in color was observed. The bio reduction of Ag^+ ion in solution was monitored using UV–visible spectrometer, FESEM and EDAX analysis. FESEM analysis showed the presence of AgNPs with the size between 17.3-61.2 nm and the shapes were spherical and some were irregular. The energy dispersive X-ray analysis (EDAX) revealed strong signal at 3 keV, in the silver region and confirmed the formation of silver nanoparticles. The extract was tested against the urinary tract pathogens like *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumonia.* The silver nanoparticles showed maximum zone of inhibition of 24 mm ± 0.3SD, 14 mm ± 0.2 SD, 12 ± 0.5 SD against *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumonia* respectively. The present research work emphasizes the use of lemon peels for the effective synthesize of AgNPs and could be used against the urinary tract pathogens which are found to develop drug resistance towards broad-spectrum antibiotics.

Keywords: Silver nanoparticles, Urinary Tract Infection, Biosynthesis, SRP, *Citrus limon* peel, EDAX, FESM.

INTRODUCTION

Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale[20]. Noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials[18]. Metal nanoparticles which have a high specific surface area and a high fraction of surface atoms have been studied extensively[4]. Ag nanoparticles are excellent nanomaterials providing a powerful platform in biomedical applications of bio molecular recognition, biosensing, drug delivery and molecular imaging[29]. Silver nanoparticles also exhibit a potent cytoprotective activity towards HIV-infected cells[30]. Nanotechnology-enhanced electrochemical detection of nucleic acids is used for the diagnosis and early stage treatment of genetic diseases[32]. Nanoparticles of silver, elicited significant loss in micro filarial motility and provide the first ever conclusive proof in support of apoptosis as a novel stratagem in anti filarial drug designing[28]. Bio synthesized silver nanoparticles has cytotoxic activity against Hep-2 cell line[11]. Urinary tract infections are the most commonly observed infections in clinical practice. They also contribute the most common nosocomial infection in many hospitals, and accounts for approximately 35 % of all hospital acquired infections[9]. The Enterobacteriaceae members are the most frequent pathogens detected, causing 84.3 % of the UTIs[7]. Multiple microbial resistances among Gram-negative organisms have been a long term and well-recognized problem with urinary tract infections. Resistance has been observed in multiple genera including *Escherichia, Enterobacter, Klebsiella, Proteus, Salmonella, Serratia* and *Pseudomonas*[19]. Nanobiotechnology is a field that inter relates both biological sciences and nanotechnology. It provides a platform for the development of eco friendly and the green synthesis of nanoparticles with the help of biological sources like plants and micro organisms[8]. Biosynthesis of nanoparticles is cost effective method when compared to physical and chemical methods and less toxic to human and environment[26]. Biosynthesis of gold and silver nanoparticles was produced from extract of banana peel extract[3], *Citrus sinensis* peel[13], *Withania somnifera, Allium* sp[17]. Literature survey has shown that naturally available agricultural wastes have not been investigated for the synthesis of silver nanoparticles. A classical example of such an abundantly available natural material is the lemon peel. Lemon is an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz., leaves, stem, root and flower) of Lemon against clinically significant bacterial strains has been reported[14]. The

peel of *Citrus* fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants[1] all of which are supposed to have a number of positive health effects in the prevention of lifestyle-related diseases, and to have anti inflammatory, anticancer, and antiviral activities based on their antioxidant activity[31,19,6]. The aim of the present study is to determine a hitherto unreported green biological route for the synthesis of silver nanoparticles using the extract derived from lemon peels and to determine its antibacterial activity against the isolated urinary tract pathogens.

MATERIALS AND METHODS

Materials

All chemicals used in this experiment were of highest purity and obtained from Sigma (Bangalore, India) and Merck (Mumbai, India). *Citrus limon* fruits for producing silver nano particles were purchased from the local market in Chennai, Tamil Nadu, India.

Sample collection, isolation and identification of urinary tract pathogens

About 25 urine samples were collected from a private hospital in Chennai, Tamil Nadu, India. Clean catch mid stream urine samples were collected in sterile container from suspected persons and transferred to the laboratory for processing. With the urine sample, a direct Gram staining was done and analyzed. The urine sample was taken in a calibrated loop and streaked on Nutrient agar, Mac Conkey agar and blood agar. Pure cultures of all morphologically suspected colonies were characterized.

Biosynthesis of silver nanoparticles using lemon peel extract

The lemon peel is washed thoroughly with double distilled water and cut into small pieces. 4 g was transferred into 40 ml double distilled water, boiled for 2 minutes. The extract obtained was filtered through Whatman No. 1 filter paper and the filtrate was collected in 250 ml Erlenmeyer flak and stored at 4°C for further use. 3 ml of the extract was added to 40 ml of 1 mM $AgNO_3$ solution and kept at room temperature for 5 hours[13].

UV-VIS spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. The UV–visible spectra of the resulting diluents were monitored as a function of reaction time and biomaterial dosage at a resolution of 1 nm and a spectrum was taken on a wavelength from 200 nm to 600 nm.

FESEM analysis of silver nanoparticles

The morphology of the AgNPs was examined using Field Emission Scanning Electron Microscopy (HITACHI SU6600 FESEM). Thin films of the samples were prepared on an aluminium foil by dropping a small amount of the sample and placed on a copper grid, extra solution was removed using a blotting paper and then allowed to dry prior measurements.

Energy Dispersive X-Ray (Edax) analysis

The reduced silver was dried on an aluminium foil coated copper grid and EDX analysis of the sample was performed using FESEM (HITACHI SU6600 FESEM) equipped with an EDAX attachment.

Screening of synthesized silver nanoparticles for antibacterial activity

Screening of antibacterial activity was performed by well diffusion technique[22]. Approximately 20 mL of molten and cooled nutrient agar was poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The isolates were grown in nutrient broth for 24 h. 1 × 105 CFU/mL was used to prepare lawns. The inoculum was spread evenly and the seeded plates were allowed to dry for 10 minutes. A standard cork borer of 5 mm was used to cut three uniform wells on the surface of the agar. The wells were labelled as a, b, c. The well 'a' was loaded with 30 µl of lemon peel extract, 'b' well was loaded with 30 µl of silver nanoparticles suspended 'hydrosol' and 'c' well loaded with 30 µl of Chloramphenicol (10 mg/ml) which was used as positive control. The plates were then incubated at 37°C for 24 hours. The plates were examined for evidence of zones of inhibition. The diameter of such zones was measured.

RESULTS

Isolation of Urinary Tract Pathogens

A total of 25 urine samples were collected from the nearest private hospital and processed for the isolation of pathogens. Based on the morphology, cultural characters and biochemical tests the isolates were identified as *Escherichia coli*, *Klebsiella pneumonia*e and *Pseudomonas aeruginosa*. Among them, *Escherichia coli* were predominant followed by *Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

Synthesis of Silver nanoparticles

When the lemon peel extract was added to 1 mM solution of $AgNO_3$, a change in colour from colourless to yellowish brown was observed in about 10 minutes. The final colour deepened with increase in time. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within an hour of reaction.

UV–VIS spectra analysis

The characteristic surface plasmon resonance band of biogenic AgNPs occured at 420 nm; control AgNPs solutions (without lemon peel extract) neither developed the brown colours nor did they displayed the characteristic peaks. (Figure 1)

FESEM analysis

The presence of AgNPs was confirmed by carrying out FESEM. The size (diameter) of the nanoparticles was between 17.3-61.2 nm and the shapes were spherical and some were irregular. (Figure 2)

Energy Dispersive X-Ray analysis

The energy dispersive X-ray analysis (EDX) revealed strong signal in the silver region and confirmed the formation of silver nanoparticles. The optical absorption peak was observed approximately at 3 keV, which is typical for the absorption of metallic silver nanocrystallites due to surface plasmon resonance. (Figure 3)

Screening of synthesized silver nanoparticles for antibacterial activity

The silver nanoparticles showed maximum zone of inhibition of 24 mm ± 0.3 SD, 14 mm ± 0.2 SD, 12 ± 0.5 SD against *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumonia* respectively. The zone produced was found to be more effective against *Pseudomonas aeruginosa* followed by *Klebsiella pneumonia* and *Escherichia coli.* The standard antibiotic showed maximum zone of inhibition of 23 mm ± 0.2 SD, 18 mm ± 0.3 SD, 17 ± 0.4 SD against *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia* respectively (Figure 4 and 5).

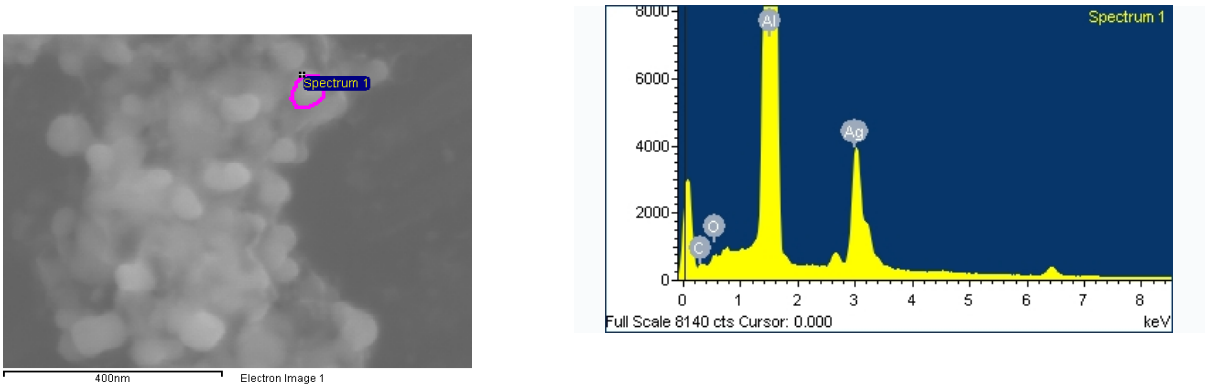


Figure 1: Absorption peak of silver nanoparticles produced from lemon peel under UV- vis absorption spectroscopy

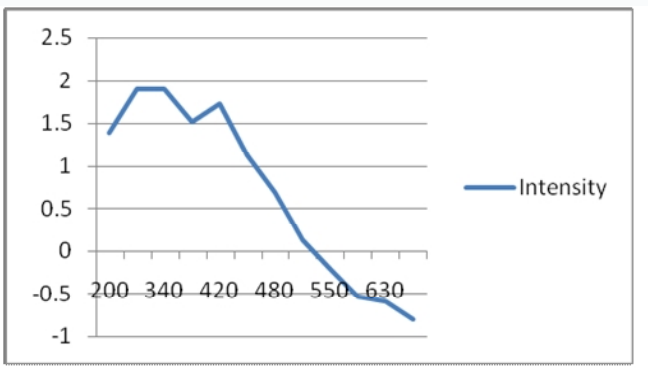
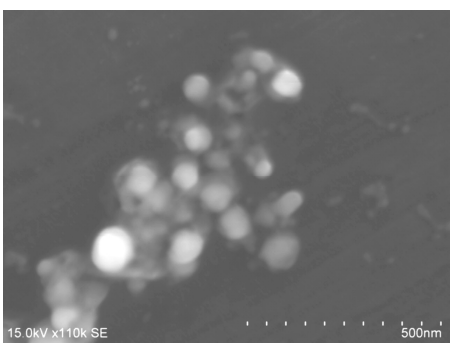
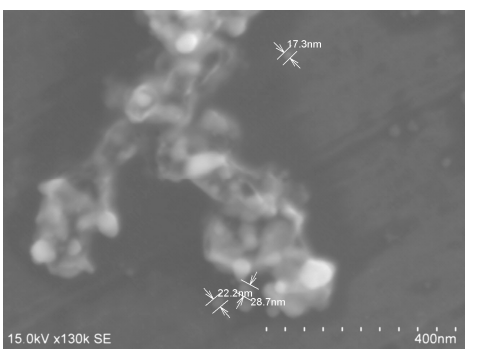


Figure 2: FESEM Analysis of Silver nanoparticles



FESEM image of the Ag nanoparticles at 500 nm



FESEM image of the AgNPs synthesized from lemon peel extract at 400 nm

Figure 3: Energy Dispersive X- ray Analysis of Silver Nanoparticles

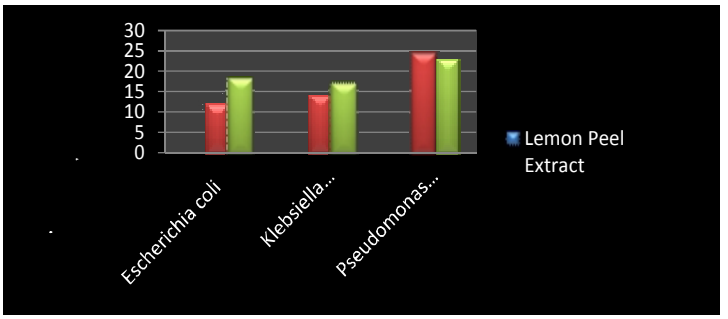


Figure 4: Sensitivity pattern of Silver nanoparticles against Urinary Tract Pathogens (Agar-Well Diffusion Technique)
Sensitivity pattern of Silver nanoparticles against the Isolates

A *B* *C*

Figure 5: Sensitivity pattern of silver nanoparticles against the urinary tract pathogens (Agar-Well Diffusion Technique)
A-*Klebsiella pneumoniae* B- *Escherichia coli* C- *Pseudomonas aeruginosa*

DISCUSSION

Uncomplicated Urinary tract infections are common in adult women across the entire age spectrum, with mean annual incidence of 15 % and 10 % in those aged 15-39 and 40-79 years, respectively. Urinary tract infection (UTI, is one of the most common but widely misunderstood and challenging infectious diseases encountered in clinical practice. Recurrent urinary tract infections (UTIs) present a significant problem for women and a challenge for the doctors who care for them. Identification of causative agent and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stages of Urinary Tract Infections[15]. Hence the present study was undertaken to analyse the antimicrobial activity of AgNPs produced using lemon peel against the urinary tract pathogens. The present report showed predominance of *Escherichia coli* followed by *Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Similar reports were given by Noor *et al*. In their study *E. coli* was the most frequent etiological agent followed by *K. pneumonia*[21]. Similarly, Inbaneson *et al*[12] revealed *E. coli* was predominant among their isolates from urine samples which are in accordance with our study. Shankar *et al* reported that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[27]. The results of present study are consistent with his report. The present investigation of UV-vis spectra of the silver nanoparticles showed a distinct absorption at around 420 nm. Metal nanoparticles such as silver and gold have free electrons, which give rise to SPR absorption band. In our study the size (diameter) of the nanoparticles lie between 17.3-61.2 nm and the shapes were spherical and few were irregular. Several earlier workers have reported similar findings[2,23]. Result of EDAX analysis is in correlation with the earlier studies of Kaviya *et al*[13] and Kulkarni *et al*[16] in which EDAX spectrum showed was high for silver signals from Ag nanoparticles. Prasad and Elumalai[24] experimented that Ag nanoparticle produced using *Moringa oleifera* leaf extract had considerable activity compared to respective antibiotic which coincides with our study. Kaviya *et al* reported that the bio synthesized Ag nanoparticles showed good antibacterial activity against *E. coli* and *Ps. aeruginosa*[13]. Similarly, antibacterial activity of papaya fruit extract mediated silver nanoparticles on urinary tract pathogens, showed high toxicity against multidrug resistance bacteria[12]. Bankar *et al*.,[3] observed antibacterial activity of Ag nanoparticles towards *E. coli*, *E. aerogenes* , *Klebsiella* sp. and *Shigella* sp. Prasanth *et al* [25] used Vasambu silver nanoparticles and showed good inhibitory activity against *Escherichia coli, Micrococcus sp., S. aureus, Corynebacterium diphtheriae* and *C. albicans.* These herbal synthesized nano particles are efficient than the chemically synthesized nanoparticles and on par with that of commercial chemical triclosan. Thus, as per the present investigation, the assayed extract of silver nano particles was as potent as the control antibiotic. The high bactericidal activity is certainly due to the silver cations released from Ag nanoparticles that act as reservoirs for the Ag^{+} bactericidal agent. Big changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria[5]. The present research work

emphasizes the use of AgNPs against the isolated urinary tract pathogens because they are found to be drug resistance development towards broad-spectrum antibiotics. The lemon peels which are considered as waste could be converted into silver nanoparticles and could be used as an excellent source against multi drug resistant microbes. This might be useful in elimination of drug resistant urinary tract pathogens which give challenges to the clinicians. Further, it follows a green chemistry path way without using any toxic chemical. Highly stable silver nano particles could be reduced using simple rapid and cost effective method. However further evaluation is required for the definite conclusions contributing to the antimicrobial activities.

Abbreviation: AgNPs - Silver nanoparticles

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