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Synthesis of the silver nanoparticles from Aloe barbadensis extract and its application against the urinogenial tract infection

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Abstract-The integration of nanoparticles with the field of biotechnology is finding the wide range of applicability in various area of medical sciences. Recently the silver nanoparticles have gained the attention because of their antimicrobial activity which offers the possibility of their use for medicinal purposes. This study aims at evaluating the antimicrobial properties of green synthesized silver nanoparticles (AgNP's) from *Aloe barbadensis* leaf extract is treated against urinary tract infection pathogens [1]. About thirty-two bacteria are isolated from the mid urine sample of 25 male and 25 female patients from erode district, Tamilnadu, India and are identified by the conventional methods. The prepared nanoparticles were characterized by FT-IR and UV– Vis spectroscopy [2]. FT-IR study showed sharp absorption peaks at 1,631 and 3,433 cm^{-1} for amide and alcoholic hydroxide groups, respectively. *Escherichia coli* was found predominant in account of 47% sensitivity. The antibacterial activity of silver nanoparticles are evaluated by the disc diffusion assay and well diffusion method. *Escherichia coli* has maximum sensitivity (11±0.58mm) followed by candida albican (8±0.49mm) at a concentration of 20µg $disc^{-1}$ and the sensitivity was highly comparable with the positive control chloramphenicol and streptomycin. *K.pneumoniae* and S.aureus showed no sensitivity to any concentrations of the silver nanoparticles. The result provided by the experiment shows that the silver nanoparticles are potentially used for the treatment of the urinary tract infections (UTIs) which are caused by the *streptococcus aureus, Escherichia coli Candia albicans, Klebsiella pneumonia.* However, microscopic observation revealed that synthesized nanoparticles caused detrimental effects on conidial germination along with other deformations such as structure of cell membrane and inhibited normal budding process of the tested species. Therefore, it has been concluded that *Aloe barbedensis* leaf extract origin silver nanoparticles have tremendous potentiality towards controlling urinary tract infection causing pathogens [3]. However, further research is needed to check the efficacy of size dependent AgNPs on different species of bacteria and fungus.

Key Words*: silver nanoparticle-Green synthesis –Aloe Vera leaf -Antimicrobial effect –disc diffusion -*streptococcus aureus-Escherichia coli -Candia albicans-Klebsiella pneumonia

INTRODUCTION

The field of nanotechnology is one of the most industrious areas of research in current material sciences. Nanotechnology is a field is improving day by day, making an impact in all spheres of human life especially medical science and biotechnology. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanoparticles of noble metals, such as gold, silver, platinum, and zinc oxide are widely applied in products that directly come in contact with the human body, such as detergent, cosmetic products, and toothpaste, besides medical and pharmaceutical applications. Nanoparticle formation has been reported using chemical and physical methods. There are various methods for NPs formation such as sol-gel process, chemical precipitation, hydrothermal method, microwave, chemical vapour deposition, the above methods involve the usage of hazardous reagents for synthesis of nanoparticles. In view of an environmental sustenance, there is an urgent need to develop an eco-friendly method of synthesis of nanomaterial Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods. Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various Nano technological applications. The use of materials like plant extract bacteria, fungi and enzymes for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Silver has long been used as a disinfectant; for example, the metal has been used in treating wounds and burns because of its broad-spectrum toxicity to bacteria [4]. Silver nanoparticles have unique catalytic, optical, electrical and antimicrobial properties, which inhibiting 650 types of microbe's growth as well as because of its reputation of limited toxicity to humans . Various methods are available for synthesis of silver nanoparticles, which sign thereto above. various plant extracts such as Cinnamon camphora, Cinnamon zeylanicum,

Geranium, Neem leaf broth, Lemongrass extract, Tamarind leaf extract and Acalyphaindica [5] . The common pathogenic bacteria which include *Escherichia coli*, *streptococcus aureus, Candia albicans, Klebsiella pneumonia* are the major causative agents of nosocomial infections. Treatment with available antibiotics leads to resistance among pathogenic bacteria which leads to greater threat[6] . The patients suffering a lot of urinogenital tract infection[7] . Silver is a very effective antibacterial agent and also possesses a strong activity against bacteria, fungi and viruses . It is also possible that silver and silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria[8]. Many researchers also proposed that Ag+ ions interact with the thiol groups in bacteria proteins, affecting the replication of DNA . The present study was made an attempt to find out the antibacterial activity of silver nanoparticles against the 6 urinary tract infectious (UTIs) bacterial isolates. This study we report a simple, effective, low cost and environmental safe synthesis of silver and other metal nanoparticles using leaves extract and measurement its therapy effect of it against the nosocomial infections, were characterized by UV-VIS spectroscopy and Fourier transform infrared spectroscopy [9].

2. Materials required:

2.1 Preparation of the plant extract:

Fresh leaves of *Aloe barbedensis* were collected from the garden in locality near Thindal, Erode district. The leaves were washed with distilled water, and after grinding, 10 g leaves was mixed with 100 ml distilled water and heated for 12 min. Then the extract was filtered through Whatmann No.1 filter paper, collected and stored in refrigerator for further processing

2.2 Preparation of the metal solution:

The different concentration of the silver nitrate is prepared by dissolving the appropriate amount of the silver nitrate in the appropriate volume of water. Initially, 1.575gram of the silver nitrate is added to the one litre of distilled water.

Table -1: Different Concentration of silver metal solution:

Different concentration of silver metal solution			
Molar concentration	Silver nitrate (mg)	Water (ml)	Aloe barbedensis extract (ml)
1×10^{-3}	0.0170	100	10
5×10^{-3}	0.085	100	10
9×10^{-3}	0.1575	100	10

3. Synthesis of the silver nanoparticles:

10 % *Aloe barbedensis* plant extract was mixed with silver nitrate solution in 1:10 proportion. All the solution of reacting material are prepared using double distilled water and kept at room temperature for 48 hours for the development of reddish brown colour and after 48hours of incubation in dark room, in order to avoid oxidation, the extract is centrifuged at 9000rpm for 5minutes. The extract is air dried up to 3 days so as to enable to obtain powdered form of silver nanoparticles

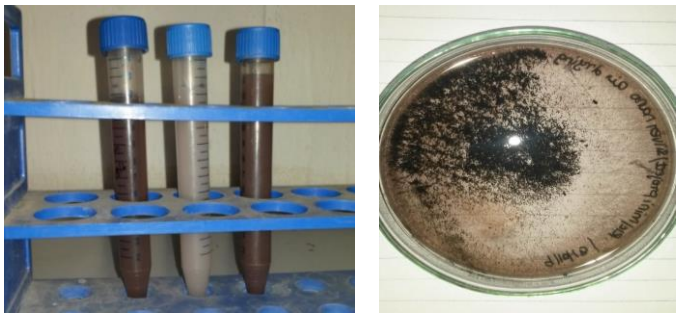


Fig .1 (a): Different concentration of silver nanoparticles; **1(b)** Air dried AgNP at room temperature.

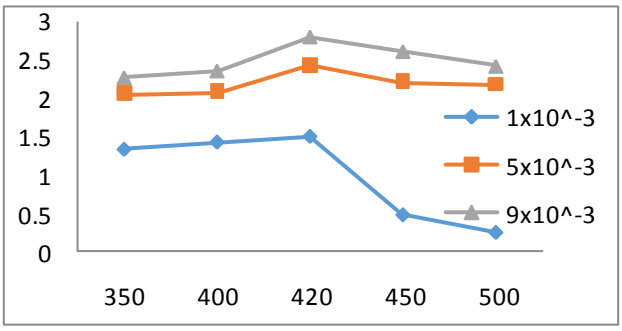
4 Characterization of the silver nanoparticles:

4.1 Colour change of nanoparticles:

(i) UV-Visible spectroscopy:

About 1 ml of resuspended silver nitrate solution was monitored in double beam UV–VIS spectrophotometer with different time intervals at different wavelength . It is the excellent method for measuring of the concentration of the nanoparticles. The optical density value was taken at wavelength of 350 to 500nm. The silver nanoparticles shows the peak in 420nm. After 8 h of incubation, the solution was centrifuged with 12,000 rpm for 20 min and their pellets were re-dispersed in sterile distilled water. The centrifugation and re-dispersion was repeated three times to ensure the complete separation of nanoparticles.

Chart -2 : UV Visible spectroscopic peaks of different concentration of AgNP at 420nm.

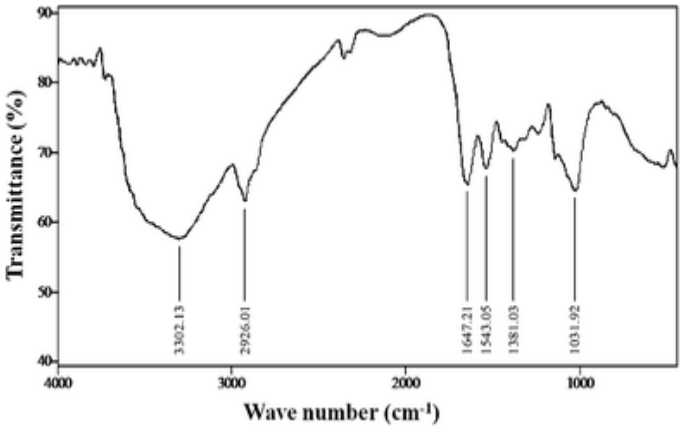


4.2 Surface morphology of nanoparticles:

FTIR studies:

Dried and powdered AgNPs were pelleted with potassium bromide (KBr) (1 : 10 proportion).The spectra were recorded in the wavenumber range of 450–2500 cm^{-1} and analyzed by subtracting the spectrum of pure KBr. FT-IR analysis was carried out in the diffuse reflectance mode operated at a resolution of 4 cm^{-1} in the range of 400 to 4,000 cm^{-1} to evaluate the functional groups that might be involved in nanoparticle formation.

Chart -2 : FTIR analysis of the AgNP at 4cm^{-1} resolution



5.Isolation of UTI bacterial pathogens:

A urine samples are collected from the patients admitted in the hospitals as UTI problems were collected from different hospitals and laboratory localities Erode District, Tamil Nadu, India in a separate sterile wide mouth bottle. Mid-stream urine was collected in a sterile wide mouthed container. For the isolation of UTI bacterial strains and fungal strains, loop full of urine samples were streaked into the nutrient agar, MacConkey agar, Blood agar and Chocolate agar plates and incubated at 37 ± 2C for 24 h. Next day individual colonies were selected and identified on the basis of morphological characteristics, gram staining, and biochemical characters.

6. Antibacterial sensitivity assay

Disc diffusion assay was performed to determine the antibacterial activity. Overnight culture of UTI pathogens were swabbed over the surface of sterile Mueller–Hinton agar plates using sterile cotton swabs. Discs impregnated with different concentrations of silver nanoparticles (5, 10, 15 and 20 µg $Disc^{-1}$) were applied on the solid agar medium by pressing slightly and incubated at 37 ± 2C for 24 hours. After incubation, the zone of inhibition was measured and expressed as zone of sensitivity millimeter in diameter.

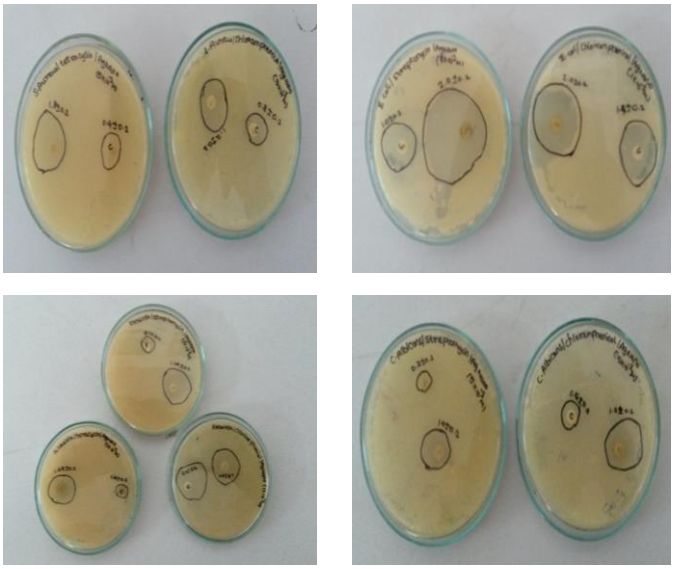


Fig -1: Antimicrobial activity of the UTI pathogens

7. Results and discussion:

Out of the collected samples, 3bacterial isolates and 1 fungal isolates were recovered and the biochemical tests revealed that, these isolates belong to 4 species. Of these E. coli is the predominant one (47%); *Klebsiella pneumonia (22%), Candia albicans (19%), streptococcus aureus (3%)*. The color intensity of the synthesized silver nanoparticles was increased with increased time duration, and the maximum intensity was observed with 420-nm wavelength. Further, FT-IR analysis was carried out in the diffuse reflectance mode operated at a resolution of 4 cm^{-1} in the range of 400 to 4,000 cm^{-1}. The silver nanoparticles is effective against *E.coli*, followed by *Klebsiella pneumonia (*8 ± 0.49 mm) at 20 µg $disc^{-1}$ concentration. *Klebsiella pneumonia, Candia albicans* showed sensitivity against all the tested concentrations (5, 10, 15 and 20 µg $disc^{-1}$). S*treptococcus aureus* showed less sensitivity against all the tested concentrations of silver nanoparticles.

8. Conclusion :

Patients with non-infectious disease who have stay in hospital have high risk to acquire nosocomial infection. It has been reported that, 10% hospital patients acquire this infection while staying in hospital. Nosocomial infectious bacteria exhibited least susceptibility to antibiotics and some of these bacteria out rightly developed multidrug resistance to these

antibiotics. *E.coli* showed maximum sensitivity at 20 µg disc^{-1} followed by *Klebsiella pneumonia.* The silver nanoparticles could be used as an effective antibacterial agent for the management of urinary tract infections caused *Escherichia coli* and Klebsiella pneumonia after successful completion of in vivo studies and clinical trials.

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