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ANTIBACTERIAL ACTIVITY OF GREEN SYNTHEZIED SILVER NANO PARTICLES AGAINST UTI CAUSING PATHOGENS

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ABSTRACT
The silver nanoparticles were synthesized by *Adhatoda vasica* leaves extract and the nanoparticles were characterized using Scanning Electron Microscope (SEM) studies. The synthesized silver nanoparticles were investigated to evaluate the antibacterial activity against urinary tract infections (UTIs) bacterial pathogens.

KEY WORDS: Green synthesis, silver nano particles, *Adhatoda vasica*, UTI causing bacteria, SEM

INTRODUCTION

A urinary tract infection, or UTI, is a bacterial infection of any part of the urinary tract, which includes the bladder, kidneys, ureters (tubes that connect the kidneys to the bladder) and the urethra (the tube that allows the bladder to be emptied). Infections of the bladder or the urethra are the most common. Most often, a UTI occurs because bacteria enter the urethra and travel up to the bladder, where they multiply. Bladder infections are typically caused by *Escherichia coli* (*E. coli*) bacteria, which are common bacteria in the human gut. Bacteria in the bladder can also move up to the kidneys and cause a kidney infection (known as pyelonephritis), which can cause permanent kidney damage. An untreated UTI in the bladder can lead to such an infection. Women are more likely than men to get a UTI. One reason for this is that women have a shorter urethra than men do, and it is closer to the anus. Both of these reasons explain why bacteria can reach the bladder more easily in women. Usually, a urinary tract infection is treated with antibiotics to prevent the infection from spreading to the kidneys. Symptoms of a bladder infection usually go away within one to two days after starting antibiotics. More than 95% of UTI are caused by single bacterial species *E. coli* which is the most frequently infecting organisms.[1] However, many other bacteria can also because an infection for example, *Klebsiella, Pseudomonas, Enterobacter, Proteus, Staphylococcus* etc. (Fig.1).[2] In complicated urinary tract infections and hospitalized patients, organisms such as *Enterococcus faecalis* and highly resistant Gram-negative rods including *Pseudomonas* spp. are comparatively more common. The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization.[3] It has recently been found that silver nanoparticles possess significant antibacterial potential. Several studies reported that, the silver and silver nanoparticles may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell.[4] It is also possible that silver and silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria. Many researchers also proposed that Ag+ ions interact with the thiol groups in bacteria proteins, affecting the replication of DNA. It has also been reported that Ag+ ions uncouple the respiratory chain from oxidative phosphorylation or collapse the proton-motive force across the cytoplasmic membrane. The present study was made an attempt to find out the antibacterial activity of green synthesized silver nanoparticles against the three UTI bacteria.

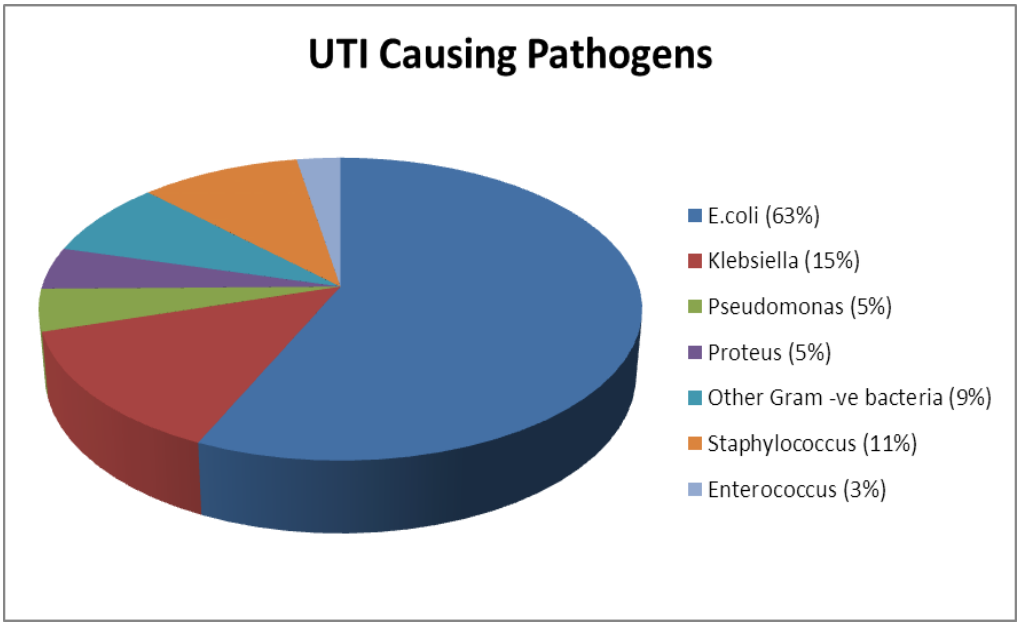

Fig.1: Percentage occurrence and distribution of bacterial pathogens in UTIs

MATERIALS AND METHODS

Preparation of Leaves extract

The leaves of *Adhatoda vasica* was shade dried at room temperature. The dried material was then homogenized to obtain coarse powder 10gm powdered plant material was soaked in 20ml of ethanol overnight and then filtered through a Whatman No.1 filter paper. The filtrate is used for the determination of antimicrobial activity.

Preparation of 1mM $AgNO_3$ and AgNPs: The fresh *Adhatoda vasica* leaves (10 g) were boiled with 100 ml distilled water and filtered. For the preparation of 1mM $AgNO_3$, 0.016 gm of $AgNO_3$ weighed accurately and made upto 100 ml using sterile distilled water. For the preparation of AgNPs, 90ml of 1mM silver nitrate solution was added to 10 ml of plant extract to make up a final solution 100 ml and centrifuged at 3,000 rpm for 10 min.

SEM analysis of silver nano particles: Scanning electron microscopic (SEM) analysis was done using VEGA3 TESCAN machine, Czech Republic.

Assay of Antibacterial Activity

The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then discs were mixed with chemical compounds separately and control discs were prepared. Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.*, [5]. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45 ºC. The cooled media was poured on to sterile petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The ethanol extract soaked discs were placed on the each petri plates and also placed control and standard (Nitrofurantoin) discs. The plates were incubated at 37 ºC for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

RESULTS AND DISCUSSION

In this study, AgNPs were synthesized using a reduction of aqueous Ag^+ with plant extract. It was generally recognized that AgNPs produced brown solution in water, due to the surface plasmon resonances (SPR) effect and reduction of $AgNO_3$. After the addition of $AgNO_3$ solution, the plant extract changed to brown colour in a few minutes (Fig.2). Thus, colour change of the solution clearly indicated the formation of AgNPs. The colour intensity of plant extract with $AgNO_3$ was sustained even after 24 hour incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation.

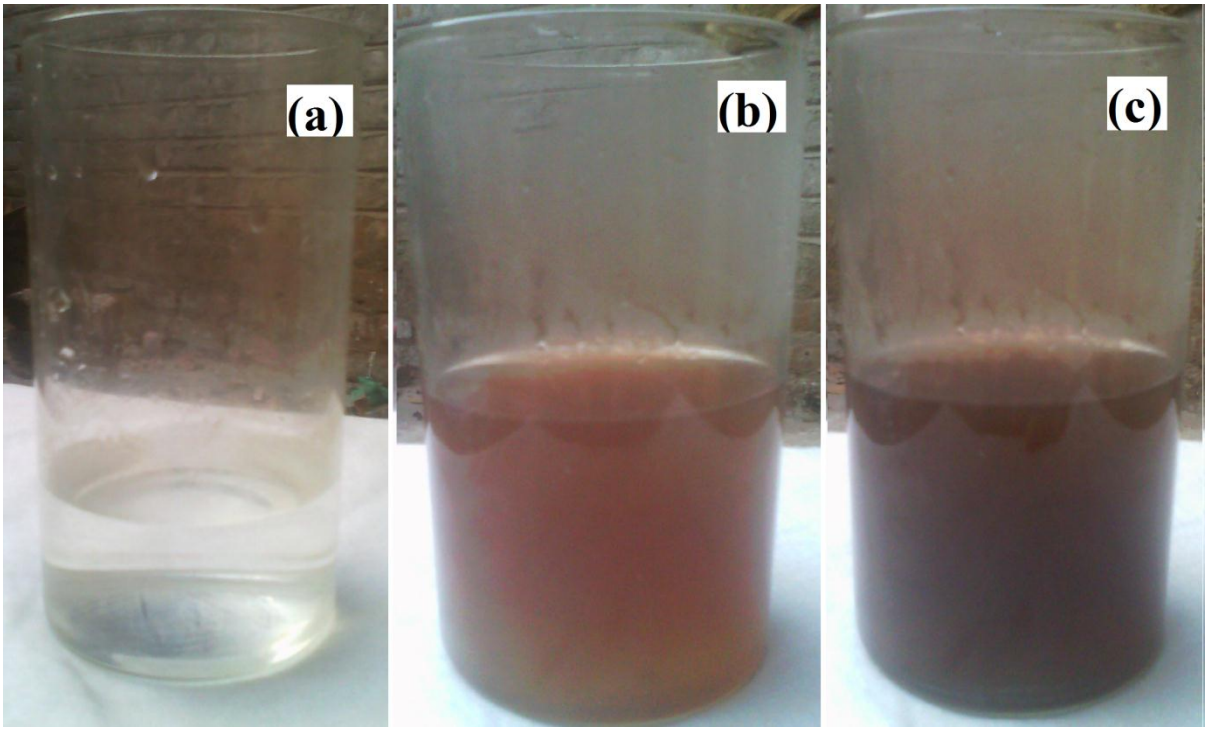

Fig.2: Visible observation of AgNPs biosynthesis. (a) Pure $AgNO_3$ solution (1 mM). (b-c) Gradual colour change appeared after the addition plant extract into $AgNO_3$ solution.

Scanning Electron Microscopy (SEM) analysis

SEM measurements were carried out to determine the morphology and shape of AgNPs. SEM micrograph (Fig.3) revealed that, the AgNPs were spherical shaped and well dispersed without agglomeration. The particle sizes of AgNPs synthesized were within 100 nm.

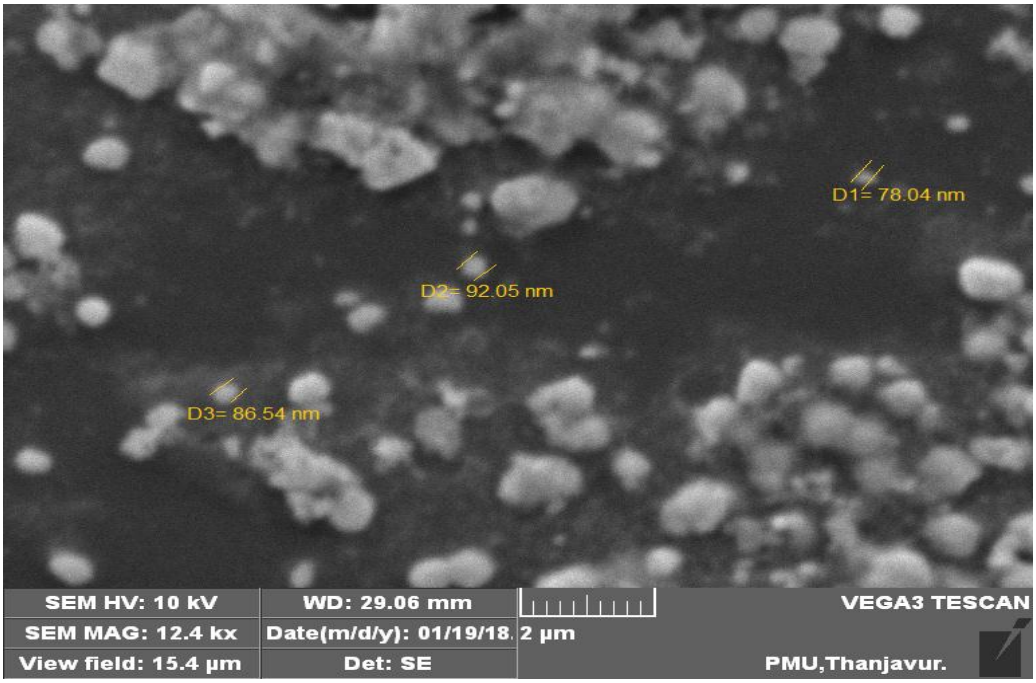

Fig.3: SSEM image of synthesized AgNPs.

Antimicrobial Activity Analysis of AgNPs

The antimicrobial activity of AgNPs UTI causing bacteria *Escherichia coli*, *Klebsiella pneumonia and Enterococcus faecalis* compared with the standard, the diameters of inhibition zones increased for all the test pathogens (Table 1 & Fig.4). The AgNPs produced could inhibit different typical pathogenic bacteria. Thus, AgNPs could be considered as excellent broad-spectrum antibacterial agents.

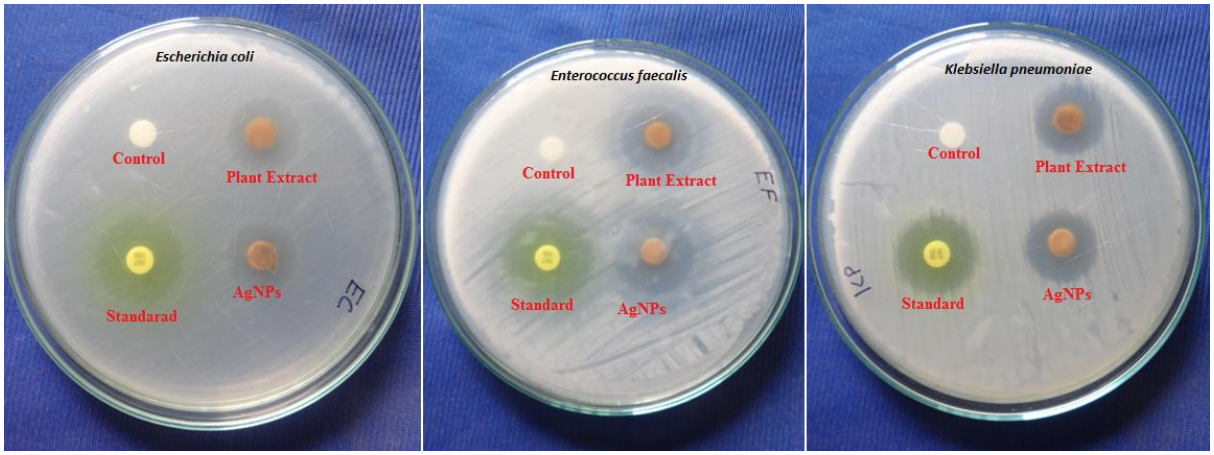

Fig.4: Antibacterial activitity of synthesized AgNPs and plant extract against UTI causing pathogens.

Table 1.Size of the inhibition zone for AgNPs and plant extract against UTI causing pathogens.

S. No.	Bacteria	Zone of Inhibition (mm in diameter)			
		Control	Standard * (Nitrofurantoin:100µg)	AgNPs (20 mg)	Plant Extract (20 mg)
1	*Escherichia coli*	-	21	17	19
2	*Klebsiella pneumonia*	-	20	17	19
3	*Enterococcus faecalis*	-	23	21	23

Bacterial infection of the urinary tract is one of the common causes for seeking medical attention in the community.[6] Effective management of patients suffering from bacterial UTIs commonly relays on the identification of the type of organisms that caused the disease and the selection of an effective antibiotic agent to the organism in question.[7]

Dowzicky and Park (2008) reported that, UTI bacterial pathogens have exhibited decreased susceptibility rates to tigecycline over the years. Antibacterial property of silver nanoparticles would be the alternative to overcome the resistance problem.[8] In this study, the application of green silver nanoparticles as an antimicrobial agent was investigated and exhibited better antimicrobial activity against UTI causing pathogens. The results (Fig.2) showed increasing colour intensity with increased time intervals and this might be due to the production of the silver nanoparticles and the formation of the brownish yellow colour might be due to the excitation of the surface plasmon vibration of the synthesized silver nanoparticles.[9] The mechanism of action may be due to the silver and silver nanoparticles attach to the surface of the cell membrane and disturbing permeability and respiration functions of the cell, moreover, due to the uptake of free silver ions followed by disruption of ATP production and DNA replication. Smaller silver and silver nanoparticles having the large surface area available for interaction would give more bactericidal effect than the larger silver and silver nanoparticles.[4] On the other hand, the main composition of bacteria cell membrane is phospholipid bilayers and protein molecules having negative electricity which make the whole cell membrane negatively charged. Therefore, the silver ions with positive electricity have the ability to attach to bacteria cell membrane quickly, which alters or damages the structures of bacteria. Moreover, Ag+ ions can be attracted to the sulfhydryl group (SH) of bacterial enzymes (respiratory enzymes), making the enzymes inactivated and even died out. The antibacterial activities of smaller AgNPs (average diameter 9–10 nm) are generally higher on the basis of equivalent silver mass content. AgNPs have tendency to get oxidized and aggregate in media with high electrolyte content, resulting in a reduction of their antibacterial activities. However, complexation with polyvinyl pyrrolidone can stabilize the AgNPs against aggregation, leading to a retention of the antibacterial activities.[10] Although the exact mechanism of action of AgNPs remains largely unknown, they have been found to anchor and penetrate the bacterial cell walls and modulating the bacterial cell signaling pathways. Another explanation that antibacterial effect of AgNPs are dependent on chemisorbed Ag+, which is readily formed owing to extreme sensitivity to oxygen. Ag+ has been reported to interfere cellular functions of bacteria by inhibiting respiratory chain enzymes, induces massive proton leakage and interacts with cytoplasmic components and nucleic acid. [10] The AgNPs antimicrobial activity depends strongly on several factors including type of microorganisms, temperature, pH, $AgNO_3$ concentration, size and shape of the nanoparticles [11,12] The bactericidal activity of the AgNPs has also been reported to be size and shape dependent as has been reported previously. It is speculated that AgNPs with the same surface area but different shape may exhibit different bactericidal effects due to different active facets.[11]

CONCLUSION

Urinary tract infections in human are considered the most serious health problems facing the world. *E. coli* is the most common bacteria causing UTI. The results of this study demonstrated that alcoholic extracts of *Adhatoda vasica* and green synthezied silver nano particles were effective against UTI causing bacteria *Escherichia coli*, *Klebsiella pneumonia* and *Enterococcus faecalis*. It is concluded from the present study that, the silver nanoparticles could be used as an effective antibacterial agent for the management of urinary tract infections caused by *Escherichia coli*, *Klebsiella pneumonia* and *Enterococcus faecalis* after successful completion of in vivo studies and clinical trials. Green synthesis of AgNPs using plant extracts has several advantages such as eco-friendliness, cost effectiveness, compatibility for medical and pharmaceutical applications. It is confirmed that green silver nanoparticles are capable of rendering high antimicrobial efficacy and hence has a great potential in the field of nano medicine.

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