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CHARACTERIZATION AND ANTIBACTERIAL POTENTIAL OF SENNA *OCCIDENTALIS* (L.) LINK SILVER NANOPARTICLES

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Abstract

Senna occidentalis (L.), Link also known as kasundi in Pakistan, is a 2m tall shrub belonging to family Fabaceae. This plant has miracle advantages and this Ayurvedic plant is very well known for its "kehwa" made of dry leaves and a "coffee" made of roasted seeds. Today, green nanoparticles are considered as best tools against microbes as well as an alternative to lengthy manufacturing processes and toxic chemicals. In the present research, two sets of experiments were setup one with the control group (water, methanol and n-hexane extracts of *S. occidentalis*) and another experimental group which includes biosynthesis of AgNPs with three extracts. Leaf extracts of *S. occidentalis* were treated by silver ions (Ag+), which were reduced to silver nanoparticles (AgNPs). The green AgNPs were characterized visually (color change of extract) and additionally by UV-Visible spectroscopy and Scanning Electron microscope (SEM). The green nanoparticles were then analyzed for their antibacterial potential against gram –ve *Escherichia coli,* and *Pseudomonas aeruginosa* and gram +ve *Streptococcus aureus.* The result indicated that the green AgNPs exhibited excellent antibacterial potential as compared to the plant extracts. The zone of inhibitions (ZOI) observed in *Streptococcus aureus* was 3.4 mm in AgNPs from water, 7 mm in AgNPs from methanol and 4 mm in AgNPs from n-hexane. The ZOI observed in *Pseudomonas aeruginosa* was 2.6 mm in AgNPs from water, 5.5 mm in AgNPs from methanol and 7 mm in AgNPs from n-hexane and the ZOI observed in *Escherichia coli* was 1.8 mm in AgNPs from water, 4 mm in AgNPs from methanol and 2 mm in AgNPs in n-hexane. The eco-friendly green synthesis of nanoparticles can serve as a simple and quick process for production of nanoparticles on large scale in future.

Key words: AgNPs, *Escherichia coli*, Green nanoparticles, *Pseudomonas aeruginosa*, SEM, *Streptococcus aureus*.

Introduction

Nanotechnology is recently discovered as a branch of science which deals with materials vary from 1-100 nm. Today it is considered as a dynamic and zestful area of research in modern material science (Britto *et al.*, 2014). The diverse applications of nanoparticles revolve around the globe in food, health care, cosmetics, mechanics, space industries, environmental health, industries, biomedicines, catalysis, optics, drug-gene delivery, light emitters, photo-electrochemical and energy science applications (Hajipour *et al*., 2012). Now a days, microorganisms (e.g., fungi, bacteria), enzymes and plants have been in great use for synthesis and manipulation of nanoparticles as they are proved to be eco-friendly comparative to chemo- physical methodologies and are proved to be advantageous. Some nanoparticles unveil antimicrobial response against some pathogenic bacteria, common examples of such nanoparticles are ZnO, CuO, Fe_2O_3, $AgNO_3$ (Pantidos and Horsfal, 2014).

This plant belongs to family *Fabaceae* and subfamily Caesalpinnaceae, has earned many common names as ant bush, coffee senna, coffee weed, septic weed**.** It is native to America, Africa, southern Asia and USA (Odeja *et al.,* 2015). It is widespread in the areas of Punjab and KPK. In a survey it was estimated that among many other plants *Senna occidentalis* have been growing around and in Lahore-Islamabad motorway (Ahmad, 2007). In India *S. occidentalis* has been regarded as herbal medicine because of its rich phytochemical profile which includes alkaloids, phlobatannins, tannins and flavanoids (Awwad *et al*., 2013). Due to these phytochemicals *S. occidentalis* have its extensive use in cure of diseases and have, anti-inflammatory anti-carcinogenic, anti-mutagenic, anti-microbial, anti-rheumatic, anti-plasmodial and hapatoprotective properties (Yadav *et al*., 2009: Vijayalakshmi *et al*., 2013: Vashishtha *et al*., 2009). The whole plant is medicinal from roots to leaf (Sadiq *et al.*, 2012).

Biocontrol of pathogens is a vital way of preventing the adverse effects caused by synthetic chemicals and antibiotics (Awwad *et al*., 2013). The exploration of this marvel has gained value due to incredible increase of bacterial resistance to antibiotics. Antibacterial agents are toxic to bacteria without damaging the surrounding tissues, nanoparticles have been considered as good antibacterial agents but with expensive and lengthy industrial routes (Krithiga *et al*., 2015). Presently, the focus has been diverted on the green synthesis of these nanoparticles which promises cost effective, ecofriendly synthesis of nano materials with maximum elimination of complex industrial synthetic routes (Akinsiku *et al*., 2015).

Antibacterial responses of silver-containing materials are useful, in pharmaceuticals to lessen infections and to resist bacterial colonization. AgNPs have been widely used as antibacterial agents due to greater surface/ volume ratio and high reactivity. Silver is generally used as a nitrate salt, but in form of silver nanoparticles its compounds are extremely toxic to diverse microorganism, which make them fascinating candidates for multiple pharmacological and medical fields (Furno *et al*., 2004). The present work reports the synthesis of eco-friendly AgNPs using the principle of bio-reduction of silver ions (Ag+) with the extract of *Se. occidentalis* as reducing agents. UV-Visible spectroscopy and SEM was used for characterization of AgNPs and then the antibacterial response was examined against *S. aureus, P. aeruginosa* and *E. coli.*

Materials and Method

Collection: Fresh green leaves of *S. occidentalis* were collected from different Gardens of Lahore. Leaves were washed and allowed to shade dried and finely crumbled.

Microwave assisted extraction of *S. occidentalis*: 10mg fine powdered leaves were dissolved in 250ml water, methanol and n hexane separately and were heated in microwave using power of 1000 watt for 10 seconds for 15 times. The mixture was then filtered using Whattman no.1 filter paper (Javad *et al.*, 2017).

Formation of Silver nanoparticles: 10 ml of 5 mM silver nitrate solution was added in the 30ml plant extract. The bio reduction of silver ions into AgNPs was monitored visually by color change (pale green to dark brown or black).

Centrifugation: The extract contain AgNPs were taken in eppendorfs and ultra-centrifuged at 10000 rpm for 5 minutes. Supernatant was removed and the pallets of AgNPs were stored at 4°C (Sreenivasulu *et al.*, 2016).

UV Visible spectroscopy and SEM: Dual beam UV- Visible spectrophotometer was used within range of 200-700nm to characterize the nanoparticles. SEM was used also used to characterize AgNPs.

Antibacterial Assay: Two sets of experiments were performed, one with the simple leaf extracts of *S. occidentalis* as control and the other with the green synthesized AgNPs as experimental. Antibacterial assay was performed using bacterial strains i.e., *S. aureus*, *P. aeruginosa* and *E. coli*, by agar well diffusion method. Under aseptic conditions 4 ml nutrient agar was poured in each Petri plate and was allowed to solidify. Bacterial strains were inoculated by streaking. Four holes were made in the agar with the help of aseptic bowered. Simple extract and AgNPs that were synthesized from methanol, n- hexane and water was poured in each of them. Zone of inhibitions were measured after incubating the plates for 24 hours at 37°C.

Statistical Analysis: All data was evaluated by one-way Analysis of Variance (ANOVA).

Results

Synthesis of AgNPs: During the research, two sets of tests were done, one with the simple leaf extracts of *S. occidentalis* and other with the green synthesized silver nanoparticles. During the synthesis of AgNPs color of plant extract began to change instantly after adding $AgNO_3$ solution which was an indication of nanoparticles synthesis. The color change visually confirmed the synthesis of AgNPs as initial color of solution was pale or green which changed to dark brown gradually as shown in figure 1. The intensity of color with time revealed increased and enhanced AgNPs synthesis; also the size of AgNPs depends on the color (Jain *et al*., 2006). Further UV-Vis spectrophotometry indicated the formation of particles. UV scan of AgNPs with water showed maximum absorbance of 8.5 at wavelength 432 nm. In case of methanol and n-hexane showed peaks at 400 nm with the absorbance of 7.5 and 8.0 respectively as illustrated in figure 2.

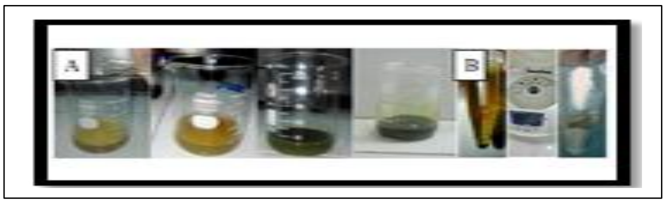

Figure 1: (A) Gradual color change confirming the synthesis of AgNPs. (B) AgNPs before and after centrifugation.

Figure 2: UV- visible spectroscopy of (A) water (B) methanol (C) n-hexane

SEM Analysis: The silver nanoparticles were analyzed under SEM) in which particles were measured ranging from 100-200 nm as depicted in figure 3.

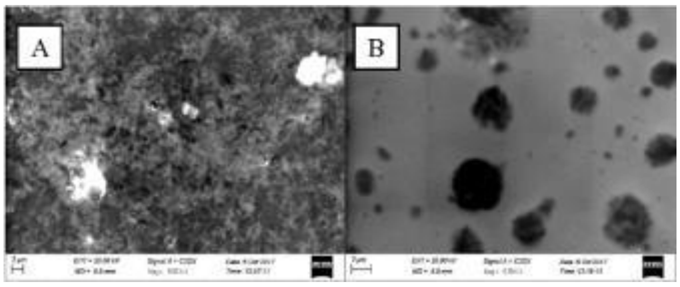

Figure 3: SEM Analysis of AgNPs synthesized from (A) water (B) methanol

Antibacterial Assay: Plant extracts of *S. occidentalis* and AgNPs were then used to examine the antibacterial activity against *S. aureus*, *E. coli* (G-) and *P. aeruginosa* (G-). After one day of incubation under 37°C significant inhibition zones were observed which showed antibacterial potential. The result recommended that AgNPs possessed highest antibacterial potential as compared to the control i.e., plant extract. Among all solvents, maximum inhibitory action against bacteria was shown by AgNPs synthesized using methanol. The figure 4 shows the zone of inhibitions and the antibacterial activity. Overall AgNPs and extract showed the highest antibacterial potential against *S. aurens* which revealed that *S. occidentalis* and AgNPs can be used as best antibacterial agents against these bacteria. The decreasing order of antibacterial agents of this experiment can be summarize as AgNPs (methanol)> AgNPs (n-hexane) > AgNPs (water) > extract (methanol) > extract (water) > extract (n-hexane).

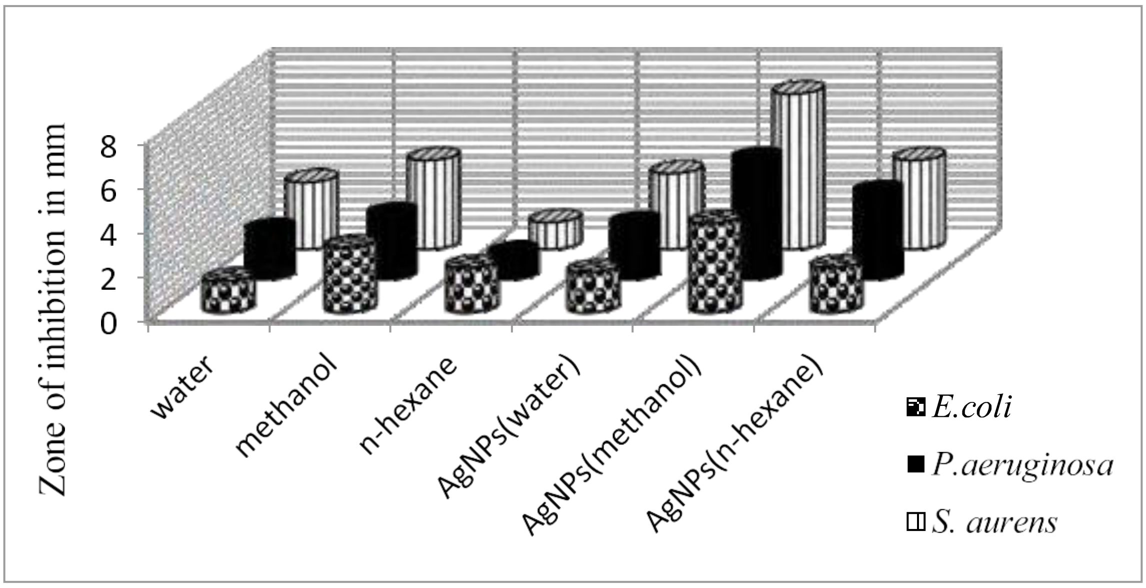

Figure 4: Illustration of antibacterial activity of *S. occidentalis* extracts and AgNPs against *E.coli*, *P. auriginose* and *S. aureus.*

Discussion

In the present investigation, environment friendly green synthetic route was used for synthesis of silver nanoparticles. AgNPs were synthesized using *Senna occidentalis* leaves without using toxic chemicals and long processing. This technique plays an important role as it was not time consuming and nanoparticles can be prepared on large quantity. The extracts of *S. occidentalis* was treated with silver nitrate salt which immediately causes the reduction of ions thus changes silver nitrate to silver nanoparticles. The color became dark and intense with time. The color change is very important because it indicated the synthesis of nanoparticles. Furthermore UV-Visible spectroscopy confirmed the synthesis of AgNPs. Similar work was done by Akinsiku *et al*. (2015) by synthesizing silver nanoparticles using *Canna indica* and *Senna occidentalis* leaf extracts. Onset of formation began as early as 5 minutes and nanoparticles thus formed were in between 400-430 nm. The silver nanoparticles synthesized from *S. occidentalis* using water as a solvent was present in literature and research but there is yet no work on synthesis of silver nitrate nanoparticles synthesized from methanol and n-hexane used as a solvent. Both of the extract showed complete change in color within 2 hours. The silver nano particles derived from *S. occidentalis* extract were analyzed for antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus aureus*. It was found that the largest zone of inhibitions was shown by AgNPs synthesized from methanolic *S. occidentalis* extract followed by n-hexane and then water. The literature shows variation in effectiveness of leaf extracts of *S. occidentalis* against *E. coli* (Sreenivasulu *et al*., 2016). It was also observed that the antimicrobial activity of silver particles was highly influenced by the dimensions of the particles, more tiny the grain, greater will be its antimicrobial response. Hence, a special importance was made to restrain and control the silver nanoparticles size in their formation as it increases the surface area and thereby antibacterial efficiency was greatly enhanced. It was also observed that AgNPs have significantly less effect on the growth of Gram-positive bacteria (*S. aureus*) than Gram negative (*E. coli* and *P. aeruginosa*). This can be explained that the wall composition of bacteria (Pal *et al*., 2007). Gram-negative bacteria have a layer of lipopolysaccharides on the outside and present below a thin layer of peptidoglycan. Although lipopolysaccharides are composed of lipids covalently bound to polysaccharides, there was a lack of rigidity of the overall s t ructural envelope. The negative charges on the lipopolysaccharides are attracted to the weak positive charge of AgNPs. On the other hand, the cell wall of Gram-positive bacteria was mainly composed of a thick layer of peptidoglycan consisting of linear polysaccharidic chains cross-linked by short peptides to form a three-dimensional rigid structure (Lin *et al*., 2010). The stiffness and the extensive cross-linking not only reduce the bacterial cell wall anchoring sites for AgNPs but also render the wall itself more difficult to penetrate. It is now clear that AgNPs possess a strong antibacterial potential by the present work and also highlighted by several studies. With these nanoparticles and extracts, there is a need for the preparation of different formulations towards ensuring acceptable dosing to *in vivo* trials. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antibacterial agents of natural origin for the treatment of bacterial infections in human beings.

Conclusion

The present work concluded that the green synthesis of nanoparticles is easy and cost effective way. It has benefit over chemical and industrial nanoparticles formation. Nanoparticles were best synthesized when water and methanol were used as solvents as a bulk of pallet was obtained after centrifugation while in case of n-hexane very small amount of AgNPs were obtained. Antibacterial activity of silver nanoparticles and plant extracts were compared that revealed silver nanoparticles showed higher antibacterial potential than the plant extract of *S. occidentalis*. Furthermore, AgNPs synthesized from methanol showed the highest antibacterial activity among all. The reason could be explain as the very small size of AgNPs with the great surface area destroys and crosses the membrane of microorganisms that cause damage. Green AgNPs can therefore be used as antimicrobials and with these nanoparticles we can deal with the challenging diseases without complex mechanisms, expenses and time. With these nanoparticles, there is a need for establishment of some drugs or compounds that could be used as patent antibacterial agents without any toxic chemicals and lengthy processes.

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