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Barleria acanthoides Vahl. Mediated Green Synthesis of Silver Nanoparticles and their Antibacterial Activity

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Authors' contributions

This work was carried out in collaboration among all authors. Author MFAS conceptualized the study, conducted experiments and prepared initial draft of the manuscript. Author KP supervised the study, reviewed, edited and communicated the manuscript. Author NAB supervised and reviewed the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Barleria acanthoides Vahl members of the Acanthaceae are plants of the old world, found in the regions of Africa, the Arab peninsula and Southeast Asia. The plant contains several phenolic compounds, antioxidants, and secondary metabolites that can assist in the synthesis of silver nanoparticles (SNPs). Therefore, in the present study, *B. acanthoides* was explored for the utilization of *B. acanthoides* extract for the synthesis of stable SNPs as well as for their antibacterial properties. The $AgNO_3$ solution (5 mM) and plant leaf extract at a ratio of 9:1 were mixed for the preparation of reaction mixture. The green synthesis of SNPs was achieved when the reaction mixture was heated at 90^oC for 5 min. The synthesized SNPs were characterized by UV-vis spectroscopy, FTIR, SEM, and DLS analysis. Furthermore, the SNPs were evaluated for their antibacterial potential by a well diffusion against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* (ATCC 6633), *Pseudomona aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922). The minimum inhibitory concentration (MIC) of the SNPs against bacteria was also evaluated. The SNP synthesis mediated by *B. acanthoides* was simple, quick, and risk-free. The characterization of SNPs by various analyses showed that the SNPs were spherical with an average size of 45.15 nm. The SNPs showed broad spectrum antibacterial properties. A maximum

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zone of inhibition was observed against *E. coli* (33.75 mm), followed by *P. aeruginosa* (16.67 mm), *S. aureus* (16.5 mm), and *B. subtilis* (14.33 mm). The MIC value of SNPs was found to be 1.56 µl /ml for *E. coli*, *P. aeruginosa*, and *B. subtilis*, while the MIC value for *S. aureus* was 3.125 µl /ml.
Conclusion: *B. acanthoides* can be explored further for the non-hazardous, eco-friendly synthesis of SNPs for medical and therapeutic uses.

Keywords: Barleria acanthoides; antibacterial activity; silver nanoparticles; MIC; green synthessis.

ABBREVIATIONS

CFU : Colony forming unit;
DLS : Dynamic light scattering;
EtOH : ethanol alcohol;
FTIR : Fourier transform infrared spectroscopy;
SNPs : Silver Nanoparticles;
MIC : Minimum inhibitory concentration;
PE : Plant Extract;
PDI : polydispersion index;
SEM : Scanning Electron Microscopy;
SPR : surface plasmon resonance

1. INTRODUCTION

Nanoparticles are well recognized for their distinctive characteristics over their sizeable forms. Nanoparticles have gained wide attention in the fields of medicine, engineering, and agriculture. The metal nanoparticles are synthesized by different procedures that include physical, chemical, and biological methods (green synthesis). The synthesis of nanoparticles by utilizing biological materials has gained much more attention in recent years due to its non-hazardous approach and economics. Generally microorganisms or plants are used [1,2]. Plant extracts are used as the mediator and capping agents for the synthesis of stable metallic nanoparticles. Usually, this process is simple, fast and does not involve hazardous chemicals or procedures [3]. Plants may be used as a unique green source for producing metallic nanoparticles that can be employed in a variety of applications and can be used instead of commonly used antibacterial agents. The antimicrobial properties of silver metal have been known since ancient times. Similarly, SNPs have been recognized for their antimicrobial properties now. Silver nanoparticles can be synthesized through the use of plant extracts. The successful synthesis of stable SNPs depends on the plant extract, other than the physical properties of the experiment such as temperature and pH. Various novel methods have been developed for the green synthesis of the SNPs by modifying the plant extracts and various parameters [4–6].

Barleria L. (Acanthaceae) is a genus of over 300 species that are predominantly found in tropical parts of the world, such as Africa, the Arab peninsula, the Indian subcontinent, and Southeast Asia [7]. Herbs and shrubs make up the majority of the species in this genus. The four-partite calyx with two large outer segments and two smaller interior segments, the globose honey-combed pollen grain, and the double cystoliths in the epidermal cells differentiate *Barleria* from other members of the Acanthaceae family. *Barleria* members are well-known for their usage in traditional medicine. Alkaloids, terpenoids, tannins, quinones, and flavonoids are abundant in *Barleria* species. *B. prionitis, B. lupulina, and B. cristata* are the most well-known for their antibacterial, anti-inflammatory, and antiviral activities [8–10]. The pharmacology and green production of nanoparticles by *Barleria acanthoides* Vahl, on the other hand, are little understood. Various research on the green production of silver nanoparticles by many *Barleria* species have recently been published [9] [11] [12]. However, the utilization of *B. acanthoides* for the synthesis of silver nanoparticles has yet to be explored. As a result, the current study explores the utilization of *B. acanthoides* extract as a mediator for the rapid and reliable synthesis of stable SNPs, as well as their antibacterial properties.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Preparation of Plant Extract

The plant samples were collected from Abha, Saudi Arabia. The plants were identified on the basis of taxonomic characteristics of the species and the identity was confirmed by the taxonomist, Dr. Najat Bukhari of King Saud University, Saudi Arabia as *Barleria acanthoides* (Fig. 1).

Fig. 1. *Barleria acanthoides* collected from Abha, Saudi Arabia

Classification

Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	Acanthaceae
Genus:	*Barleria*
Species:	*Barleria acanthoides* Vahl

The collected samples were washed with water to remove dirt and soil particles. Leaves were separated from the branch and rinsed twice with deionized water. The wet leaves were spread on blotting paper, covered with tissue paper, and left to dry at room temperature on a clean lab bench. Dried leaves were ground into a homogenous powder by an electric grinder. The powder (25 g) was mixed with 100 ml of deionized water and boiled (100 ^{o}C) for 15 min. on a hot plate. After that, the mixture was cooled down to room temperature and filtered using Whatman filter paper no.1. The resulting filtrate (plant extract) was kept refrigerated at 4 ^{o}C until the green synthesis of SNPs was performed.

2.2 Green synthesis of Silver Nanoparticles(SNPs)

The reaction mixture for the synthesis of SNP was made by mixing (9:1) silver nitrate solution (5 mM) prepared in deionized water and plant extract. The prepared reaction mixture was heated at 90^{o}C for 5 min. The change in color to dark brownish was considered a positive result, i.e., the formation of SNPs [5].

2.1.1 Characterization of SNPs

Various analyses were carried out for the determination of the formation, stabilization, morphology, and dimensions of SNP [5,13].

Initially, to determine the formation of SNPs, the suspension of SNPs was analyzed on a UV-visible spectrophotometer (Shimadzu, Tokyo, Japan), UV-visible spectrometric measurements were performed at a wavelength range of 320 to 600 nm.

For the confirmation of the capping and stabilizing of the SNP, Fourier transform infrared spectroscopy (FTIR) was performed on the FT-IR (Nicolet 6700 FT-IR Spectrometer, Waltham, MA, USA).

Scanning Electron Microscopy (SEM) analysis was done to characterize the shape and size of the SNP. A SNP suspension (10 µL) was dropped onto grids (200 mesh) with a carbon support film (Agar Scientific, London, UK) and then the sample was dried. After that, the dried sample was rinsed with ethanol alcohol (EtOH), dried again, and fixed on the SEM holder. Images were captured using a SEM (JEOL 7500FA JEOL, Peabody, MA, USA) at a 30 kV accelerating voltage.
For confirmation of the size and distribution of SNP, a dynamic light scattering (DLS) instrument (DynaPro-TC-04) equipped with ZetaSizer (Malvern, UK) was used. The SNP suspension was sonicated for 20 min and DLS measurements were taken.

2.3 Antibacterial Activity of SNPs

The SNPs were evaluated for their antibacterial activity by an agar well diffusion assay. Four bacterial strains, i.e., *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Pseudomona aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were obtained from the Department of Botany and Microbiology,

King Saud University, Saudi Arabia. An aliquot of 10 ml of nutrient broth was inoculated with the bacterial pathogens and incubated at 37°C for 24 h. The next day, the cultured broth was adjusted to a turbidity of 0.5 Mac Farland standards (10^8 CFU/ml). To obtain uniform inoculums, 100 µl bacterial suspensions were evenly spread over the entire surface of the nutrient agar plate. Four wells per plate were made, and each well was filled with 50 µl of SNP, plant extract, and denoised water (negative control). While the antibacterial activity of an antibiotic, Augmentin (10 µg/ml) was also tested against all bacteria (positive control).

2.4 Determination of Minimum Inhibitory Concentration (MIC) of SNPs

The synthesized SNPs were also evaluated for the minimum inhibitory concentration (MIC) against all bacteria. The MIC was determined by the microbroth dilution method on 96 well plates [14].

2.5 Statistical Analysis

There were three replicates for each experiment. Each test was performed twice and Microsoft Excel 2013 was used for calculation of data and standard error.

3. RESULTS AND DISCUSSION

Plants contain a variety of primary and secondary metabolites, each with its own set of characteristics. SNPs are now being studied intensively for their potential relevance in the management of pathogens. A reducing agent is required for the synthesis of SNPs. These are mostly toxic compounds. Plant extracts have developed as an alternative to these hazardous compounds as a reducing agent. In general, phytochemicals have complex compositions, and these compounds act as very good reducing agents for the synthesis of SNPs. *B. acanthoides* has been reported to contain various phytochemicals and metabolites that can assist in the synthesis of SNPs [10]. Therefore, the plant extract of this plant was investigated for the synthesis of SNPs. The SNP were synthesized by heating the reaction mixture of $AgNO_3$ (5 mM) and a plant extract of *B. acathoides* at 90 ^oC for 5 min. The visible color change of the reaction mixture to reddish-brown was the first indication of efficient synthesis of SNPs (Fig. 2).

The biosynthesized SNPs were also analyzed using UV-visible spectroscopy. The wavelength of the SNP suspension was measured using UV-visible spectroscopy to detect the synthesis of SNPs. There was a surface plasmon resonance phenomena, as seen by the peaks in Fig. 3. Furthermore, at 450 nm, the UV-vis absorption band was found, which is an indication of the presence of silver nanoparticles in the reaction mixture (Fig. 3).

The phytochemicals and metabolites in the *A. acanthoides* extract worked as an electron donor, reducing silver ions and causing the synthesis of SNPs; also, the surface plasmon resonance (SPR) phenomenon caused the color shift to reddish-brown [15]. The UV-vis spectroscopy confirmed the synthesis of SNPs by recognizing the SPR feature in absorption spectrum bands [16]. The absorption peak is determined by the biochemical properties of plant extracts as well as the concentration of the initial substrate used for SNP synthesis [17].

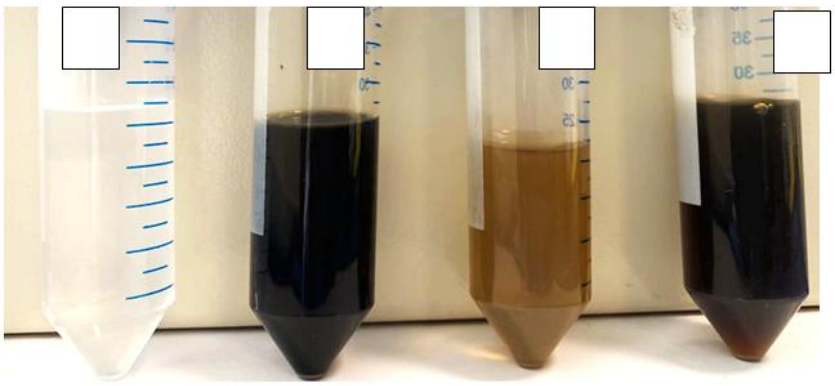

Fig. 2. *B. acanthoides* mediated SNPs synthesis. a) $AgNO_3$ (5 mM) b) aqueous *B. acanthoides* extract (PE) c) Reaction mixture 9 ml $AgNO_3$ (5 mM) + 1ml PE d) Synthesized SNPs

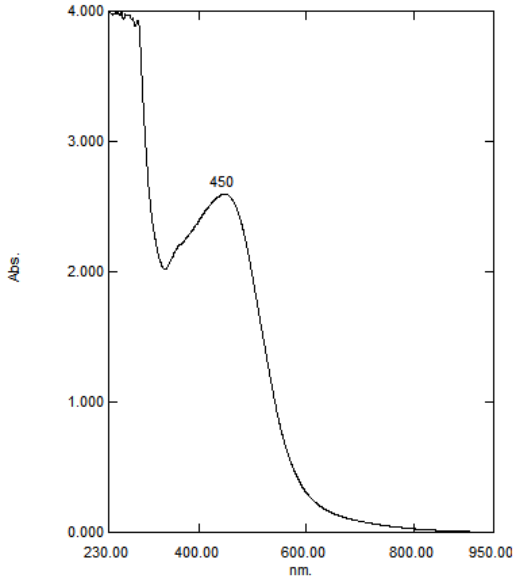

Fig. 3. UV–vis absorbance spectra of SNPs, synthesized by using PE of *B. acanthoides*

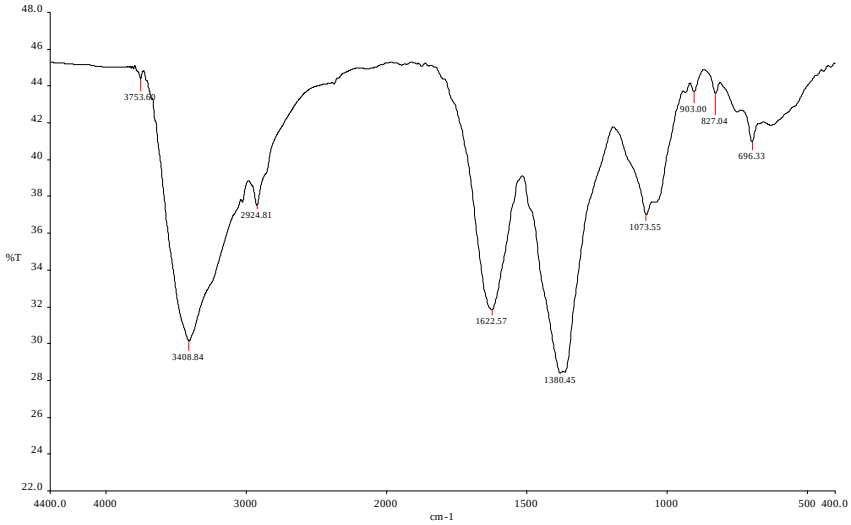

Fig. 4. FT-IR absorbance spectra of SNPs, synthesized by using PE of *B. acanthoides*

The presence of functional groups in the biosynthesized SNPs solution was confirmed using Fourier-Transform Infrared Spectroscopy (FT-IR). The absorption bands can be seen in the FTIR spectra in ranges spanning from 3753.60 to 696.33 cm-1 (Fig. 4). Furthermore, strong absorption peaks can be found at 3408.84, 2924.81, 1622.57, 1380.45, 1073.55, 903.00, 827.04, and 696.33 cm-1 in the spectrum. These peaks show that the various functional groups present in the solution, such as alcohols, carboxylic acids, amides, alkynes, alkanes, alkyl amines, halogen, and cycloalkanes, have served as capping and stabilizing agents (Fig. 4).

B. acanthoides extract, which was utilized as a reducing agent in the synthesis of SNPs, was the source of these functional groups. SNPs were most likely reduced, capped, and stabilized by these compounds and proteins in the plant extract. The FT-IR chromatograph revealed a significant band at 3408.84 cm-1, indicating the presence of -OH groups. It shows that plant extract has a high affinity for the surface of SNPs, resulting in the stability of biosynthesized SNPs [12].

The synthesized SNPs were also subjected to a zeta potential analysis to determine the average size and polydispersion of the synthesized nanoparticles (Fig. 5 and Fig. 6). The average particle size of the SNPs synthesized by *B. acanthoides,* was 45.15 nm, the polydispersion index (PDI) 0.435 (Fig. 5). The zeta potential was -17.1mV, for the SNPs synthesized from *B. acanthoides* furthermore, the results indicate high stability the resulting SNPs (Fig. 6).

The size of the nanoparticles is primarily controlled by the molarity of $AgNO_3$ and the concentration of the reducing agent. In addition, the plant species and type of extract used are important factors in the determination of SNP dimensions. Researchers have successfully synthesized SNPs from various plant extracts, such as from *B. cristata* extracts with an average size of 39 nm [18], *Pulicaria glutinosa* extract SNPs of 40–60 nm [19], *Calligonum comosoma* extract with the SNPs average size of 105 nm [20], *Berberis vulgaris* with an average size of 30–70 nm [21], and *Malva pyriflora* leaf of 50.6 nm [5].

The morphological features of the nanoparticles were determined using scanning electron microscopy (SEM). The biosynthesized SNPs were primarily spherical in shape, although there were a few irregularly shaped ones as well. The nanoparticles were fabricated in sizes ranging from 25.3 nm to 48.8 nm (Fig. 7). The results of present study are in agreement with the findings of Ghosh et al. [11] that have reported the synthesis of spherical SNPs of 10-20 nm sizes with help of *B. prionitis*. While another group of researchers reported the production of 2.4 nm spherical SNPs by *B. longiflora* [12]. Previous research has shown spherical SNPs when synthesis was aided by date seed extract, *Artemisia princeps, M. pyriflora* [5,22,23].

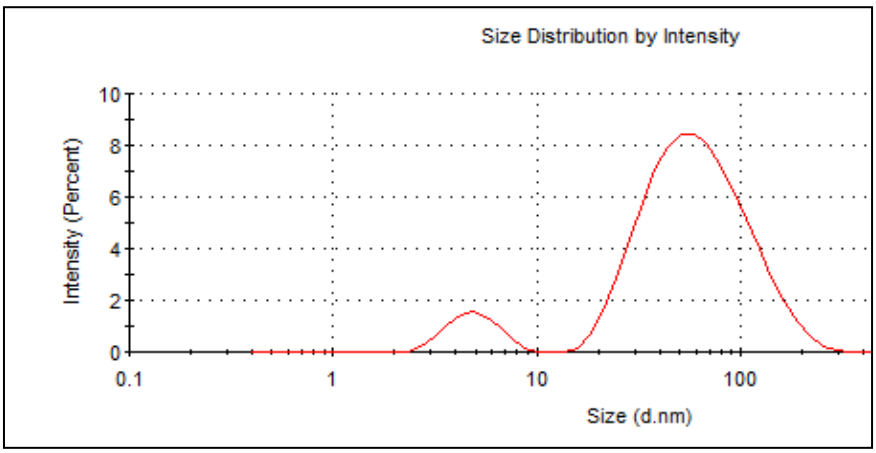

Fig. 5. Particle size distribution of SNPs, synthesized by using PE of *B. acanthoides*

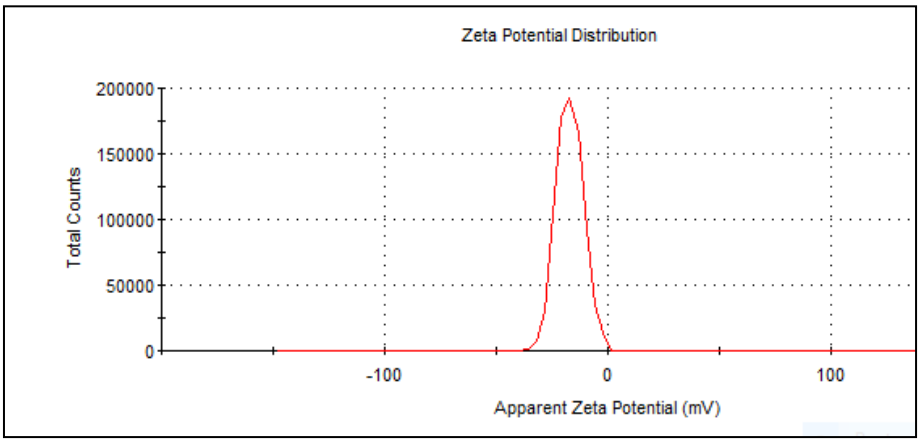

Fig. 6. Zeta potential of SNPs, synthesized by using PE of *B. acanthoides*

The antibacterial activity of *B. acanthoides*-mediated SNPs is depicted in Fig. 8. The SNPs were revealed to cause different zones of inhibition against the pathogens examined. On the contrary, the aqueous *B. acanthoides* extract did not show any antibacterial activity. The tested bacteria were inhibited to varying degrees by SNP suspension. Against *E. coli,* the maximum zone of inhibition (33.75 mm) was found followed by *P. aeruginosa* (16.67mm), *S. aureus* (16.5mm), and *B. subtilis* (14.33 mm). MIC value of SNPs was found to be ≤ 1.56 µl/ml for *E. coli, P. aeruginosa B. subtilis*, while, MIC value for *S. aureus* was ≤ 3.125 µl/ml (Fig. 9).

Silver nanoparticles carry new and improved properties based on their size, shape, and distribution, so nanoparticles in general and silver nanoparticles in particular have become, at present, a focus of attention to overcome the emergence of bacteria. Synthesized silver nanoparticles were produced from the leaf extract of *B. longiflora*. SNPs demonstrated higher antibacterial activity against *Enterococcus* sp. and P aeruginosa and lower antibacterial activity against *S. aureus* and *Neurococci* when compared to pharmaceutical preparations of chloramphenicol [12].

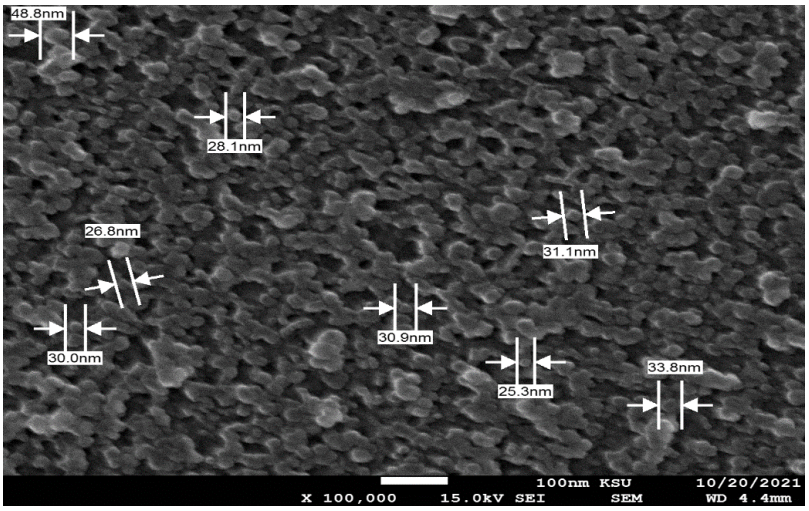

Fig. 7. SEM image of SNPs, synthesized by using PE of *B. acanthoides.* The image shows that SNPs size varies from 25.3 nm to 48.8 nm and SNPs were spherical in shape

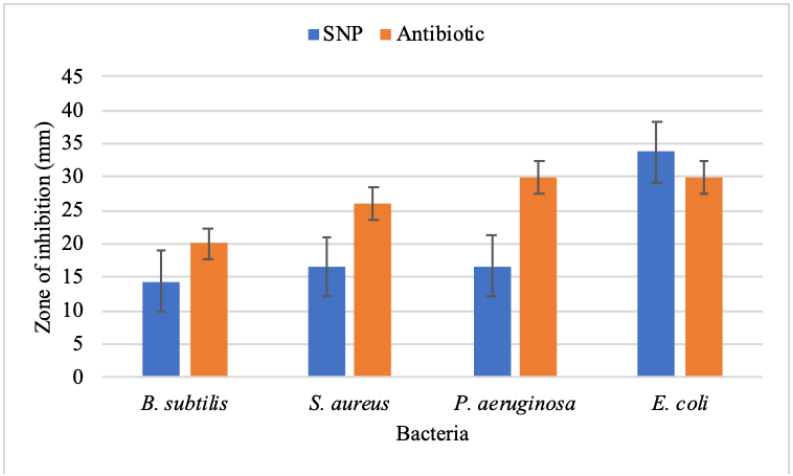

Fig. 8. Antibacterial activity of SNPs against bacteria. Aqueous extract of *B. acanthoides* did not give any results. Antibiotic Augmentin (10µg/ml) was used as a positive control while negative control was sterile distilled water

The vertical bars represent ± standard error (n=3)

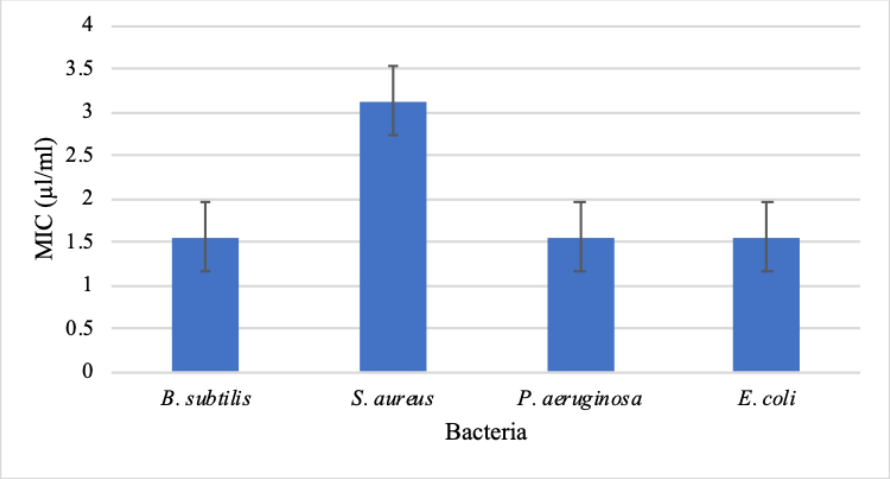

Fig. 9. Minimum inhibitory concentration (MIC) of SNPs against bacteria.
The vertical bars represent ± standard error (n=3)

Silver ions as well as SNPs were known to have strong antimicrobial activities. Several mode of action have been suggested by researchers. The inhibition of bacterial growth was reported affected by the concentration of SNPs and bacteria used in the experiments [16]. As the particle size lowers, the surface area-to-volume ratio of SNPs rises. SNPs with a size of 10-100 nm have a high antibacterial impact against gram-positive and gram-negative bacteria [24,25]. The MIC values from prior investigations indicated a wide range of variance. Moreover, the MIC values of gram positive and gram-negative values also differ. The disparity in MIC values might be explained by differences in cell wall structure and composition [13]. It can be presumed that, because of their minute particle size, SNPs may readily cling to the cell surface and penetrate inside bacterium cells, increasing their antimicrobial action. The nanoparticles' entrance into the plasma membrane was presumably aided by the SNPs' small size, shape, and form. The nanoparticles may have hampered the normal functioning of proteins in the cell membrane, causing the cells to implode.

4. CONCLUSION

B. acanthoides mediated the successful synthesis of SNPs. The green synthesis of SNPs was easy, fast, and eco-friendly. The SNPs showed broad spectrum antibacterial property, so it can be explored further for the non-hazardous, eco-friendly production of silver nanoparticles for medical and therapeutic uses.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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