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Research Article

ANTIBACTERIAL ACTIVITY OF *TABERNAEMONTANA DIVARICATA* (APOCYNACEAE) SECONDARY METABOLITES CAPPED SILVER AND GOLD NANOPARTICLES

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ABSTRACT

Objective: To study the antibacterial activity of *Tabernaemontana divaricata* (Apocynaceae) secondary metabolites capped silver nanoparticles (SNPs) and gold nanoparticles (AuNPs).

Methods: In the present investigation, SNPs and AuNPs were synthesized using an aqueous extract of *T. divaricata* leaves. Leaf aqueous extract was mixed with 1 mM silver nitrate and chloroauric acid for the biosynthesis of nanoparticles, and the same was analyzed using ultraviolet-visible (UV-Vis) spectrophotometer and particle size analyzer. The antibacterial activity of SNPs and AuNPs was determined against various bacterial cultures including laboratory isolates using the agar well diffusion method.

Results: The results recorded from UV-Vis spectrum supported the biosynthesis and characterization of SNPs and AuNPs. The SNPs when compared to AuNPs, showed the highest antibacterial activity against Gram-positive and Gram-negative bacteria.

Conclusion: The present study envisions on the biosynthesis of SNPs from *T. divaricata* plant which are emerging as antibacterial therapy in modern medical applications.

Keywords: Antibacterial activity, Gold nanoparticles, Silver nanoparticles, *Tabernaemontana divaricata*.

INTRODUCTION

Medicinal plants have numerous applications in the field of medical sciences because most of the prescribed drugs are obtained from herbs, trees, and shrubs. Plants are well-known as a potential source of modern medicine [1]. *Tabernaemontana divaricata*, commonly known as pinwheel flower belongs to the family Apocynaceae, is a beautifully shaped evergreen shrub which blooms in spring but flowers may appear sporadically throughout the year and distributed throughout Bangladesh and other parts of the South East Asia. The phytochemistry, alkaloids and non-alkaloid constituents such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes from the leaves, stems, and roots have been reported. In folklore practice, it is used to treat fever and diarrhea. The plant is also used as a tonic to the brain, liver, and spleen. It is reported that plant extract possesses antinociceptive, antioxidant, anti-inflammatory, and reversible acetylcholinesterase inhibition activities [2-4]. The flower juice can be mixed with oil and used as eye drops. The milky juice of the leaves along with oil is applied over the forehead for pain in the eyes. The roots are ground along with water and given internally for intestinal worms. Nanobiotechnology is an emerging field that applies the nanoscale principle and techniques to understand and transform bio systems (living and non-living). Nanoparticles have expressed significant advances owing to a wide range of applications in the field of biomedical, sensors, and antimicrobials. Synthesis of nanoparticles from plant extract is advantageous because they are of low cost, fast, efficient and generally lead to the formation of crystalline nanoparticles with a variety of shapes and sizes. In view of the medicinal applications of this plant, the present study was designed to investigate the antimicrobial activity of silver nanoparticles (SNPs) and gold nanoparticles (AuNPs) synthesized from *T. divaricata* to examine the pharmacological basis of the use of the plant in folk medicine for the treatment of infectious diseases.

METHODS

Preparation of the extract

Flowers of *T. divaricata* were washed thoroughly with autoclaved distilled water and dried in shade for a week and ground using a mixer to coarse powder. The powder was used for preparing the aqueous extract. 1 g of leaf powder was boiled in 10 ml of deionized water for 10 minutes. It was cooled and filtered through Whatman No. 1 filter paper, and the filtrate was stored at 4°C until further use.

Synthesis of SNPs

Silver nitrate ($AgNO_3$) of analytical grade (AR) was purchased from Merck (India). To synthesize SNPs, 1 ml of the aqueous extract of *T. divaricata* flower was added to 100 ml of 1 mM $AgNO_3$ solution in 150 ml glass beaker. Then the beaker was incubated for 24 hrs at room temperature on a magnetic stirrer in the dark place for the reduction of SNPs. The color change from light yellow to dark orange indicates the formation of SNPs. An initial setup was also maintained as flower extract without the addition of $AgNO_3$.

Synthesis of AuNP

Chloroauric acid was purchased from Sigma–Aldrich Chemicals. To synthesize AuNPs, 1 ml of the aqueous extract of *T. divaricata* flower was added to 100 ml of 1 mM gold chloride solution in 150 ml glass beaker. This mixture was kept for 24 hrs at room temperature on a magnetic stirrer at 100 rpm. The color change from light yellow to purple indicates the formation of AuNPs. An initial setup was also maintained as flower extract without the addition of gold chloride.

Particle size analysis and zeta potential determination

The particle sizes were determined using particle size analyzer (Malvern Zetasizer nanosizer). Particle sizes were calculated based on measuring the time dependent fluctuation of scattering of laser light by the nanoparticles.

Test microorganisms of interest

Bacillus subtilis (MTCC 441), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (MTCC 1771), *Vibrio cholera* (ATCC 14035), *Bacillus licheniformis* strain 018, *Bacillus tequilensis* strain ARMATI, and *B. subtilis* strain AK were used for antibacterial assay.

Antibacterial activity

Antibacterial activity was carried out using the well diffusion method on Mueller Hinton Agar (MHA) plates. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 minutes. The aqueous extract of *T. divaricata* flowers, synthesized *T. divaricata* SNPs and AuNps were used at two different concentrations (100 µl and 150 µl per well). The wells were loaded and left for 30 minutes at room temperature for compound diffusion. $AgNO_3$ and gold chloride solutions were used as control. The plates were incubated for 24 hrs at 37°C, and the zone of inhibition was measured in millimeters (mm).

Minimum inhibitory concentration (MIC)

MIC of SNPs and AuNPs were determined against *B. tequilensis* strain ARMATI, *B. subtilis* strain AK, *B. subtilis*, *S. aureus*, *B. licheniformis* strain 018, and *V. cholera*. The bacterial suspension was prepared, and 100 µl of MHA broth was added to the microtitre plate and incorporated with different concentration (500, 250, 125, 62.5, 31.25, 15.625, 7.81, 3.90, 1.95, 0.976, 0.48, 0.24 µl) of SNP and AuNPs. The microtitre plate was incubated at 37°C for 24 hrs.

RESULTS AND DISCUSSION

Synthesis

Nanotechnology has become a very active and vital research topic which is rapidly developing in industrial sectors and spreading to almost every field of science and engineering. Nanostructured inorganic, organic, and biological materials may have existed in nature since the evolution of life started on earth. Rapid synthesis and excellent reducing capping of SNPs and AuNPs through the plant extract mediated process has a time-saving advantage over microbial synthesis and the laborious and lengthy procedures involved with the mare the environmental friendly. SNPs and AuNPs biosynthesis may open new process in pharmaceutical chemistry, microbial biotechnology, biomedical and material science, nanotechnology, and biotechnology fields. In the present study, the green synthesis of SNPs through plant extracts were carried out. The appearances of yellowish-orange in the reaction vessels suggest the formation of SNPs (Fig. 1). It is well-known that SNPs exhibit yellowish-brown in aqueous solution due to excitation of surface plasmon vibrations in SNPs [5]. $AgNO_3$ is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions, when exposed to herbal extracts, were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of the change in color varies from plant to plant. In the present study, *T. divaricata* synthesized SNPs after 24 hrs of reaction. The extracellular synthesis of AuNPs occurred during the exposure of *T. divaricata* flower extract to 1 mM gold chloride solution (Fig. 2). The complete reduction of gold ions was observed within 1 hr. The color change in the reaction mixture was observed during the incubation period because the formation of AuNPs is able to produce the particular color in the reaction mixtures due to their specific properties. The appearance of dark purple is a clear indication of the formation of AuNPs.

Antibacterial activity by Agar well diffusion assay

The antibacterial effects of biologically synthesized SNPs and AuNPs have been investigated against Gram-positive and Gram-negative strains; *E. faecalis*, *S. aureus*, *Staphylococcus epidermis, E. coli, B. tequilensis* strain ARMATI, *B. subtilis* strain AK, *B. subtilis, P. vulgaris, V. cholera* and *B. licheniformis* strain 018. A clear zone of growth inhibition was observed against strain ARMATI, *B. subtilis, S. aureus*, *V. cholera* and strain 018. It confirms the antibacterial activity of biologically synthesized nanoparticles. The highest zone of inhibition was observed in the well loaded with SNPs, and less zone of inhibition was observed in the well loaded with AuNPs. On the other hand, there

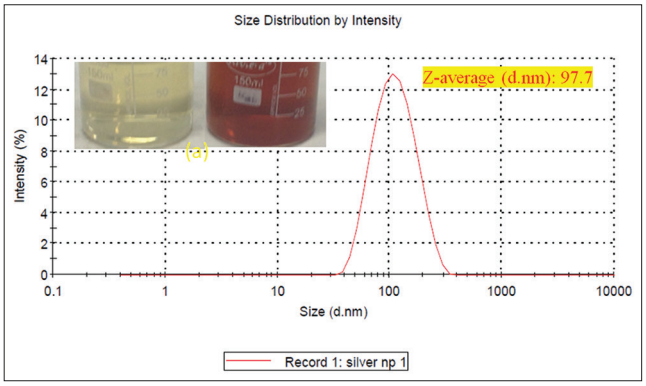

Fig. 1: Optical photograph of synthesized silver nanoparticles and zeta size distribution of synthesized silver nanoparticles

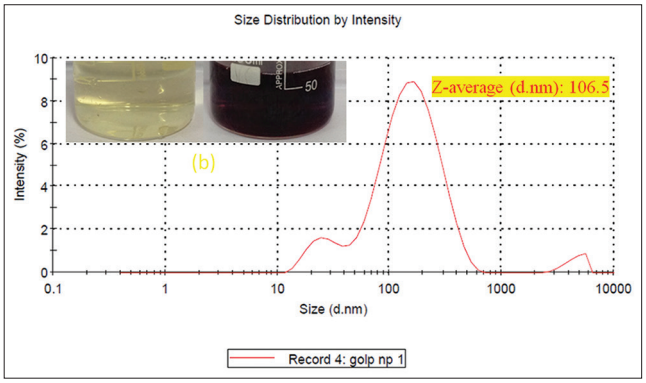

Fig. 2: Optical photograph of synthesized gold nanoparticles and zeta size distribution of synthesized gold nanoparticles

Table 1: Antibacterial activity of synthesized nanoparticles

S. No.	Bacterial culture	Zone of inhibition (mm)				
		Flower extract	$AgNO_3$	SNP	Gold chloride	Gold NP
01	*S. epidermis*	-	6	-	-	-
02	*E. coli*	-	-	-	-	-
03	*B. tequilensis* strain ARMATI	-	5	5	-	-
04	*P. vulgaris*	-	-	-	-	-
05	*B. subtilis* strain AK	-	5	-	-	-
06	*B. subtilis*	-	5	4	-	-
07	*E. faecalis*	-	-	-	-	-
08	*S. aureus*	-	5	5	-	-
09	*V. cholera*	-	3	3	-	3
10	*B. licheniformis* strain 018	-	5	5	-	6

V. cholera: Vibrio cholera, S. epidermis: Staphylococcus epidermis, E. coli: Escherichia coli, B. tequilensis: Bacillus tequilensis, P. vulgaris: Proteus vulgaris, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. faecalis: Enterococcus faecalis, B. licheniformis: Bacillus licheniformis, SNPs: Silver nanoparticles, $AgNO_3$: Silver nitrate

Table 2: MIC of synthesized SNPs and gold nanoparticles

S. No.	Nanoparticles	Bacterial cultures	MIC (µg)
1.	SNPs	*B. tequilensis* strain ARMATI	62.5
2.		*B. subtilis* strain AK	31.25
3.		*B. subtilis*	15.63
4.		*S. aureus*	7.81
5.		*V. cholera*	7.81
6.		*B. licheniformis* strain 018	3.9
7.	Gold nanoparticles	*V. cholera*	250
8.		*B. licheniformis* strain 018	125

SNPs: Silver nanoparticles, MIC: Minimum inhibitory concentration,
V. cholera: Vibrio cholera, B. tequilensis: Bacillus tequilensis,
S. aureus: Staphylococcus aureus, B. licheniformis: Bacillus licheniformis

was no zone of inhibition observed in the well loaded with flower extract (Table 1).

In the present investigation, nanoparticles show higher inhibition against the Gram-positive *S. aureus* (5 mm) compared to other Gram-negative strain *V. cholera* (3 mm), employed in this antibacterial assay. Savithramma and Rao [5] demonstrated the antibacterial effect of SNPs and the growth of *Pseudomonas* and *Rhizopus* species were inhibited maximum by the SNPs synthesized from leaf extract of *Svensonia hyderobadensis*, indicating that the SNPs may have an important advantage over conventional antibiotics. AuNPs also have potential activity against microbial pathogens, and it mainly depends on the size and shape of the particles. Consequently, the interaction between Gram-positive bacteria and SNPs were certainly stronger than that of Gram-negative bacteria. The cell wall of gram-negative bacteria consists of an outer membrane composed of lipid, protein, and lipopolysaccharides which act as a barrier and provide effective protection against the antibacterial agent. However, the cell wall of the Gram-positive bacteria lacks an outer membrane [6].

MIC of biologically synthesized SNPs and AuNPs

The synthesized SNPs and AuNPs were effective in inhibiting the bacterial growth. The MIC was checked against Gram-positive (*B. tequilensis* strain ARMATI, *B. subtilis* strain AK, *B. subtilis, B. licheniformis* strain 018, and *S. aureus*) and Gram-negative (*V. cholera*) bacteria. The SNPs and AuNPs were used in different concentration such as 500, 250, 125, 62.5, 31.25, 15.63, 7.8, 3.9, 1.95, 0.976, 0.48, 0.24 µl in order to determine the MIC. The SNPs showed MIC value of 62.5 µl for strain ARMATI, 31.25 µl for strain AK, 15.63 µl for *B. subtilis*, 7.81 µl for *V. cholera,* 3.9 µl for strain 018, and 7.81 µl for *S. aureus*. The AuNPs showed MIC value of 250 µl for *V. cholera* and 125 µl for strain 018 (Table 2).

CONCLUSION

Freely water soluble terpenoids, steroids, alkaloids, and proteins of *T. divaricata* are responsible for the biosynthesis and stability of spherical shaped nanoparticles in an aqueous medium. The SNPs showed the highest antibacterial activity against both Gram-positive and Gram-negative bacteria. The AuNPs were not found to be a potential bactericidal agent. The SNPs showed the MIC concentration ranging from 62.5 to 3.9 µg against bacteria. The AuNPs showed the MIC concentration of 250 µg and 125 µg against *V. cholera* and *B. licheniformis* strain 018, respectively.

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