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# Antibacterial Studies *Tabernaemontana divaricata* (Apocynaceae) Secondary Metabolites Capped Silver Nanoparticles

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**Abstract:** To study the antibacterial activity of *Tabernaemontana divaricata* (Apocynaceae) secondary metabolites capped silver nanoparticles (SNPs). In the present investigation, SNPs were synthesized using an aqueous extract of *T. divaricata* flowers. Flower aqueous extract was mixed with 1 m M silver nitrate for the biosynthesis of nanoparticles. The antibacterial activity of SNPs was determined against various bacterial cultures including laboratory isolates using the agar well diffusion method. The SNPs showed the highest antibacterial activity against Gram-positive and Gram-negative bacteria. 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, Silver nanoparticles, *Tabernaemontana divaricata*.

## INTRODUCTION

Plants are well-known as a potential source of modern medicine [1]. Tabernaemontana divaricata Linn is a species of plant under Apocynaceae family commonly known as chandani in Hindi. It is a popular medicinal plant widely distributed in tropical countries including Brazil, Egypt, India, Sri Lanka, Vietnam, Malaysia and Thailand. This herb is reported to have various pharmacological actions such as Anxiolytic [2], Antidiabetic [3], antiulcer, anticancer and Anticonvulsant [4] activities. It is also used in Chinese, ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery. It bears white fragnant flowers which also have medicinal value in the treatment of eye infections. The flower juice can be mixed with oil and used as eye drops.

Emerging infectious diseases and the increase in incidence of drug resistance among pathogenic bacteria have made the search for new antimicrobials inevitable. In the current situation, one of the most promising and novel therapeutic agents are the nanoparticles. The unique physiochemical properties of the nanoparticles combined with the growth inhibitory capacity against microbes has led to the upsurge in the research on nanoparticles and their potential application as antimicrobials. Silver compounds have been used to treat burns, wounds and infections. Various salts of silver and their derivatives are used as antimicrobial agents [5, 6]. Recent studies have reported that nanosized silver particles exhibit antimicrobial properties [7, 8]. Nanoparticles of silver have been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating materials [9-11].

Plant-mediated synthesized NPs are biodegradable, non-toxic, and biocompatible that show quick action by entering into cell membrane and act as an alternative system of herbal medicine to treat infections [12].

The present study was designed to investigate the antimicrobial activity of silver nanoparticles (SNPs) 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 flower 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 $AgNO_3$.

**Test microorganisms of interest**

Ten bacterial strains isolated from eye infected cases examined in Sarojini Devi Eye Hospital, Hyderabad, were used in the study. Of which nine were gram-positive, bacteria viz., *Staphylococcus aureus, S. epidermidis, Gardnerella vaginalis, Enterococcus faecalis, S. agalactiae, Propionibacterium acnes, Corynebacterium macbinleys, Bacillus serus, B. subtilis* and one gram- negative viz., *E. coli*. All the bacterial strains were from patients with eye diseases. The bacteria were initially identified by streak plate method in blood agar medium and specifically identified at Royal Life Sciences Laboratory using enzyme assay method (VITEK 2 COMPACT) and maintained on nutrient agar slants at $4^0$C.

**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 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. $AgNO_3$ solution was 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 were determined against all the strains. 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. The microtitre plate was incubated at 37°C for 24 hrs.

## RESULTS AND DISCUSSION

**Antibacterial activity by Agar well diffusion assay**

The antibacterial effects of biologically synthesized SNPs have been investigated against Gram-positive ocular pathogens - *Staphylococcus aureus, S. epidermidis, Gardnerella vaginalis, Enterococcus faecalis, S. agalactiae, Propionibacterium acnes, Corynebacterium macbinleys, Bacillus serus, B. subtilis a*nd one gram- negative viz., *E. coli*. A clear zone of growth inhibition was observed against the isolated strains. 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 only flower extract (Table-1).

In the present investigation, nanoparticles showed higher inhibition against the Gram-positive pathogens compared to other Gram negative strain *E. coli*, employed in this antibacterial assay. Savithramma and Rao [13] 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. 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 [14].

**MIC of biologically synthesized SNPs**

The synthesized SNPs were effective in inhibiting the bacterial growth. The MIC was checked against Gram-positive (*Staphylococcus aureus, S. epidermidis, Gardnerella vaginalis, Enterococcus faecalis, S. agalactiae, Propionibacterium acnes, Corynebacterium macbinleys, Bacillus serus, B. subtilis*) and Gram-negative (*E. coli*) bacteria. The SNPs 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 *Enterococcus faecalis*, *Propionibacterium acnes*, 31.25 µl for *Staphylococcus epidermidis*, *Gardnerella vaginalis*, *Staphylococcus agalactiae* 15.63 µl for *Bacillus cereus*, *Bacillus subtilis* 7.81 µl for E.Coli, *Corynebacterium macbinleys* and *S. aureus.*

**Table-1: Results of antibacterial activity**

| S.No | Ocular pathogen | Flower Extract | AgNO3 | SNP 50 µg/ml | SNP 100 µg/ml |
|---|---|---|---|---|---|
| 1 | *Staphylococcus aureus* | 14±0.03 | 13±0.03 | 21±0.13 | 28±0.33 |
| 2 | *Staphylococcus epidermidis* | 14±0.21 | 13±0.33 | 21±0.33 | 28±0.33 |
| 3 | *Gardnerella vaginalis* | 11±0.13 | 10±0.13 | 15±0.33 | 22±0.13 |
| 4 | *Enterococcus faecalis* | 14±0.24 | 13±0.24 | 21±0.14 | 28±0.04 |
| 5 | *Staphylococcus agalactiae* | 11±0.33 | 11±0.33 | 16±0.43 | 22±0.33 |
| 6 | *Propionibacterium acnes* | 12±0.12 | 11±0.12 | 17±0.3 | 24±0.14 |
| 7 | *Corynebacterium macbinleys* | 11±0.03 | 10±0.03 | 16±0.02 | 22±0.03 |
| 8 | *Bacillus cereus* | 12±0.13 | 11±0.13 | 17±0.13 | 24±0.13 |
| 9 | *Bacillus subtilis* | 14±0.33 | 13±0.33 | 18±0.33 | 24±0.13 |
| 10 | *Escherichia Coli* | 12±0.12 | 11±0.12 | 17±0.12 | 24±0.12 |

**Table-2: MIC of SNPs**

| S.No | Ocular pathogen | MIC SNP |
|---|---|---|
| 1 | *Staphylococcus aureus* | 7.81 µl |
| 2 | *Staphylococcus epidermidis* | 31.25 µl |
| 3 | *Gardnerella vaginalis* | 31.25 µl |
| 4 | *Enterococcus faecalis* | 62.5 µl |
| 5 | *Staphylococcus agalactiae* | 31.25 µl |
| 6 | *Propionibacterium acnes* | 62.5 µl |
| 7 | *Corynebacterium macbinleys* | 7.81 µl |
| 8 | *Bacillus cereus* | 15.63 µl |
| 9 | *Bacillus subtilis* | 15.63 µl |
| 10 | *Escherichia Coli* | 7.81 µl |

Several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect.

Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, silver nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag+) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or can react with sulfur-containing proteins, leading to the inhibition of enzyme functions [15, 16]. The general understanding is that Ag nanoparticle of typically less than 20 nm diameters get attached to sulfur-containing proteins of bacterial cell membranes leading to greater permeability of the membrane, which causes the death of the bacteria [17].

The antibacterial activity of plant based silver nanoparticles of *Ocimum sanctum* and *Vitex negundo* were tested against *Staphylococcus aureus, Vibrio cholerae, Proteus vulgaris* and *Pseudomonas aeruginosa*, for which significant results were observed [18]. Antibacterial activity of silver nanoparticles against *Staphyloccocus aureus, Pseudomonas aeruginosa* and *Escherichia coli* has been investigated [19]. The antibacterial properties of the biosynthesized silver nanoparticles when incorporated on textile fabric were investigated [20]. Silver impregnated medical devices like surgical masks and implantable devices showed significant antimicrobial efficiency [21]. The current investigation suggests that, use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc [22]. Antibacterial activity of cotton fabric coated silver nanoparticles showed distinct bactericidal effect against *Staphylococcus aureus* and *E.coli* with all the tested concentration [23]. Seven Apocynaceae members were studied for their antibacterial activity against ten pathogens, of which *Plumeria alba* showed efficient antibacterial activity and *Rauvolfia tetraphylla* showed moderate activity against most of the pathogens [24]. But in this study the plant based Silver nanoparticles synthesized from *T. divaricata* was active against the pathogens studied. Hence the plant based Silver nanoparticles are found to be more efficient than the plant extracts that have been used since time immortal.

## CONCLUSION

The silver nanoparticles synthesized and investigated in this study establish a stronger antibacterial potency which was efficient against most of the ocular pathogens studied. The green chemistry approach addressed in the present work on the synthesis of silver nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and

reproducible. This approach can be further capitalized to rapidly screen plants used in traditional medicines for ailments resulting from microorganism as well as in the extraction of potential molecules that could be used in future therapeutics.

**Conflict of interest**

The authors declare no conflict of interest.

**Authors' Declaration**

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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