

## PHARMACOLOGICAL EVALUATION OF SILVER NANOPARTICLE OF INDIAN TRADITIONAL HERBS

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### ABSTRACT

Herbal medicine is still the main stay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents. The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly. Silver nanoparticles are one of the metal nanoparticles have received significant consideration because they are effective antimicrobial agent/s that exhibits low toxicity. The aim of present study is to synthesize silver nanoparticles by using neem seed extract, tulsi extract and turmeric extract, characterization of these silver nanoparticles &

Converts its gel formulation as well as to observe the antimicrobial activity.

**KEYWORDS:** Nanoparticles, Antimicrobial, Antifungal, Silver Nanoparticles.

### INTRODUCTION

Herbal drugs are playing a vital role in health care system. This is because they are being cheap and locally available. The activity of herbal medicines depends on overall function of a variety of active components, as all the constituents provide synergistic action and thus enhance the therapeutic value.<sup>[1]</sup> Herbal medicines are now in great demand in the developing

world for primary health care not because of inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects.<sup>[2]</sup>

The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by combination of bio-molecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures.<sup>[3]</sup> The protocol for the nanoparticle syntheses involves: the collection of the part of plant of interest from the available sites was done and then it was washed thoroughly twice/thrice with tap water to remove both epiphytes and necrotic plants; followed with sterile distilled water to remove associated debris if any. These; clean and fresh sources are shade-dried for 10–15 days and then powdered using domestic blender. For the plant broth preparation, around 10 g of the dried powder is boiled with 100 mL of deionized distilled water (hot percolation method). The resulting infusion is then filtered thoroughly until no insoluble material appeared in the broth. To AgNO<sub>3</sub> solution, on addition of few mL of plant extract follow the reduction of pure Ag(I) ions to Ag(0) which can be monitored by measuring the UV–visible spectra of the solution at regular intervals.<sup>[4]</sup>

The medical properties of silver have been known for over 2000 years. Silver is generally used in the nitrate form to induce antimicrobial effect but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbes to be exposed to. Silver nanoparticles synthesized using plant extracts (from different sources) have been used for analysing their antimicrobial activities against different microbes.<sup>[5]</sup>

## OBJECTIVE

The objectives of present study is to synthesize silver nanoparticles by using neem seed extract, tulsi extract and turmeric extract, characterization of these silver nanoparticles & Converts its gel formulation as well as to observe the antimicrobial activity.

## MATERIALS AND METHODS

**Collection of plant Material:** Plant materials of *Azadirachta indica*, *Curcuma longa* & *Ocimum santum* were collected from Shirpur region of Dhule district (Maharashtra).

**Extraction methodology:** The extractions of powdered material were done by using Soxhlet apparatus. In solvent extraction, dried material is extracted with methanol. For extraction, 250 gm of powdered material were packed in thimble containing filter paper and extracted with methanol in Soxhlet apparatus for the period till all the substances and others were extracted. The extract thus obtained was concentrated with the help of rotary vacuum evaporator.

**Synthesis of silver nanoparticles:** For synthesis of silver nanoparticles, the conical flask containing 100 ml of AgNO<sub>3</sub> (1mM) was reacted with 12 ml of the methanolic extract of *A. indica*, *C. longa* & *O. santum*. This setup was incubated in dark (to minimize the photoactivation of silver nitrate), at 37 °C under static condition.<sup>[6]</sup>

## PROCEDURE

### Evaluation of Antimicrobial & Anti-fungal activity by agar diffusion method<sup>[7]</sup>

1. About 20 ml of Muller Hinton agar medium for bacteria and Potato dextrose agar for fungi was allowed to set in empty sterile Petri plate.
2. About 0.1 ml of fungal inoculums was made in petri plates preset for spore count, cell density and bacterial inoculums in respective Medias.
3. The well of 6 mm diameters were bored on the agar media using sterile borer and each plate was filled with 0.5 ml of plant extracts.
4. The plates containing bacteria were incubated at 37°C for 24 hours and those containing fungi were incubated at 30°C for 48 hours.
5. The positive antimicrobial activity was read by measuring zone of inhibition (in mm) which was produced after incubation.
6. All the tests were repeated in triplicates.

## RESULT

### a) Antimicrobial activity

SNPs & extract of The Neem Seed, Turmeric & Tulsi were screened for antimicrobial activity by agar-well diffusion method.

Table: Antimicrobial activity of SNPs &amp; Extract.

Micro-organisms	Diameter of Zone of inhibition(mm)						Standard
	Silver Nanoparticles			Extract			
	Neem Seed	Turmeric	Tulsi	Neem Seed	Turmeric	Tulsi	
<b>Gram Negative bacteria</b>							
<i>Pseudomonas aeruginosa</i>	8.3	7.4	6.7	4.3	5.7	4.9	12.46
<i>Escherichia coli</i>	6.5	7.9	8.8	3.7	4.9	5.1	29.12
<b>Gram Positive bacteria</b>							
<i>Staphylococcus aureus</i>	9.1	7.7	8.4	5.4	6.1	4.6	15.32
<i>Bacillus subtilis</i>	7.5	6.6	7.9	4.9	3.9	4.4	18.02
<b>Fungi</b>							
<i>Candida albicans</i>	3.8	4.2	4.6	2.1	3.4	2.6	11.59
<i>Aspergillus niger</i>	4.6	5.2	5.8	3.5	2.4	3.7	12.10

Diameter in mm calculated by Vernier Caliper; '-' means no zone of inhibition; Well diameter= 6 mm; NCIM-National Collection of Industrial Micro-organisms; Standard-Chloramphenicol

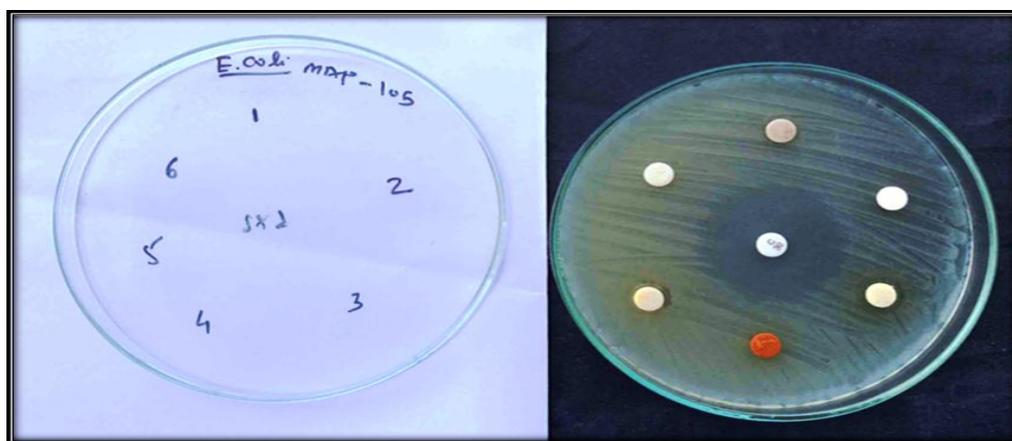
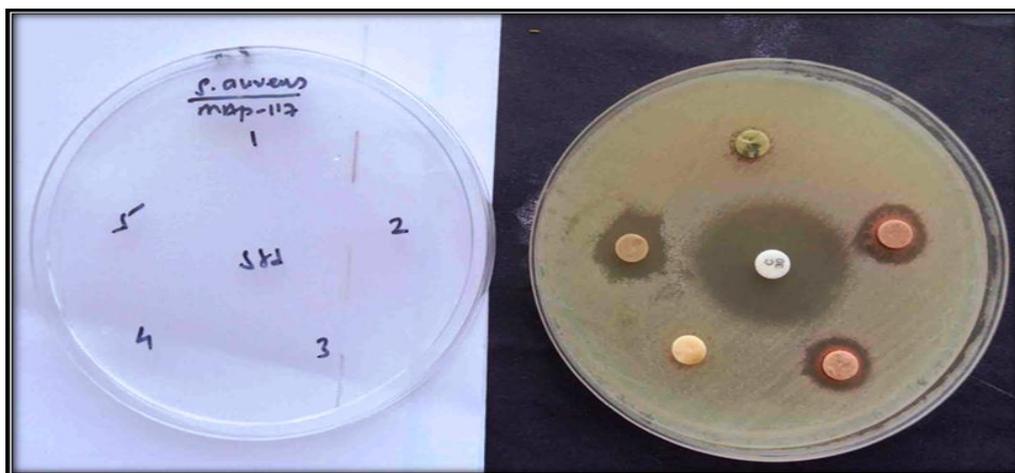


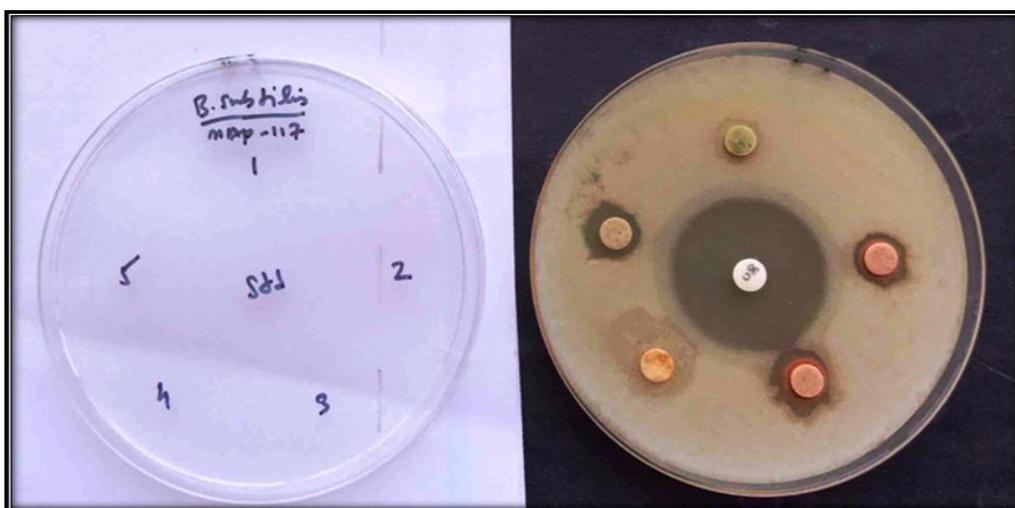
Figure 43: Antimicrobial activity of SNPs & Extract of Tulsi, Neem Seed & Turmeric against: *Escherichia coli*.



Figure 44: Antimicrobial activity of SNPs & Extract of Tulsi, Neem Seed & Turmeric against: *Pseudomonas aeruginosa*.



**Figure 45: Antimicrobial activity of SNPs & Extract of Tulsi, Neem Seed & Turmeric against: *Staphylococcus aureus*.**



**Figure 46: Antimicrobial activity of SNPs & Extract of Tulsi, Neem Seed & Turmeric against: *Bacillus subtilis*.**

The antimicrobial activity of silver nanoparticles was evaluated against Gram negative *Escherichia coli* & *Pseudomonas aeruginosa* and Gram positive bacteria namely, *Staphylococcus aureus* & *Bacillus subtilis* by disc method. The zone of inhibition of synthesized SNPs was found to be more than that of plant extract. Neem and Tulsi SNPs exhibited good antimicrobial activity against gram negative bacteria such as *Escherichia coli* & *Pseudomonas aeruginosa* with zone of inhibition 8.3 mm & 6.7 mm and 6.5 mm & 8.8 mm respectively. Looking toward the activity of extracts Turmeric extract was most effective against *Pseudomonas aeruginosa* & Tulsi extract gives positive effect against *Escherichia coli*. Neem and Tulsi SNPs exhibited good antimicrobial activity against gram positive bacteria such as *Staphylococcus aureus* & *Bacillus subtilis* with zone of inhibition 9.1 mm &

8.4 mm and 7.5 mm & 7.9 mm respectively. Looking toward the activity of extracts Turmeric extract was most effective against *Staphylococcus aureus* & Neem seed extract gives positive effect against *Bacillus subtilis*.

## CONCLUSION

In the present study, dried powdered Tulsi leaves, Neem seeds & Turmeric rhizomes were subjected to extraction with methanol. SNPs of all tree extract were prepared using standard method.

Since the antimicrobial effects of silver and its compounds are known from several years back, the antimicrobial activity of the synthesized silver nanoparticles was studied using the renowned agar well diffusion method. All SNPs were evaluated for its possible anti-microbial & antifungal properties by standard *in vitro* models. The antibacterial efficacy was tested against the Gram negative bacteria namely, *Pseudomonas aeruginosa* & *Escherichia coli* as well as Gram positive bacteria namely, *Staphylococcus aureus* & *Bacillus subtilis*. The results of antibacterial study suggest that the synthesized SNPs are very effective antibacterial agents. They exhibited very good activity against all tested bacterial strains.

In conclusion, it has been demonstrated that the extract of Tulsi leaves, Neem seeds & Turmeric rhizomes are capable of producing Ag nanoparticles extracellularly and the Ag nanoparticles are quite stable in solution. The formed silver nanoparticles shows considerable antimicrobial & antiseptic activity compared to the respective antibiotics. These biosynthesis silver nanoparticles prove to be potential candidates for medical applications where antimicrobial activity is essential.

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