EVALUATION OF ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM *Leucas aspera* FLOWER

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ABSTRACT

In the present study to investigate the antibacterial activity of silver nanoparticles from Leucas aspera flower extract. Silver nanoparticles biosynthesized from Leucas aspera flower extract was tested individually against test organisms for antibacterial activity by agar disc diffusion method. For this study both Gram positive (Staphylococcus aureus, and Bacillus subtilis) and Gram negative (Escherichia coli) organisms were used. After 24 hours of incubation, the inhibitory effect of AgNPs was significant as compared to Leucas aspera flower extract alone and standard chloramphenicol. Zone of inhibition (ZoI) was used as a measure for comparing bactericidal activity of these $AgNO_3$. AgNPs from Leucas aspera flower extract showed about 3.28 mm zone against the test organisms: E. coli. Similarly the AgNPs from Leucas aspera flower showed 2.30 mm and 2.11 mm ZoI against test organisms: S. aureusan and Bacillus subtilis respectively and considering the advantage of the microbicidal activities of the silver.

Keywords: *Leucas aspera flower, Antibacterial activity, Silver nanoparticles*

INTRODUCTION

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, (1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, aridness, UV exposure and pathogenic attack are called as phytochemicals (Gibson *et al.*, 1998: Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics Meagher and Thomson, (1999) and About 150 phytochemicals have been studied in detail. In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000).

Antimicrobials are typically liquids. Antimicrobial liquids kill or inhibit the growth of microorganisms such as bacteria, fungi and protozoans. Antimicrobial drugs (e.g. penicillin) are selective and kill microbes (micro biocidal) or prevent their growth (micro biostatic).

Disinfectants are non-selective antimicrobial substances (e.g. bleach) and are used on non-living objects or the outside of the body. With the emergence and increase of microbial organisms resistant to multiple antibiotics and the continuing emphasis on health-care costs, many researchers have tried to develop new effective antimicrobial reagents free of resistance and cost. The most important problem caused by the chemical antimicrobial agents is multidrug resistance (Hadacek and Greger, 2000). In the present study to investigate the antibacterial activity of silver nanoparticles from *Leucas aspera* flower extract.

MATERIALS AND METHODS

Collection of plant materials

The mature *Leucas aspera* flowers were collected in May 2019 from Thanjavur, Tamil Nadu, India. The flowers were identified and authenticated by Botanist, Prof. Dr. S. John Britto, Director, The Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

Preparation of flower extract

The dried flowers were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of methanol and water separately. The mixture was kept in 24 hrs. After 24 hrs. the flower extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Determination of Antibacterial Activity

The microbial strains employed in the biological assays were Gram -positive bacteria: *Staphylococcus aureus* (MTCC 3106), *Bacillus subtilis* (MTCC 2423) and Gram - negative bacteria: *Escherichia coli* (MTCC 732) Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), chandigarh, India.

Preparation of plant extract, AgNPs and Standard solutions for the experiment

The flower extract, AgNPs and Standard were weighed (10mg/10ml) and dissolved in sterile distilled water. AgNPs (1mM silver nitrate was added to plant extract to make up a final solution 200 ml and centrifuged at 18,000 rpm for 25 min. The collected pellets were used in this study) and Standard solution as Chloramphenicol (25mg/ml distilled water) were used. They were kept under refrigerated condition unless they were used for the experiment.

Antimicrobial assay

Antimicrobial activity was done by the method of NCCLS, (1993) and Awoyinka *et al*., (2007) using disc diffusion method. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. A sterile cotton swab is dipped into standardized bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* and were spread on solidified nutrient agar plate and allowed to dry for 10 mints. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30µl of plant extract, AgNPs and Standard solution as Chloramphenicol were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 hr for the bacteria. Each sample was tested in triplicates. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS AND DISCUSSION

Antibacterial activity of *Leucas aspera* and Silver Nanoparticles

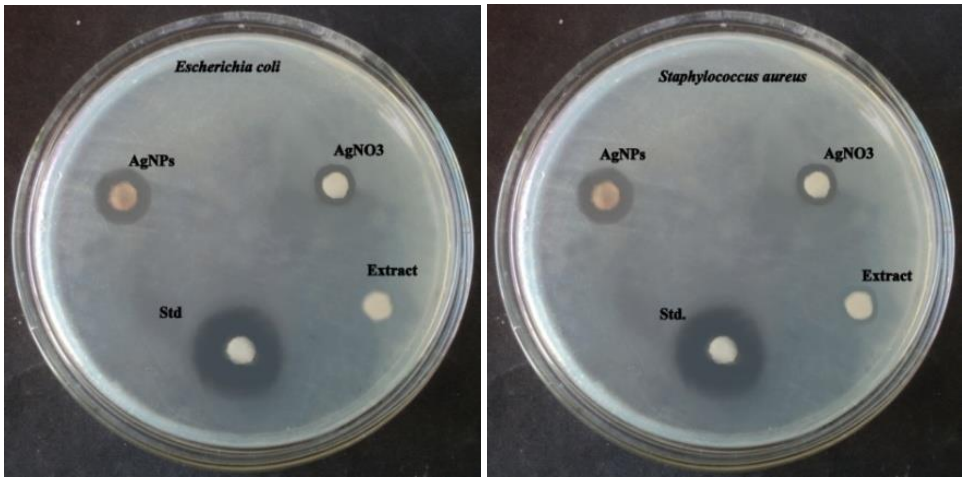
Silver nanoparticles biosynthesized from *Leucas aspera* flower extract was tested individually against test organisms for antibacterial activity by agar disc diffusion method. For this study both Gram positive (*Staphylococcus aureus*, and *Bacillus subtilis*) and Gram negative (*Escherichia coli*) organisms were used. This was performed by determining ZoI (zone of inhibition) which is rapid and inexpensive to determine the susceptibility of a particular test organism as antimicrobial agent. This was executed by measuring the zone of inhibition using a vernier caliper.

After 24 hours of incubation, the inhibitory effect of AgNPs from *Leucas aspera* flower extract was significant as compared to *Leucas aspera* flower extract along and standard chloramphenicol. Zone of inhibition (ZoI) was used as a measure for comparing bactericidal activity of these $AgNO_3$. AgNPs from *Leucas aspera* flower extract showed about 3.28mm zone against the test organisms: *E. coli*. Similarly the AgNPs from *Leucas aspera* flower showed 2.30 mm and 2.11 mm ZoI against test organisms: *S. aureusan* and *Bacillus subtilis* respectively. (Table 1 and Plate 1).

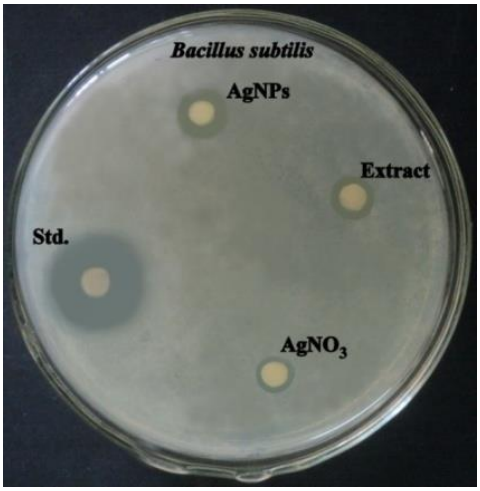
Table.1: Anti-bacterial activity of AgNPs, $AgNO_3$ and *Leucas aspera* flower extract

Samples	Doses	*Escherichia coli* (mm)	*Staphylococcus auerus* (mm)	*Bacillus subtilis* (mm)
$AgNO_3$	30µl/ml	1.58±0.11	1.76±0.12	1.68±0.11
Erythrina indica	30µl/ml	1.06±0.07	0.82±0.05	0.75±0.05
AgNPs	30µl/ml	3.28±0.22	2.30±0.16	2.11±0.14
Standard (chloramphenicol)	30µl/ml	6.29±0.44	5.78±0.40	5.71±0.39

Values were expressed as Mean ± SD for triplicate.



Escherichia coli ***Staphylococcus auerus***



Bacillus subtilis

Plate.1: Shows the Antibacterial activity of AgNPs for *Leucas aspera* flower extract

Antibacterial Activity of *Leucas aspera* and Silver Nanoparticle

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Gardea-Torresdey *et al*., 2003). The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria.

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Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions (Pal *et al*., 2007). Ahmad *et al*. (2011) mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth. The growth of microorganisms was inhibited by the green synthesized SNPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or

lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria (Ahmad *et al*., 2011).

The SNPs synthesized from plant species are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications. Similar observation was found in *Allium cepa* (Saxena *et al*., 2010), *Argimone Mexicana* (Khandelwal *et al*., 2010), *Artocarpus heterophyllus* (Thirumurgan *et al*., 2010). Warisnoicharoen et *al*. (2001) found that silver nanoparticles have an ability to interfere with metabolic pathways. Sereemaspun et *al*. (2008) findings suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane. The use of silver ions as preventing agents in cosmetics was tested by a challenged list in a set of cosmetic dispersions with the addition of known preservative inhibitors or microorganism's growth promoters.

Silver has more microbial efficacy and more effective in the presence of proteinaceous material and inorganic binding proteins that associated with inorganic structures *in vivo* using routine molecular biology techniques. The silver nanoparticles synthesized from flower extract showed higher toxicity than that of bark extracts. The reason could be that the flower extract synthesized higher concentration of silver nanoparticles. Moreover green flowers are the site of photosynthesis and availability of more H^+ ions to reduce the silver nitrate into silver nanoparticles. The molecular basis for the synthesis of these silver crystals is speculated that the organic matrix contain silver binding proteins that provide amino acid moieties that serve as the nucleation sites (Prabhu *et al*., 2010). The efficiency of various silver based antimicrobial fillers in polyamide toward their silver ion release characteristics in an aqueous medium was also investigated and discussed in number of plants including algae, yeast and fungi (Arya *et al*., 2010).

Sondi and Salopek-Sondi (2004) reported that the antimicrobial activity of silver nano- particles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticle, and was closely associated with the formation of dpitsT in the cell wall of bacteria. Then, Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. Amro *et al*. (2000) suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins. Also, Sondi and Salopek-Sondi speculate that a similar mechanism may cause the degradation of the membrane structure of *E. coli* during treatment with Ag nanoparticles .

CONCLUSION

Silver nanoparticles synthesised from *Leucas aspera* flower extract have great promise as antimicrobial agents. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

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