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EVALUATION OF ANTI-MICROBIAL ACTIVITY OF SILVER NANOPARTICLES FROM *CISSUS VITIGINEA* LEAF EXTRACT

I. Gnanasundaram*[1] and K. Balakrishnan[2]

[1*]Research Scholar, Post Graduate and Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India.

[2]Associate Professor, Post Graduate and Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India.

ABSTRACT

Drug-resistance infections have increased extremely quickly in the past years, emerging as a serious health problem in the world. Novel and better antimicrobial agents are still being developed to control associated microorganisms. However, this still represents a great challenge for antimicrobial agents. The aim of this study was to evaluate their antimicrobial properties against various Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis)* Gram - negative bacteria: (*Escherichia coli)* and fungi (*Candida albicans*, *Aspergillus flavus)*. The results of this experiment suggest that biologically synthesized AgNPs are fairly ideal candidates for the development of new antimicrobial drugs against bacteria and fungi.

KEYWORDS

Cissus vitiginea, Silver nanoparticles and Antimicrobial activity.

Author for Correspondence:

Gnanasundaram I,

Department of Chemistry,

A.V.V.M. Sri Pushpam College (Autonomous),

Poondi, Thanjavur, Tamil Nadu, India.

Email: igsundaram@yahoo.com

INTRODUCTION

Microorganisms such as bacteria, molds, yeasts, and viruses present in their living environments often causes to human being. Because of the emergence and increase in the number of multiple antibiotic-resistant microorganisms and the continuing emphasis on health-care costs, many scientists have researched methods to develop new effective antimicrobial compounds that overwhelmed the fighting of these microorganisms and are also cost-effective. Such problems and needs have led to resurgence in the use of silver-based antiseptics that

may be linked to a broad-spectrum activity and considerably lower propensity to induce microbial resistance compared with those of antibiotics[1].

Silver nanoparticles (SNPs) are currently used in a large number of medical applications ranging from diagnosis and drug delivery to medical device dressing and, more notably, development of new generation of antibiotics[2]. Furthermore, potent antibacterial, antifungal and antiviral properties of SNPs have nominated them as a promising alternative to commercially available antibiotics[3]. In the recent decades, increased development of green synthesis of nanoparticles is inevitable because of its incredible applications in all fields of science. Many work have been reported based on the plant mediated synthesis of nanoparticles. AgNP has been synthesized by using the plant broth from a wide variety of plants such as *Bacopa monnieri*[4]. And *Catharanthus roseus*[5]. Keeping in view, in the present study to evaluate the antimicrobial activity of silver nanoparticles using *Cissus vitiginea* leaf.

MATERIAL AND METHODS

Chemicals

All the experiments were conducted at room temperature. Chemicals used in this study are AR grade silver nitrate ($AgNO_3$) obtained from Merck, India.

Collection of plant materials

The Cissus vitiginea leaves were collected in March 2016 from Thanjavur, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, Director, Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of leaf extract

The dried leafs were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Synthesis of Ag nanoparticles using leaf extracts

45ml of 1mM aqueous $AgNO_3$ solution taken in a 250 ml Erlenmeyer flask and add 5 ml of Cissus vitiginea leaf extract. The flask was then incubated in the dark at 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The obtained Ag nanoparticle solution was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze and dried for using SEM analysis[6].

Antimicrobial assay

Antibacterial activity was done by the method of[7,8] using disc diffusion method. The microbial strains employed in the biological assays were Gram - positive bacteria: Staphylococcus aureus (MTCC 3160), Bacillus subtilis (MTCC 2423) and Gram - negative bacteria: Escherichia coli (MTCC 732) and fungi Candida albicans (MTCC 183), Aspergillus flavus (MTCC 1783) were obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing bacteria specie were spread on Nutrient agar plates and fungus strains were spread on potato dextrose agar. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30µl of plant extract, $AgNO_3$, AgNPs and Standard solution as Chloramphenicol and fluconazole were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hours for yeasts

strains. Each sample was tested in triplicate. 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

Our earlier report[9] confirmed the synthesis of silver nanoparticles using leaf extract from *Cissus vitiginea*. Water soluble organic compounds present in the leaf extract was mainly responsible for synthesis of silver nanoparticles by reducing silver ions to nanosized silver particles. The UV-visible spectroscopy, FTIR and SEM studies of the synthesized silver nanoparticles elucidated that the silver nanoparticles were crystalline in nature, spherical in shape with size ranging between 10 and 40nm and stable. This nanoparticle further evaluated in antimicrobial activity.

Antimicrobial activity of *Cissus vitiginea* and Silver Nanoparticles

Antimicrobial studies on pathogen is an important applications in Nanomedicine. 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[10]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria.

Silver nanoparticles biosynthesized from *Cissus vitiginea* leaves extract was tested individually against test organisms for antimicrobial activity by agar disc diffusion method. For this study Gram positive (*Staphylococcus aureus,* and *Bacillus subtilis*) Gram negative (*Escherichia coli*) bacterial specie and *Candida albicans, Aspergillusflavus* of fungus strains 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 *Cissus vitiginea* leaves extract was significant as compared to *Cissus vitiginea* leaves extract along and standard chloramphenicol. Zone of inhibition (ZoI) was used as a measure for comparing bactericidal activity of these $AgNO_3$. AgNPs from *Cissus vitiginea* leaves extract showed about 7.10mm zone against the test organism *E. coli.* Similarly the AgNPs from *Cissus vitiginea* leaves showed 6.30mm and 6.70mm ZoI against *S. aureusa* and *Bacillus subtilis* respectively. (Table No.1 and Figure No.1a, b and c). The zone of inhibition of AgNPs is nearest to standard Chloramphenicol.

After 48 hours of incubation, the inhibitory effect of AgNPs from *Cissus vitiginea* leaves extract was significant as compared to *Cissus vitiginea* leaves extract along and standard fluconazole. Zone of inhibition (ZoI) was used as a measure for comparing fungicidal activity of $AgNO_3$. AgNPs from *Cissus vitiginea* leaves extract showed about 2.20mmzone against the test organisms: *C. albicans.* Similarly the AgNPs from *Cissus vitiginea* leaves showed 4.30mm ZoI against *A.flavus*. (Table No.2 and Figure No.2 a and b).

Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions[11,12] Ahmad *et al.* (2011) reported 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 and is a complex structure due to contains teichoic acids or lipoteichoic acids which possess 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[12].

The AgNPs synthesized from various plants are toxic to microorganisms. It shows that they have

great potential in biomedical applications. Similar observation was found in *Allium cepa*[13], *Argimone Mexicana*[14], *Artocarpus heterophyllus*[15,16] found that silver nanoparticles have an ability to interfere with metabolic pathways. [17]Sereemaspun *et al*., (2008) suggested that the by penetration of metallic nano sized particles across the microsomal membrane due to inhibition of oxidation based biological process.

Silver has more antimicrobial 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 *Cissus vitiginea* leaf extract showed higher toxicity. The reason could be that the leaf extract synthesized higher concentration of silver nanoparticles. Moreover leafs 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[18]. The effectiveness of 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[19].

[20]Sondi *et al* (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 pits in the cell wall of bacteria. AgNPs interfere in the bacterial membrane caused the permeability, resulting in cell death. [21]Amro *et al*., (2001) 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. Sondi and Salopek-Sondi also reported a similar mechanism may cause the degradation of the membrane structure of *E. coli* during treatment with AgNPs.

Table No.1: Antibacterial activity of AgNPs, $AgNO_3$ and *Cissus vitiginea* leaves extract

S.No	Samples	Doses	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Bacillus subtilis* (mm)
1	$AgNO_3$	30µl/ml	0.30±0.02	0.40±0.02	0.20±0.01
2	*Cissus vitiginea*	30µl/ml	2.40±0.16	2.60±0.18	1.80±0.12
3	AgNPs	30µl/ml	7.10±0.49	6.30±0.44	6.70±0.46
4	Standard	30µl/ml	11.20±0.78	10.80±0.75	11.30±0.79

Values were expressed as Mean ± SD for triplicate

Table No.2: Antifungal activity of AgNPs, $AgNO_3$ and *Cissus vitiginea* leaves extract

S.No	Samples	Doses	*Candida albicans* (mm)	*Aspergillus flavus* (mm)
1	$AgNO_3$	30µl/ml	0.10±0.01	0.20±0.01
2	*Cissus vitiginea*	30µl/ml	0.30±0.02	0.50±0.03
3	AgNPs	30µl/ml	2.20±0.15	4.30±0.30
4	Standard	30µl/ml	10.40±0.72	10.60±0.74

Values were expressed as Mean ± SD for triplicate

Bacterial standard: Chloramphenicol

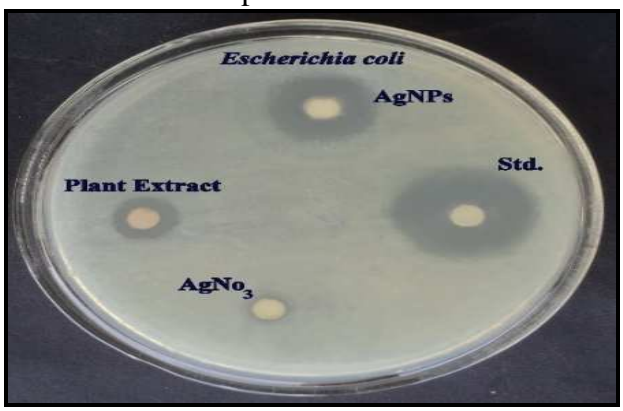

Figure No.1a: ***Escherichia coli***

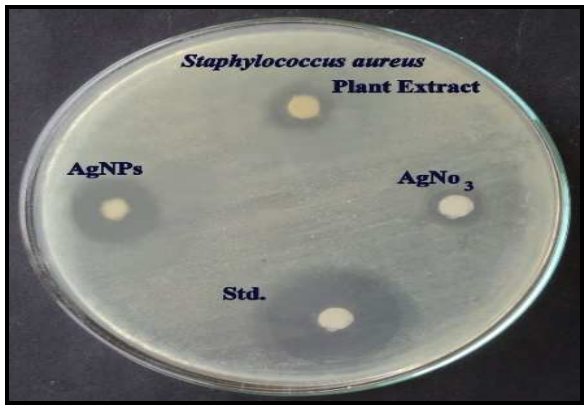

Figure No.1b: ***Staphylococcus auerus***

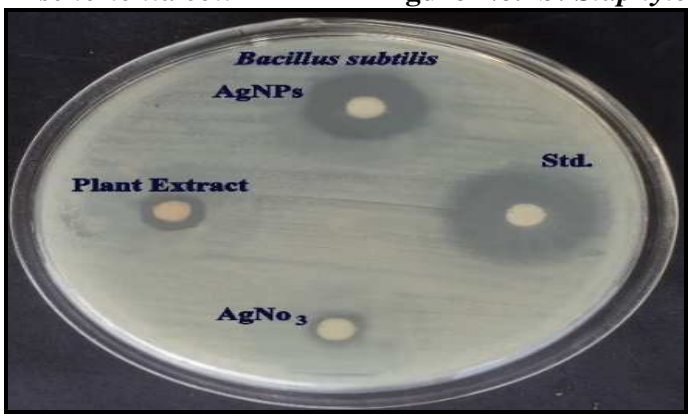

Figure No.1c: ***Bacillus subtilis***

$AgNO_3$ = Silver Nitrate; AgNPs = Silver Nanoparticles

Figure No.1a, b and c: Shows the antibacterial activity of AgNPs, $AgNO_3$ and *Cissus vitiginea* leaves extract

Fungal standard: Fluconazole

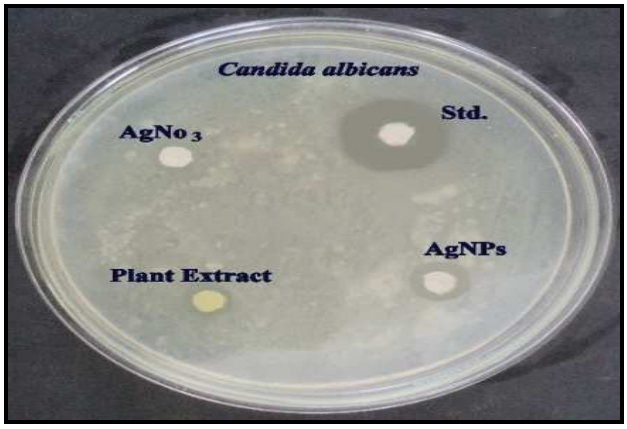

Figure No.2a: ***Candida albicans***

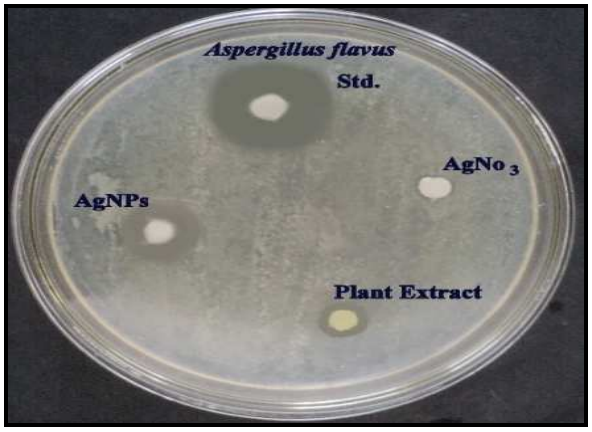

Figure No.2b: ***Aspergillus flavus***

$AgNO_3$ = Silver Nitrate; AgNPs = Silver Nanoparticles

Figure No.2a, and b: Shows the antifungal activity of AgNPs, $AgNO_3$ and *Cissus vitiginea* leaves extract

CONCLUSION

The results of this experiment suggest that synthesized SNPs from *Cissus vitiginea* leaves extract are fairly ideal candidates for the development of new antimicrobial drugs against bacteria and fungi. 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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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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