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Research Article

ANTIBACTERIAL ACTIVITY OF BIOGENIC SILVER NANOPARTICLES FROM *SPHAERANTHUS AMARANTHOIDES*

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ABSTRACT

Objective: The biogenic silver nanoparticles synthesised from plants origin are well known for antibacterial activity. The current study was aimed to identify the antibacterial potential of *sphaernathus amaranthoides* areal parts. But the mechanism of antimicrobial activity of silver particle is not clear. Plant extract reduces the aqueous silver ions in a short period of time.

Methods*:* Incubation of plant extract with the silver will change the colour this indicates the reduction of the silver and confirms the silver nanoparticles with the UV-Vis spectroscopy. The shape, size and stability were studied by the SEM and XRD. The plant was already proved for its antimicrobial activity.

Results: The antimicrobial activity of biogenic silver nanoparticles was proved for *Bacillus subtilis*, *Proteus vulgaris and Escherichia Coli*, by agar well method using mullerhington agar. *E.coli*, *Bacillus subtilis* strains may cause diarrhoea and *Proteus ulgagris* causes the urinary infection. The antimicrobial activity of biogenic silver nanoparticles was calculated with the zone of inhibition and MIC. The antimicrobial activity was plotted as concentration against OD.

Conclusion: These results of biogenic silver nanoparticles from *sphaeranthus amaranthoides* suggest that they can be used as effective growth inhibitors in various microorganisms.

Keywords: *Sphaeranthus amaranthoides;* Silver nanoparticles; MIC; Antimicrobial activity; Uv-vis spectroscopy.

INTRODUCTION

With wide usage of antibiotics there was an increase in the microbial resistance, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective and biogenic antimicrobial agents free of resistance and to reduce cost. The properties exhibited by the silver nanoparticals are due to their specific characteristics like reduced size, shape, distribution and morphology this increases the biocompatibility. The antimicrobial nature of the silver particles was observed from ancient time [1]. Currently silver is used to control the microbial growth in a variety of applications, including dental work, catheters, and burn wounds [2]. previous reports says that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects[3]. They are non toxic to humans [3]. Moreover, many metals and salts of metal are effective antimicrobial agents known from decades. These properties of silver are exploited in medical industry in different ways like topical applications, burns and wounds and also in surgery. The biological synthesis of silver nanoparticles enhances their antimicrobial activity.

Due to the strong resistance of microorganisms against to the existing antimicrobial agents, there is an immediate need for the research for the new antimicrobial agents. India is the greatest source for medicinal plants; this is the good natural source for the drugs.[3]. *Sphaeranthus amaranthoides* is a siddha holistic plant which is widely available during rainy seasons in the south India as a weed in the paddy fields. *Sphaeranthus amaranthodies* belongs to the family asteraceae. It's a small shrub with purple flowers. This plant is already proved for its antimicrobial activity, hepatoprotective activity[4] (swarna latha). The current study is a small attempt to find out the antimicrobial activity of biogenic silver nanoparticles synthesised from leaf of *sphaeranthus amaranthoides*. The results obtained proved that silver used for the preparation of nanoparticles enhanced the antimicrobial activity of *sphaeranthus amaranthoides* leaf extract.

MATERIALS AND METHODS

Preparation of extract and silver nanoparticles

Sphaeranthus amaranthoides Burm. F (Asteraceae) plant leaf was collected from Tirunelveli district. The collected leaf was shade dried for three weeks to get consistent weight and made in to coarse powder and is used for further studies. This Plant was examined and botanically identified by a botanist V. Chelladurai Research Officer-Botany. The leaf powder was soaked in the petroleum ether for two days to dissolve the chlorophyll and then the leaf material was transferred in to the ethanol for five days. On fifth day leaf material was filtered. The filtered extract was subjected to rota vapour to remove the ethanol and to get the concentrated extract in powder form.

To prepare silver nanoparticles 1 gram of ethanol extract was dissolved in 10ml of sterile water. After dissolving completely the plant extract was transferred into 90 ml of aqueous solution of 1mM silver nitrate for reduction into Ag+ ions and kept for incubation period of 24 h at room temperature. Here plant extract act as reducing and stabilizing agent for 1mM of $AgNO_3$. The reduction of Ag2+ ions were monitored by measuring the UV-Vis spectrum after diluting a small aliquot of the sample in distilled water by using systronic 118 UV Vis Spectrophotometer between 200-700nm .

Microorganisms

The pure culture of *Bacillus subtilis*, Proteus vulgaris and Escherichia Coli were obtained from department of microbiology, MMM hospital, Chennai. The current antimicrobial work was carried out at department of Biotechnology, Sathyabama University.

Antibacterial activity

The antibacterial activities of silver nanoparticles were carried out by agar well diffusion method [5]. Muller Hinton agar medium was prepared, sterilized and poured in the plates then allowed to solidify and then wells are made with a cork borer in proper diameter ensuring that wells are evenly distributed in the petriplate. After wells are made bacterial cultures were swabbed on these plates. With the sterile pipette silver nanoparticles solution (1 mg/ml) was placed in the agar well and kept for incubation at 37ºC for 24 hours. Then, the plates were left at room temperature for 2 hours to allow diffusion of test sample and incubated face upwards at 37ºC for overnight. The diameter of the zones of inhibition was measured with scale; the experiments were repeated thrice and mean values of zone diameter were presented.

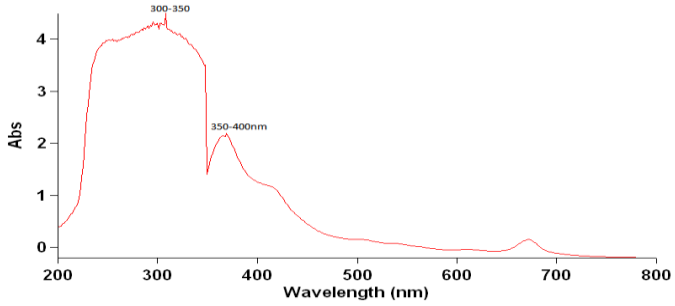
Minimum inhibitory concentration

For each bacterial culture six test tubes were taken, and in all the tubes 2 ml of Nutrient Broth was added initially, and then loop full of active cultures were added to the test tubes. Different concentrations of silver nanoparticles were added to the test tubes as 0ml, 0.2ml, 0.4ml, 0.6ml, 0. 8ml and 1ml. incubate it for one day. To establish the antimicrobial activity of silver nanoparticles on the bacterial growth, the Minimum Inhibitory Concentration [MIC] for Bacillus and Staphylococcus were determined by Optical Density of the bacterial culture solution containing different concentrations of sliver nanoparticles after 24 hours. Culture containing test tube was considered as blank for taking OD.

RESULTS

The synthesis of silver nanoparticles was confirmed by measuring UV-Vis spectro-photometer of reaction medium. The UV-Vis spectrum of *sphaeranthus amaranthoides* silver nanoparticles has the absorbance peak at 200-700 nm.

UV-Vis spectroscopy of *sphaeranthus amaranthoides*



Antibacterial activity of *sphaeranthus amaranthoides*

The colour intensity increases with the increase in the time duration. The results of agar well diffusion assay showed the maximum zone of inhibition with the *E.coli*(3.2±0.1mm), followed by *proteus ulgagris* (2.4±0.05mm) and *Bacillus subtilis* (1.3±0.1mm). Table .1 showed the zone of inhibition of the antibacterial activity of *sphaeranthus amaranthoides* on solid medium.

The zone of inhibition was more for *E.coli* than the Bacillus and proteus. But the *sphaeranthus amaranthoides* silver nanoparticles showed significant antibacterial activity against the *E.coli* and *Proteus* than *Bacillus*.

Table 1: Antibacterial activity of *sphaeranthus amaranthoides*

S. No.	Test organism	Zone of inhibition(cm)
1.	Escherichia coli	3.2±0.1
2	Proteus vulgaris	2.4±0.05
3	Bacillus subtilis	1.3±0.1

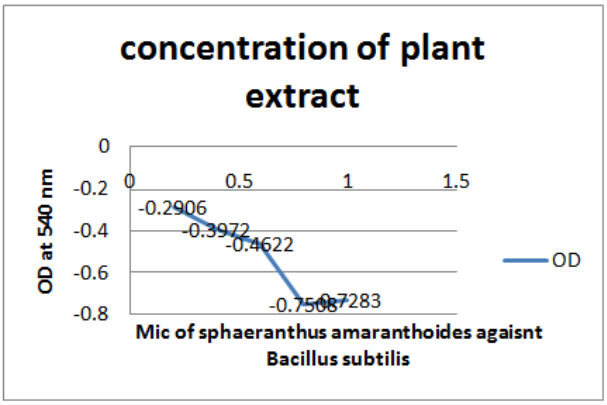


Fig. 1: MIC of *sphaeranthus amaranthoides against Bacillus subtilis*

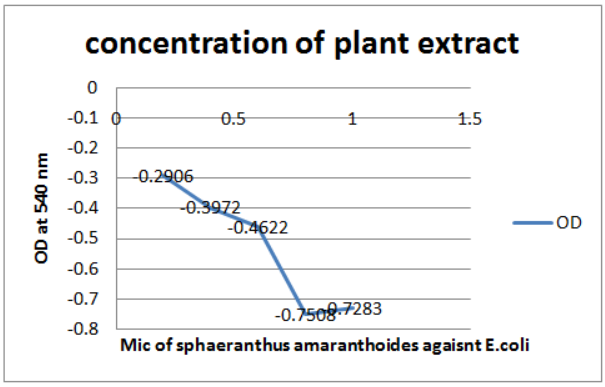


Fig. 2: MIC of *sphaeranthus amaranthoides against E.coli*

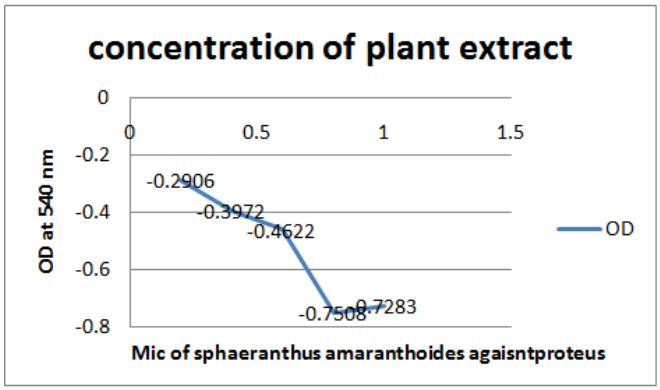


Fig. 3: MIC of *sphaeranthus amaranthoides against Proteus*

Silver nanoparticles are effective against gram –ve bacteria than the gram +ve bacteria. Further MIC values showed that the 0.6µg/ml for *E.coli*, 0.65µg/ml for *proteus ulgagris* and 0.7µg/ml for *Bacillus subtilis*. The MIC was performed against the concentration versus OD.

DISCUSSION

The biogenic synthesis of silver nanoparticles using plant extracts *sphaeranthus amaranthoides* were carried out. The reduced silver nanoparticles will look in yellowish-brown colour. This is due to the excitation of surface Plasmon vibrations in silver nanoparticles[6]. The appearance of yellowish – brown confirms the formations of silver nanoparticles in the flask [7].

The aqueous silver nitrate solution when exposed to plant extract was reduced in solution, there by leads to silver hydrosol formation. Silver has good disinfecting character; this was enhanced with the *sphaeranthus amaranthoides* ethanolic extract reduction. The bioactive compounds present in the plant extract will show the week absorption peaks in the spectrum; these will react with silver ions. Due to the various distinctive properties of silver like good conductivity, catalytic and chemical stability silver is a good reducing agent. The bioactive compounds present in the plants are responsible for the reduction of the silver ions.

In view of this, the present study aimed to make an attempt to identify the synthesis and antibacterial properties of silver nanoparticles using whole plant of *sphaeranthus amaranthoides* plant. The silver nanoparticles from this plant showed a maximum activity on *E.coli* bacteria than other two species. This antimicrobial activity of silver might be enhanced due to the presence of high content of secondary metabolites such as polyphenols, flavonoids and tannins [8, 9&10]. Moreover the present study also proved to have potential antibacterial activities with the *sphaeranthus amaranthoides* extract synthesised silver nanoparticles and this might be due to denaturation of bacterial cell wall, leaking the sugars from the cell wall this may lead to the blocking of bacterial respiration, destabilization of outer membrane and depletion of intracellular ATP [11]. Further the variation in the sensitivity between the gram +ve and –ve against to the nanoparticles varies greatly. This might be due to the membrane permeability [12]. In spite of this permeability barrier, the biogenic silver nanoparticles exhibited the strong inhibition with gram-ve bacterial strains than the gram+ve strains. The size of the nanoparticles also can be controlled by varying concentration due to this the biogenic nanoprtocles play an important role in many of the pharmaceutical applications.

CONCLUSION

In this study it is concluded that, the biogenic nanoparticles synthesised using *sphaernathus amaranthodies* showed a potential antibacterial activity with various bacterial pathogens which could be further used as potential antibacterial agents.

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