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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACT OF *STEMODIA VISCOSA* AND ITS EVALUATION OF ANTIMICROBIAL ACTIVITY

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ABSTRACT

Silver nanoparticles are prepared from aqueous extract of *Stemodia viscosa*. The silver nanoparticles (AgNPs) were characterized by UV-Visible spectrometer. The synthesized silver nanoparticles were subjected to phytochemical screening and these particles were tested against selected clinical pathogens [*E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella flexneri, and Pseudomonas fluorescence*]. The aqueous leaf extract of *Stemodia viscosa* was pale yellow in colour, and after the addition of aqueous silver nitrate, the colourless to honey brown within the 1 hours. It indicates the formation of silver nanoparticles. The UV- Vis analysis from 400-800 nm revealed that maximum absorbance. UV-Vis spectra of synthesized AgNPs, which give a sharp band at around 500 nm confirmed the presence of nanoparticles. The results of antimicrobial activity revealed that the AgNPs of *Stemodia viscosa* was found to be most effective against *S.aureus* with 27 mm in diameter, followed by *P. aeruginosa* with 25mm in diameter. From the above results, it was found out that AgNPs of *Stemodia viscosa* exhibit a remarkable antimicrobial activity. Hence, AgNPs of *Stemodia viscosa* can be used for the treatment of various diseases. This study suggests that this plant is used for the development of antimicrobial drug.

KEYWORDS: *Stemodia viscosa,* silver nanoparticle, gram positive and gram negative bacteria.

INTRODUCTION

The biosynthesis of nanoparticles has been proposed as a cost effective and environmental friendly alternative to chemical and physical methods. Plant mediated synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and plant biotechnology.[1] There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones.[2] Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms.[3] Medicinal plants have already no side effects but when we synthesize nanoparticles from this plants it activity is high in minimum amount of dosage and also enhances the fast recovery.

Stemodia viscosa, is a genus of about 40 species belonging to the family *Scrophulariaceae* occurring in tropical and subtropical regions of the world. The chemical investigation of this genus is restricted to five species from which flavonoids, diterpenes, and diterpenes derivatives with a rare tetracyclic skeletal, named stemodane, were isolated.[4,5,6] *Stemodia viscosa Roxb*., an aromatic weed found in cultivated fields of India, is also refered to as sticky blue rod, Pintye etc., It is an erect, aromatic, viscidly pubescent herb with quadrangular stem reaching up to 60 cm high. Leaves are 4 cm long, sessile, oblong and amplexicaul. Flowers axillary, violet in colour and bilipped. The fragment leaves of this herb are placed in pillows to induce a restful sleep, or crushed and mixed with fat to make rubbing medicine to treat cold and flu symptoms.[5] Glandular- hairy, ascending,herb to 50(100)cm. The analgesic, anti microbial, anti diarrhoeal, anti pyretic, hepatoprotective, anti hyper lipidemic and anti ulcer activities of the aerial parts has been reported in *Cleoma viscosa*. It also has antiseptic, anti inflammatory and wound healing activity.[7]

However very little information has been published on this plant and there is no scientifically proven data to show whether the silver nanoparaticle of s*temodia viscosa* aqueous plant extract has antimicrobial activity or not. Therefore we have undertaken the present study to explore the effects of the above extract on antimicrobial activity.

MATERIALS AND METHOD

Collection of sample

For the synthesis of silver nanoparticles, *Stemodia viscosa* was collected from the village Mallampatti, Madurai Distrist, Tamil Nadu, India during March 2016 and was authenticated by Karunai selvi, Assistant Professor, Department of Botany, V.V.Vanniaperumal college for women, Virudhunagar, Tamil Nadu, India. Silver nitrate [$AgNO_3$] was purchased from Merck Limited, India. The nutrient media used was supplied by Hi–Media Laboratories.

Preparation of the plant extract

Extract have been prepared by using fresh plant of *Stemodia viscosa*, weighing 20grams. The plant was washed thoroughly thrice in distilled water and it was cut into fine pieces, transferred into a 500 ml Erlenmeyer flask with 100ml of distilled water and boiled for 10 minutes. It was then filtered to obtain the plant extract.

Synthesis of silver nanoparticles

In a typical synthesis of silver (Ag) nanoparticles, the plant extract 40 ml was added to 10 ml of 10mM $AgNO_3$ aqueous solution and kept at room temperature. The experiment was done in triplicate for reproducibility. After 1 hour the colour of the extract changed from colourless to honey brown indicating the formation of silver nanoparticles.

UV-Vis spectra analysis

The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the sample, compared with 1ml of distilled water was used as blank.

Preliminary phytochemical analysis of *Stemodia viscosa*

The synthesised silver nanoparticles were used for the qualitative analysis for the identification of plant phytoconstituents.

a) Test for tannins

Take 1ml of the AgNP and add 2 drops of 5 % ferric chloride solutions. Dirty green precipitate indicates the presence of tannin.

b)Test for flavonoids

The AgNP (50 mg) was dissolved in distilled water and 3ml of lead acetate solution was added. A bulky white Lead precipitate indicated the test is positive.

c) Test for alkaloids

The given sample was added with Mayer's reagent (0.68g of mercuric chloride in 30ml of distilled water, 2.5g of potassium iodide in 5ml of distilled water) solution. Formation of whitish yellow (or) cream coloured precipitate indicated the absence of alkaloids.

d) Test for phenols

About 1ml of AgNP was dissolved in 5ml of alcohol was treated separately with a few drops of neutral ferric chloride (ammonium hydroxide + ferric chloride) solution. The change in colour to blue green or black colouration indicated the presence of phenols.

e) Test for protein

Two drops of Ninhydrin solution (10 mg of Ninhydrin in 200ml of acetone) were added to 2ml of sample. A characteristic purple colour indicated the presence of amino acid.

f) Test for carbohydrates

Iodine test

AgNP was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Few drops of AgNPsolution were mixed with 2ml of Molisch's reagent and shaken well. Then 2ml of concentrated sulphuric acid poured along the sides of the test tube. Appearance of violet colour indicates the presence of carbohydrates.

g) Test for terpenoids

The sample was added with chloroform and sulphuric acid. Red violet colour indicates the presence of terpenoids.

h)Test for glycosides

2ml of glacial acetic acid containing one drop of ferric chloride solution was added to 5ml of the sample. 1ml of concentrated sulphuric acid was poured along the sides of the tube. Formation of a brown ring at the interface indicates the presence of glycosides.

i) Test for quinones

The sample was mixed with 2ml of concentrated HCl and heated gently. A yellow coloration indicated the presence of the quinines.

j) Test for anthraquinone

0.5 g of the sample was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted into another test tube. The resulting solution was observed for colour changes to violet indicating the presence of anthraquinine.

ANTIMICROBIAL ACTIVITY

Antibacterial susceptibility testing of antibiotics was performed by disc diffusion method.[8] For susceptibility testing, a sterile cotton swab was dipped into the standardized inoculums and rotated firmly against the upper inside wall of the test tube to remove excess inoculum from swab. Entire sterile and dried Muller Hinton agar surface of the plate was streaked with the cotton swab. For antibacterial susceptibility testing of plant extracts the sterile disc of 6mm diameter (SD067,

Hi - Media, Mumbai) was impregnated with 40µl of AgNP (200 mg/ml). The plates were incubated at 37^0C for 24 hrs. The assessment of antibacterial activity was done by measuring the diameter of the growth inhibition zone formed around disc. Test was done in duplicate.

RESULTS

UV-VISBLE SPECTRUM OF SILVER NANOPARTICLES

The aqueous leaf extract of *Stemodia viscosa* was pale yellow in colour, and after the addition of aqueous silver nitrate, the colourless to honey brown within the 1 hours. It indicates the formation of silver nanoparticles. The formation of silver nanoparticles of H^+ ions to reduce the silver. The UV- Vis analysis from 400-800 nm revealed that maximum absorbance.

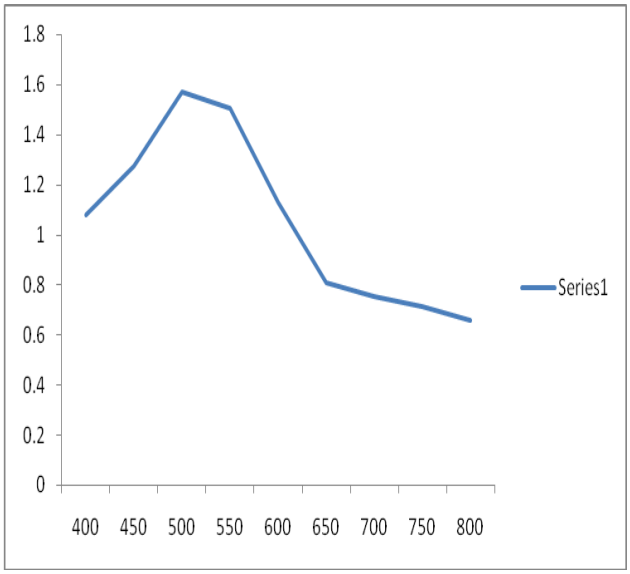

Figure 1:UV -Visible spectrum of silvernanoparticles.

HYTOCHEMICAL ANALYSIS

The phytochemical constituents of the silver nanoparticles of *Stemodia viscosa* were summarized in the table 1. The results revealed that alkaloids, tannins, flavanoids, phenols, protein, carbohydrates, terpenoids, and quinone phytoconstituents were present in this plant.

Table 1: Preliminary phytochemical analysis of silver nanoparticles of *Stemodia viscosa*.

S. NO.	Phytochemical Test	Nanoparticles of *Stemodia viscosa*
1.	Tannins	Positive
2.	Flavonoids	Positive
3.	Alkaloids	Negative
4.	Phenol	Positive
5.	Protein	Positive
6.	Carbohydrates	Positive
7.	Terpenoids	Positive
8.	Glycosides	Negative
9.	Quinones	Positive
10.	Anthroquinone	Negative

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of silver nanoparticles of aqueous extract of *Stemodia viscosa* was studied with different clinical pathogens such as *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella flexneri, and Pseudomonas fluorescence*. Antimicrobial activity was performed by disc diffusion method. The results were summarized in Tables 2. The results revealed that the silver nanoparticles of aqueous extract of *Stemodia viscosa* was found to be effective against gram positive bacteria *Staphylococcus aureus* with 27mm in diameter. The extract was also found to be effective against *Pseudomonas* aeruginosa with 25 mm in diameter. The extract was also effective against *Pseudomonas fluorescence* with 19 mm in diameter followed by *E.coli* with 13 mm in diameter and *Shigella flexneri* with a minimum zone of 12 mm in diameter.

Table 2: Antimicrobial Activity of Silver Nanoparticles of Aqueous Extract of *Stemodia Viscosa* Against Selected Clinical Pathogens.

Microorganism	Zone of inhibition (in mm)		
	Control	Standard	Extract
E.coli	0	15	13
Staphylococcus aureus	0	19	27
Pseudomonas aeruginosa	0	16	25
Shigella flexneri	0	11	12
Pseudomonas fluorescence	0	16	19

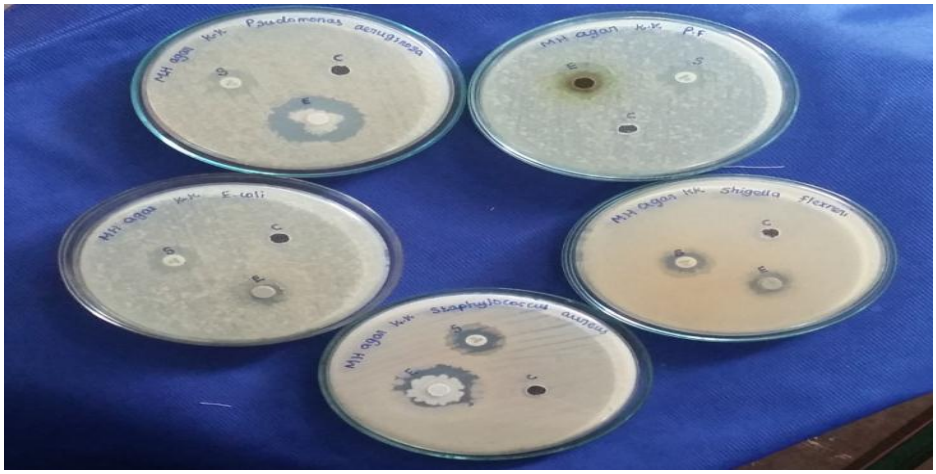

Figure 2: Antimicrobial activity of S*temodia viscosa* of silver nanoparticles against selected pathogens.

DISCUSSION

The formation of silver nanoparticles of H^+ ions to reduce the silver. The UV- Vis analysis from 400-800nm revealed that maximum absorbance peak was 500nm.Our results coincides with the findings of Ankanna *et al.*, 2010.[9] The herbal leaves and medicinal properties of nanoparticles synthesized plant Stemodia viscosa were analysed by phytochemical constituents which revealed the presence of alkaloids exhibited the many bioactivities. Further the presence of terpenoids, protein, flavanoids, and tannins possess the different kind of bioproperties.

The antimicrobial activity extract of Stemodia viscosa for this study with different clinical pathogens, such as *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa*, *Pseudomonas fluorescence*, and *Shigella flexneri*. It was found out that Stemodia viscosa proved to be effective against gram positive rods *Pseudomonas aureus* and *Pseudomonas fluorescence* followed by *E.coli*. The antimicrobial activity of phytochemical constituents may involve in differents modes of action. Delibrate research is in prognoses for alternative to compact further spread of antibiotic resistant pathogens was reported by [10] The presence of proteins, are peptide may act directly on microorganisms on results in growth inhibition by distrupting cell membrane synthesis of essential enzyme. This may lead to inhibit the growth of pathogens taken into an account for the study. A similar result was carried out Devendra *et al*, 2011. where in her emphasized the inhibitory activity of *Moringa olifera* against the pathogens *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The minimum zone of inhibition was found against Shigella flexneri was noted.[11] However to our knowledge there is no data available on *Stemodia viscosa*. Hence the present investigation on nanoparticle synthesized plant extract which is an important approach of apperant synergy employed by the plant *Stemodia viscosa*.

SUMMARY AND CONCLUSION

Stemodia viscosa, is a genus of about 40 species that belong to the family Scrophulariaceae occurring in typical and subtropical regions of the world. The phytochemical analysis of the plant extract revealed the presence alkaloids, tannins, flavonoids, phenols, protein, carbohydrates, terpenoids and quinine. These phytochemical constituents possess effective biological properties. UV-Vis spectra analysis was carried in the range of 600-800 nm to observe the appearance of specific SPK peak of plant mediated silver nanoparticles. UV-Vis spectra of synthesized AgNPs, which give a sharp band at around 500 nm confirmed the analysis of nanoparticles. In the present investigation, Stemodia viscosa was analyzed for its antimicrobial property against bacterial pathogens such as *E.coli*, *S.aureus*, *P.aeruginosa*, *S.flexneri*, and *P. fluorescence.* The results revealed that the extract was found to be most effective against *S.aureus* with 27 mm in diameter, followed by *P. aeruginosa* with 25mm in diameter. From the above results, it was found out that Stemodia viscosa exhibit a remarkable antimicrobial activity. Hence, Stemodia viscosa can be used for the treatment of various diseases. Further research in this pursuit, focusing on the pharmacologic activity like antidiabetic and wound healing activity is under investigations in our laboratory**.**

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