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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF SILVERNA NOPARTICLE STEM EXTRACT OF ROTHECA SERRATA (L.) STEANE & MABB

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ABSTRACT

The search for new antimicrobial agents has accelerated due to the rise in antibiotic-resistant bacterial infections, and green-synthesized silver nanoparticles (AgNPs) have emerged as a viable substitute. The stem extract of *Rotheca serrata* (L.) Steane & Mabb., a medicinal plant recognized for its bioactive phytoconstituents, was used in this study to create silver nanoparticles. AgNP production was verified by UV-Vis spectroscopy, which revealed a distinctive surface plasmon resonance peak at about 400 nm. The presence of alkaloids, tannins, saponins, and glycosides was verified by phytochemical screening of the stem extract; these compounds most likely helped to lower the silver ions and stabilize the nanoparticles. Using the agar well diffusion method, the antibacterial activity of the biosynthesized AgNPs was assessed against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) microorganisms. Significant antibacterial activities were demonstrated by the AgNPs; at different concentrations.

Graphical Abstract

INTRODUCTION

The development of new antimicrobial drugs is required due to the rise of multidrug-resistant (MDR) bacterial infections, which has become a significant worldwide health concern. [1] Researchers are looking into alternative strategies, such as nanotechnology and plant-derived bioactive chemicals, while traditional antibiotics are becoming less effective.[2] Because of their strong antiviral, antifungal, and antibacterial qualities, silver nanoparticles (AgNPs) have drawn a lot of attention among these[3]An environmentally acceptable, economical, and biocompatible substitute for chemical and physical approaches is the manufacture of AgNPs using plant extracts.[4] The rise of antibiotic-resistant bacterial strains has necessitated the quest for new antimicrobial medications derived from natural sources, particularly medicinal plants. [1]Plants include a variety of bioactive compounds with potential medical uses, including alkaloids, flavonoids, tannins, saponins, and terpenoids, all of which have substantial antibacterial properties.[5] Known by many as "Bharangi," *Rotheca serrata* (L.) Steane & Mabb. Moon is a medicinal shrub that grows throughout tropical and subtropical regions of Asia, including Malaysia, Sri Lanka, and India. According to Kirtikar and Basu [6], it belongs to the Lamiaceae (previously Verbenaceae) family.Fever, microbial infections, inflammation, and asthma are just a few of the ailments that *R. serrata* has long been used to cure in Ayurveda and traditional medicine.[7] Several plant parts, including the roots, leaves, and stems, have been shown to exhibit pharmacological activity, including anti-inflammatory, antioxidant, hepatoprotective, and antibacterial qualities.[8] Although many research have focused on the leaves and roots of *R. serrata*, little is known

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about the phytochemical composition and antibacterial properties of the stem.

Finding the bioactive substances that convert silver ions (Ag NPs) into nanoparticles (Ag NPs) and stabilize them requires phytochemical screening. [9] Because of their large surface area, distinct physicochemical characteristics, and cooperative relationships with phytochemicals, plant-mediated Ag-NPs have stronger antibacterial effects.[10]Assessing *R. serrata*-derived AgNPs' antibacterial activity against harmful bacteria like Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus could give their application in the fight against resistant illnesses a solid scientific foundation.Phytochemical screening is a crucial step in identifying the bioactive ingredients that give plant extracts their medicinal properties. (Harborne, 1998). [11] Additionally, evaluating plant extracts' antibacterial effectiveness against dangerous bacteria could provide a scientific basis for their long-standing application in the treatment of infections.[12] (Rios &Recio, 2005). Given the increasing need for new antimicrobial agents, the purpose of this study is to investigate the phytochemical makeup and antibacterial activity of *Rotheca serrata* (L.) Steane & Mabb. stem extract against common pathogenic bacteria.

MATERIALS AND METHODS

Plant Material Selection

The plant currently known as *Rotheca serrata* was previously classified under the genus *Clerodendrum* and was known as *Clerodendrum serratum* (L.) Moon. This reclassification was based on phylogenetic studies that led to the revival of the genus *Rotheca* in 1998. The change was supported by molecular analyses demonstrating that the inclusion of *Rotheca* within *Clerodendrum* rendered the latter polyphyletic [13] Stevens (2012).

Figure 1. *Rotheca serrata* (L.) Steane&Mabb.

Collection of Sample

Rotheca serrata (L.) Steane&Mabb.(Fig. 1) stem was collected from Botany Department of Dr. Ambedkar College, Deekshabhoomi, Nagpur affiliated to R.T.M. Nagpur University, Nagpur. The sample was ensured to be free from diseases and wash for removal of dust particles. The sample was then shade dried and powdered.

Preparation of Extract

The stem of the plant *Rotheca serrata* (L.) Steane & Mabb. was dried in the sunlight using grinder it can be convert in the powder form. 50g of powder filled in the small cotton bag by using the Soxhlet extractor extraction proceed. The extraction ethyl acetate to be used in 250ml round bottom flask.100ml ethyl acetate containing in the round bottom flask place on the heating element. The solvent heated to reflux. The solvent vapour travels up the distillation arm and flood into chamber housing the thimble of solid. The function of condenser is ensuring that any solvent vapour cool. Solvent vaporized and cool fall down on the solid material drop by drop. When Soxhlet chamber is almost full the chamber is emptied by the siphon. This cycle repeats many times we have done 15 times and finally get the plant extract.

Phytochemical Screening

The presence of bioactive compounds is identified in steam extract of *Rotheca serrata* (L.) Steane & Mabb. by using standard procedures.[14]

Test of Phenol

$FeCl_3$ test: crude extract was mixed with 2ml of 2% solution of $FeCl_3$.A blue green or black colouration the presence of phenol.[15]

Liebermann's test: Take small amount of extract and few of sodium nitrite in a dry test tube and heat gently for a minute. Cool and slowly add 0.5ml conc. H_2SO_4 properly a deep green or blue colour will be developed. Dilute the mixture with distilled water. The solution turns red. Then add excess of dilute NaOH solution. The mixture again become green or blue indicate the presence of phenol.

Test for Tannins

Gelatine test: Extract of 5gm powered plant material by boiling in 100ml of distilled water. The extract was filtered after 30 min. 2ml of 2% gelatine was added to 5ml filtrate. Curdy white precipitated foam indicates the presence of tannins.[16]

Ferric Chloride test: To the filtrate,5 drops of 5% ferric chloride solution was added. Formation of black or green blank coloration indicated the presence of tannin.

Potassium Iodide test: To the filtrate, few drops of saturated solution of potassium iodide was added if pink colour forms which changes to brown on standing, which indicate the presence of tannins like gallic and ellagic acid.

Test for Alkaloids

Mayer's reagent: It is used for the detection of alkaloids. 2-3 ml ofplant extract, few drops of Meyers's reagent. [potassium mercuric iodide] It will formation of yellow cream precipitation. This confirms presence of alkaloids.[17]

Wagner's reagent: In 2 ml of filtrate, Wagner's reagent [Iodide in potassium iodide] was added. Reddish brown precipitation appeared, indicates presence of alkaloid.

Test for Steroids

Salkowski test: In 2 ml of plant extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added and shaken well. The appearance This confirms the presence of sterols.[18]

Liebermann-Burchard Test:2 ml of ethanolic plant extract was mixed with chloroform. 1-2 ml acetic anhydride and 2 drops of concentrated H_2SO_4 from the side of the test tube was added in the mixture. First red, then blue and finally green colour indicates the presence of sterols.

Test for Terpenoids

Terpenoid are a group of complex compounds composed of 5-carbon until called isoprene. Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this,2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A greyish colour indicates the presence of terpenoids.[19]

Test for Carbohydrate

Liebermann-Burchard Test:2 ml of ethanolic plant extract was mixed with chloroform. 1-2 ml acetic anhydride and 2 drops of concentrated H_2SO_4 from the side of the test tube was added in the mixture. First red, then blue and finally green colour indicates the presence of sterols.[20]

Test for Flavonoids

Shinoda Test: Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCL was added drop wise. Pink Scarlet colour appeared after few minutes which indicated the presence of flavonoids.[21]

Alkaline Reagent Test: Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Saponins

Foam Test: A small amount of extract is shaken with little quantity of water. The foam produced persists for 10 minutes. [22]

Detection of Oil and Fats

Stain test: Small quantity of extract have to be pressed between two filter paper an oily stain on filter paper indicate that presence of fix oil.[23]

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening : The qualitative phytochemical analysis done, by using phytochemicals test like Liebermann, gelatine, Shinoda test etc. The phytochemicals which is various therapeutic uses in medicine, some of the phytochemical we have to find out from plant *Rotheca serrata* (L.) Steane & Mabb.given in Table 1.

Table 1. Qualitative phytochemical analysis test result

Sr. No.	Phytochemical tests	Result
1	TEST FOR PHENOL	
	$FeCl_3$ Test	-
	Liebermann's Test	-
2	TEST FOR TANNINS	
	Gelatine Test	-
	Ferric Chloride Test	-
	Potassium Iodide Test	-
3	TEST FOR FLAVONOIDS	
	Shinoda Test	+ +
	Alkaline Reagent Test	+ +
4	TEST FOR STERIODS	
	Salkowski Test	+
	Liebermann Burchard's Test	+
5	TEST FOR TERPENOIDS	
	Chloroform Test	+ +
6	TEST FOR ALKALOIDS	
	Mayer's Test	+
	Wagner's Test	+
7	TEST FOR SAPONINS	
	Foam Test	+
8	TEST FOR FIX OIL [STAIN TEST]	+

Synthesis of Silver Nanoparticle

Reduction Method

In the reduction method, silver nitrate used as a starting material and trisodium citrate and plant extract used as a reducing and capping agent.All the chemical solutions can be prepared using double distilled water. The concentration of the trisodium citrate and plant extract consider on the basis of order to observed the parameters for the size as well as morphology of silver nanoparticle. Addition of plant extract in the silver nitrate then addition of 1% of trisodium citrate in this solution drop by drop. Keep in the sunlight (heating source) By vigorously mixed the solution after the time colour change was observed (pale brown). Finally, remove the heating source and keep at the room temperature.Preparation silver nanoparticle,[24] we have prepared in proportion 1:9 by using reduction method. We have taken to two proportion for preparation of 1mM of silver nanoparticle 0.017g silver nitrate in 100ml deionised water.

1. The sample A containing 9ml of $AgNO_3$ and 0.2 ml of plant extract also adding 0.8ml of sodium citrate. Silver nitrate solution (1 mM) was prepared in amber bottle and stored at dark place.
2. The sample B containing 9ml of $AgNO_3$ and 0.5 ml of plant extract also adding 0.5ml of sodium citrate. Silver nitrate solution (1 mM) was prepared in amber bottle and stored at dark place. Using micropipette take 9ml of silver nitrate in the test tube the prepared stem bark extract was mixed with 1 mM $AgNO_3$ and Also add trisodium citrate 0.5ml solution become 1:9 proportions and kept in sunlight for two hours.

Table 2. Concentration of sample A and sample B

Proportion (A) (10 ml)	9 ml of $AgNO_3$ + 0.8 ml plant extract + 0.2 ml trisodium citrate
Proportion (B) (10 ml)	9 ml of $AgNO_3$ + 0.5 ml plant extract + 0.5 ml trisodium citrate

It was then shifted in dark for next 18 hour. Upon incubation, relative changes in the absorbance recorded at 0 hour and 24 hours were recorded for plasmon resonance via spectrophotometer AgNps screened under single beam UV-Visible spectrophotometer of company labtronics model LT-291 link with software UV-professional able to control via computer having wavelength range 300-600 nm. In addition to that the change in colour for the solution apparent due to formation of silver nanoparticles recorded via naked eye polymorphism.

Naked Eye Confirmation of Nanoparticle

Along with the UV-Visible spectrophotometer formation of silver nanoparticle conformed bye change in colour to dark brown as a transformation from its original colour.

Characterization of Silver Nanoparticle

UV-Visible Analysis: [25]

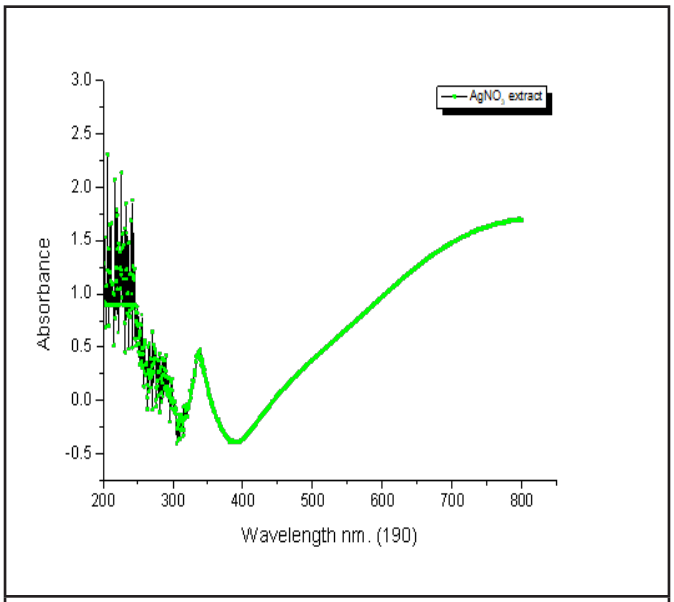

Figure 2. UV-visible spectra of Ag-NPs synthesized by *Rotheca serrata* (L.) Steane & Mabb.

The optical property of AgNPs was determined by UV-Vis spectrophotometer. After the addition of $AgNO_3$ to the plant extract, the Spectra's were taken in after 24hr. The spectra will be observed in the range of wavelength 300nm to 550 nm, it will show maximum absorption at the point of higher peak will be appear. In the chemical synthesis method, Reduction of silver ion into silver nanoparticle subjection on to plant extract was exhibition observed that change in colour of solution. The change in colour, occur due to the surface plasmon resonance phenomenon. It was observed optical measurement in the UV-Visible spectrophotometer, analysing showing absorbance peak at the around 400nm (Fig. 2). From the study of this spectra shows SPR peak for *Rotheca serrata* at 360nm. So, it can be concluded that the *Rotheca serrata* stem extract has more potential to reduce Ag ion into the silver nano particle.

Antibacterial Activity Assay: [26]

The antibacterial activity of AgNPs were carried out by agar well diffusion method against Gram negative bacteria such as E. coli (MTCC 443) and K. Pneumonia [Gram positive bacteria such as S. aureus (MTCC 3160). Agar plates were prepared, sterilized and solidified. Each bacterial strain (1 × 105 CFU/mL) was swabbed uniformly on the prepared individual petri plates using sterile cotton swabs. Four wells of size approximately 6 mm are made on prepared plates using gel puncture.

Methodology

Different pathogens were inoculated into the nutrient agar by swabbing technique.Then the 3 well were bored into the agar plate containing different pathogens.Different concentration such as 25µ, 50µ, and 100µ of extract A and extract B were loaded in wells respectively (Fig. 2 and Fig. 3).Then the plate was incubated at 37°C for 24 hours at incubator.After incubation the plates were observed.Zones were measure with zone scale.

Biological Activity of Silver Nanoparticle

Agar Well Diffusion [27]: We have to study the green synthesis of silver nanoparticle in the two proportion of the solution plant extract. By using Agar well diffusion to examine the antibacterial activity of the both proportion of

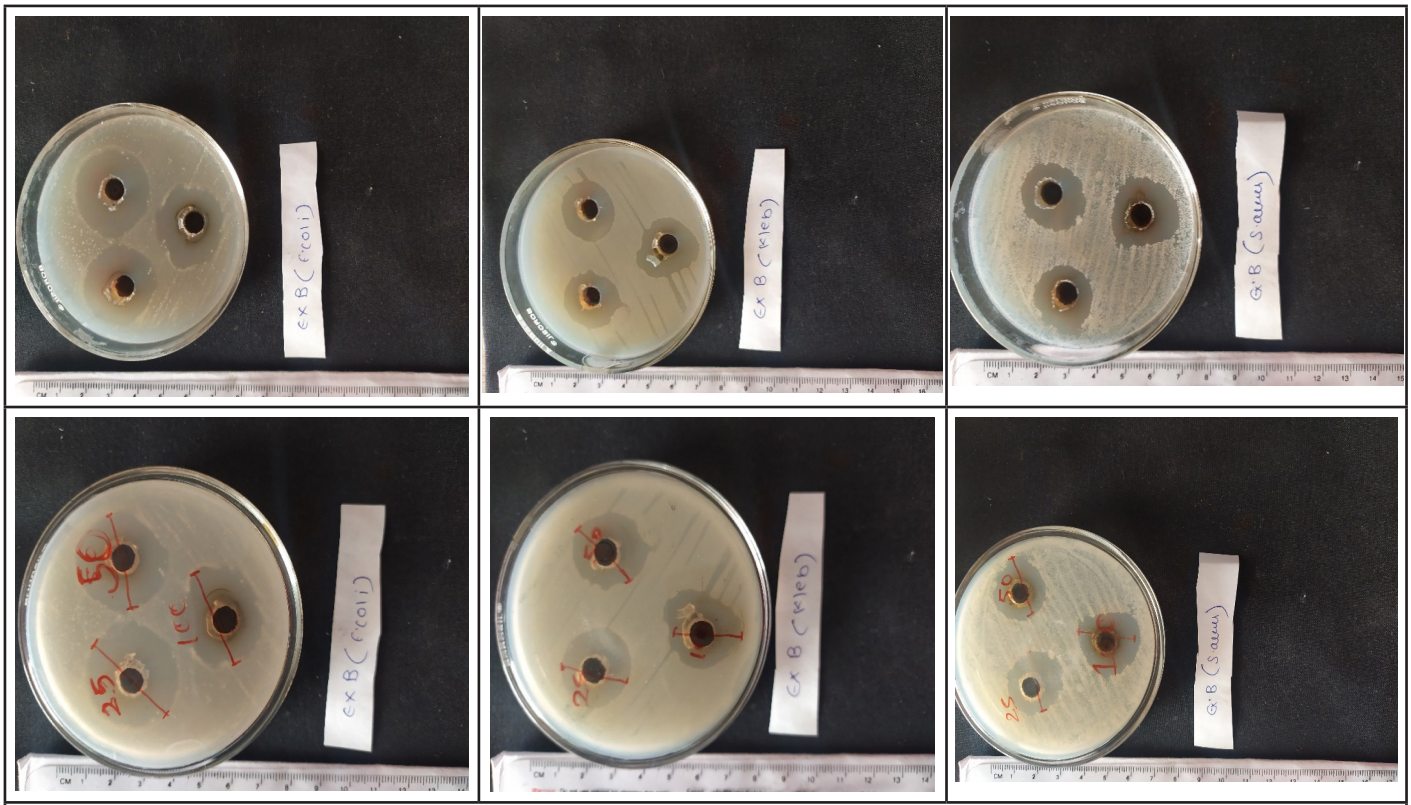

Figure 3. Zone of inhibition 25µl, 50µl,100µl all three bacteria of the sample (B)

the silver nanoparticles. For these Method some of the gram-positive and gram – negative bacteria used, *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae* etc.

Table 3. Inhibition zone by *E. coli*, *S. aureus* and *k. pneumonia* at different extract concentration

Proportion of plant extract	Volume of leaf extract (µl)	Zone of inhibition shown byE. coli (mm)	Zone of inhibition shown byS. aureus (mm)	Zone of inhibition shown byK. pneumonia(mm)
Plant extract 0.2ml(A)	25µl	22mm	16mm	18mm
	50µl	22mm	16mm	18mm
	100µl	23mm	17mm	18mm
Plant extract 0.5 ml(B)	25µl	26mm	22mm	21mm
	50µl	24mm	20mm	22mm
	100µl	24mm	24mm	22mm

From Table 3, both proportion of silver nanoparticle can be observed that minimum inhibition concentration is at 25µl for *E. coli*, S. *aureus* and *K. pneumonia* showing 26 mm, 22mm and 21mm zone diameter respectively.

We have studied the *Rotheca serrata*plant extract mediated silver nanoparticles as possible antibacterial agents. The plant extract and those mediated silver nanoparticles were closely tested for respective antimicrobial activities towards both gram positive (*S. aureus*) and gram negative (*E. coli*) (*K. pneumonia*) bacterial strains show the zones of inhibition. On the zone of inhibition produced in the both of proportion A and B synthesized silver nanoparticles prove to exhibit good antibacterial activity against *E. coli, S. aureus* and *K. pneumonia*. But in the case of proportion according to zone of inhibition (B) showing good result against the antibacterial activity. It would be concluded that the plant *Rotheca serrata* having good antibacterial property.

CONCLUSION

This study described a straightforward green synthesis of stable silver nanoparticles utilizing stem extract from A. *Rotheca serrata* at room temperature. The reduction procedure produced the nanoparticles without the use of external stabilizers or reducing chemicals. It turns out to be a quick, environmentally friendly method that produces silver nanoparticles in an efficient and economical manner. The produced silver nanoparticles shown effective antibacterial properties against *S. aureus, K. pneumonia* and *E. coli.* Utilizing plant extract for synthesis has several advantages, including cost and energy efficiency, environmental and human health protection, less waste, and safer goods. This environmentally benign process has the potential to be a cost-effective substitute for the traditional physical/chemical techniques of producing silver nanoparticles. As a result, it may find usage in biomedical applications and will soon play a significant role in opto-electronics and medical devices. Further studies are recommended to explore the mechanisms of action, cytotoxicity, and in vivo efficacy of these nanoparticles for potential therapeutic use.

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