



Research Article

Antimicrobial Activity and Characteristics of Silver Nanoparticles Biosynthesized from *Cheilocostus speciosus* Rhizome Extracts

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ABSTRACT

In recent years' synthesis of metal nanoparticle using plants has been extensively studied and recognized as a non-toxic and efficient method applicable in biomedical field. The aim of this study is to investigate the role of medical plant *Cheilocostus speciosus* rhizome on synthesizing silver nanoparticles and characterize the produced nanoparticle. Our study showed that silver nanoparticles (AgNP) synthesized via rhizome extract. *Cheilocostus speciosus* has been found to possess antimicrobial properties, which can help fight off bacterial, fungal, and viral infections. A study investigated the antimicrobial activity of *Cheilocostus speciosus* against various bacterial and fungal strains. Five different extracts of this plant rhizome were used for this namely REA (ethyl acetate), RE (Chloroform), REE (Ethanol), REM (Methanol), REW (Water) and biosynthesised AgNP.

Keywords: *Cheilocostus speciosus*, metal nanoparticle, antimicrobial activity.

INTRODUCTION

In recent times researchers are shown much interests in the preparation of noble metal nanoparticles due to their novel and different features as compared with those of macroscopic phase, that allow attractive applications in a variety of fields like electronics, optoelectronics, magnetic, and information storage.^{1,2} For the preparation of metal nanoparticles, a variety of different physical and chemical techniques are used. But many of these methods require a vast sum of toxic chemicals and high temperature conditions. So, it becomes very mandate to find an alternate method.³ The biological approach to nanoparticles synthesis offers a simple, eco friendly, reliable and non-toxic method for nanoparticle synthesis. In this approach, various microorganisms, enzymes, and plant extracts are utilized for the synthesis of nanoparticles. The usage of the plant materials for the synthesis of nanoparticles exceeds other biological methods by reducing the complex process of maintaining cell culture.^{4,5} It is fast, inexpensive, green, and a single step process.⁶ The plant extracts provide both reducing and stabilizing agents for the formation of nanoparticles simultaneously with bringing their medicinal values to the particles, which augment nanoparticles biomedical applications.⁷ At present, there have been various studies which exploited plants to synthesize metal nanoparticles. For instances, *Glycyrrhiza uralensis* root extract,⁸ Cardamom fruits,⁹ *Indigofera tinctoria* leaf extract,¹⁰ *Aerva lanata* leaf extract,¹¹ *Parkia roxburghii* leaf biomass,¹² *Myxopyrum serratum* A.W. Hill leaf extract¹³ *Guazuma ulmifolia* L. bark extract,¹⁴ *Paederia foetida* Linn leaf extract¹⁵ etc. have been reported to synthesize silver nanoparticles. Comparing to chemical methods, the biological methods for nanoparticles synthesis is slow. This problem is conquering by using microwave assisted synthesis. The microwave assisted method has numerous

attractive features like small reaction time, lesser energy utilization and better product yield.¹⁶ In this method, uniform heating of the reaction medium occurs and this gives a homogeneous nucleation and growth condition for nanoparticle formation.¹⁷ Herein, we report the synthesis of silver nanoparticles by using rhizome extract of *Costus speciosus* by the microwave assisted method. *Costus speciosus* is an important medicinal plant used to heal different diseases and is known as Crepe ginger, belongs to the family Zingiberaceae (Costaceae). The plant has many pharmacological properties like antioxidant, anti-inflammatory, antibacterial, antifungal, analgesic, antipyretic, antidiuretic, larvicidal, and anti-stress activities. The rhizome of *Costus speciosus* is febrifuge, purgative, bitter, astringent, acrid, cooling, tonic, improves digestion, and is a stimulant herb that clears toxins. The rhizomes are also given in diseases such as pneumonia, asthma, rheumatism, jaundice, bronchitis, leprosy, anemia, dropsy, and urinary diseases.¹⁸ The rhizomes extract contains phytochemicals like phenols, carbohydrates, glycosides, vitamin C, vitamin E, flavonoids, saponins, and alkaloids.¹⁹ The synthesized silver nanoparticles were characterized using different analytical techniques. The biosynthesized nanoparticles tested for their antimicrobial properties against microbial pathogens by agar well diffusion method. By performing the catalytic reduction of 4-nitrophenol and methylene blue, the catalytic efficacy of synthesized nanoparticles was demonstrated.

MATERIALS AND METHODS

Silver nitrate (AgNO₃), 4-nitrophenol, methylene blue, ascorbic acid was purchased from Merck Chemicals (India) and used without further purifications. Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O) and sodium borohydride (NaBH₄) were purchased from Sigma-Aldrich.



All the chemicals used in the current study were in analytical grade. Double distilled water was used for the preparation of all aqueous solutions.

Preparation of *Costus speciosus* rhizome extract

The fresh rhizome of *Costus speciosus* collected and taxonomically identified. Then it was washed with distilled water and cut into small pieces. They were air dried and powdered. Then the 5 g of the dried samples were taken in a round bottom flask fitted with a condenser and boiled with 100 mL double distilled water for 20 min. It was then cooled and filtered through Whatman number 1 filter paper. For the further studies, the rhizome extract was kept at 4 C in the refrigerator.

Synthesis of silver nanoparticles

For the synthesis of silver, 90 mL (1 mM) of silver nitrate (AgNO₃) solution was taken in a 250 mL beaker. Then, 10 mL of rhizome extract of *Costus speciosus* was added to it and stirred well. Afterward, this solution was subjected to microwave irradiation by the help of a domestic microwave oven (Sharp R-219 T (W)). Its operating power was 800 W and frequency was 2450 MHz. The formation of nanoparticles was examined by using UV-vis. spectrophotometer. The prepared silver and gold nanoparticles were denoted as AgNP-speciosus respectively. Antimicrobial studies by using the agar well diffusion method, the antimicrobial properties of *Costus speciosus* rhizome extract and synthesized nanoparticles were examined.²⁰ Both bacterial and fungal strains were used as test specimens. *Bacillus subtilis* and *Streptococcus* sp. were used as the representative gram positive bacteria, and *E. coli*, and *Pseudomonas* sp. were used as the representative gram negative bacteria. The fungal strains used were *Penicillium* sp. and *Aspergillus flavus*. All these microorganisms were obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. Petri plates containing 20 mL media (nutrient agar for bacteria and potato dextrose agar (PDA) for fungi) were seeded with a matured culture of microorganisms using a sterile swab under aseptic conditions. Wells of 6 mm diameter were created using a sterile cork borer and 50 mL of samples was added into the well. The plates were then incubated at room temperature for nearly one week (fungi) and at 37°C for 24–48 h (bacteria). The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

Anti-microbial activity

Cheilocostus speciosus has been found to possess antimicrobial properties, which can help fight off bacterial, fungal, and viral infections. A study investigated the antimicrobial activity of *Cheilocostus speciosus* against various bacterial and fungal strains. Five different extracts of this plant rhizome were used for this namely REA (ethyl acetate), RE (Chloroform), REE (Ethanol), REM (Methanol), REW (Water) and biosynthesised AgNP.

Anti-bacterial activity

Anti-bacterial property of plant extracts and AgNP were tested against 5 different bacterial cultures namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* in three different quantities (20, 30, 40 µl). For this purpose, overnight grown cultures were spread plated on Muller – hinton agar plates using swab. The experiment is conducted by agar well diffusion method, with the addition of extracts in the respective wells and incubated overnight at 37°C. The size of the inhibition zone indicated the anti-bacterial property of the extracts.

Anti-fungal activity

Anti-bacterial property of plant extracts and AgNP were tested against 2 different fungal cultures namely *Candida albicans* and *Fusarium proliferatum* in three different quantity (20, 30, 40 µl). For this purpose, overnight grown cultures were spread plated on Muller – hinton agar plates using swab. The experiment is conducted by agar well diffusion method, with the addition of extracts in the respective wells and incubated overnight at 37°C. The size of the inhibition zone indicated the anti-fungal property of the extracts.

Statistical analysis

All biological experiments were performed in triplicate. For each measurement, the data were recorded as the mean ± standard deviation (SD). The data were analyzed using oneway analysis of variance (One Way ANOVA) followed by Tukey's test (post-hoc test). The results with p < 0.05 were regarded as statistically significant. The data were statistically analyzed using the statistical package, Graph Pad prismV5.

RESULTS AND DISCUSSIONS

The antimicrobial activities of *Costus speciosus* rhizome extract, AgNP-speciosus analyzed by using agar well diffusion method against both bacterial (gram positive and gram negative) and fungal strains. The antibacterial activity of rhizome extract of *Costus speciosus* was already reported.²¹ The photograph antimicrobial activities of *Costus speciosus* rhizome extract, AgNP-speciosus are shown in the Figure 1-7. The antimicrobial activities of nanoparticles were significantly higher than that of rhizome extract. The synthesized AgNP-speciosus showed significant antimicrobial action on both the bacterial and fungal strains. Several mechanisms have been proposed for the antimicrobial activities of nanoparticles. The nanoparticles can bind with sulfur and phosphorus containing moieties of the microbial membrane this leads to DNA damage and protein dysfunctioning.⁹ Also, the nanoparticles bind to the surface of the cell membrane and perturb its power functions such as permeability and respiration.²² In the case of gram classes of bacteria, the zone of inhibition is higher in the case of gram negative strains of bacteria (*E. coli* and *Pseudomonas* sp.) with thin cell walls than gram positive strain bacteria (*Bacillus*

subtilis, and *Streptococcus* sp., *Klebsiella pneumoniae*) and fungal bacteria *Candida albicans*, *Fusarium ploriferatum* with a thick cell wall.

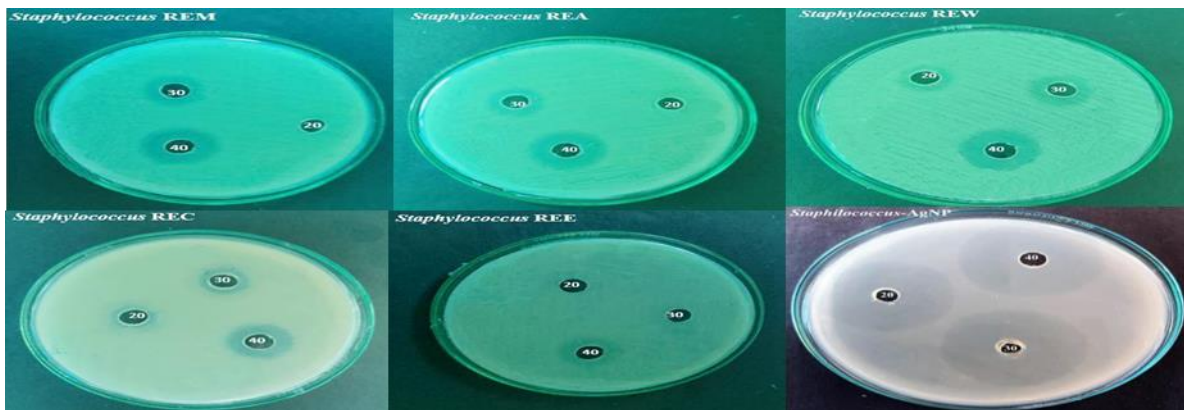


Figure 1: Well diffusion study indicating anti-microbial activity by different extracts in *Staphylococcus aureus*

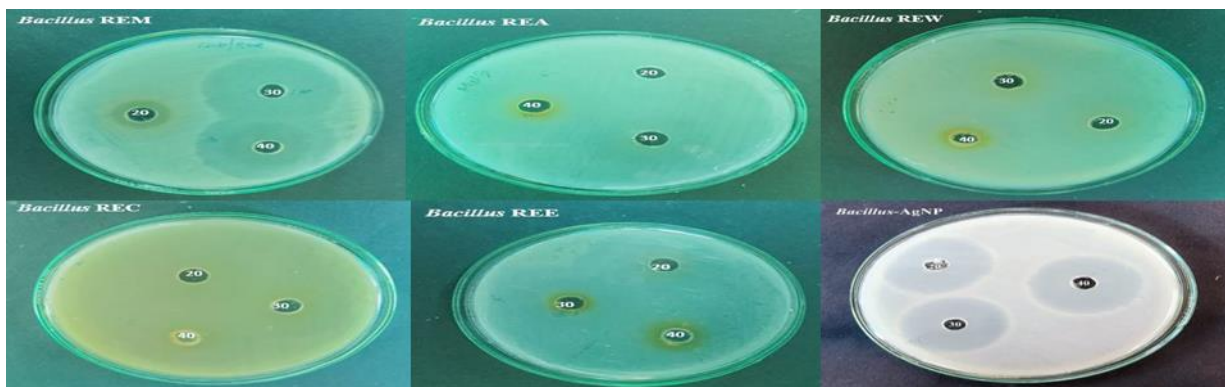


Figure 2: Well diffusion study indicating anti-microbial activity by different extracts in *B. subtilis*

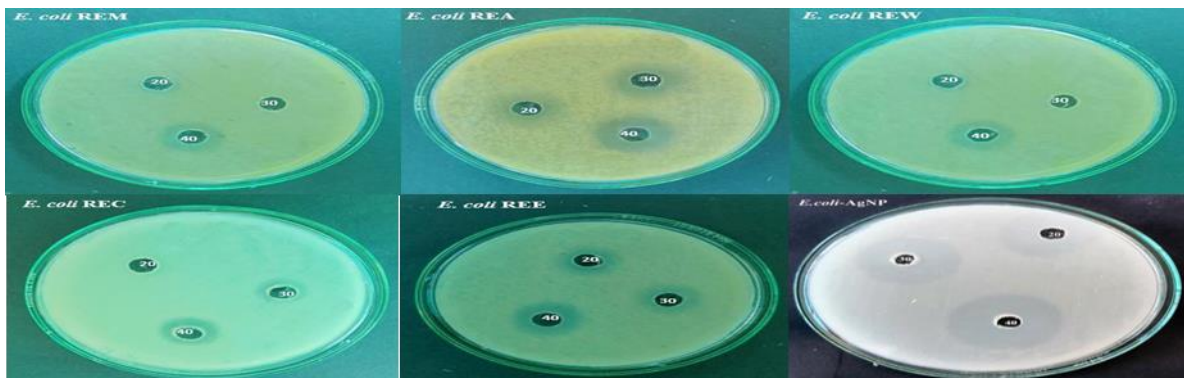


Figure 3: Well diffusion study indicating anti-microbial activity by different extracts in *E. coli*

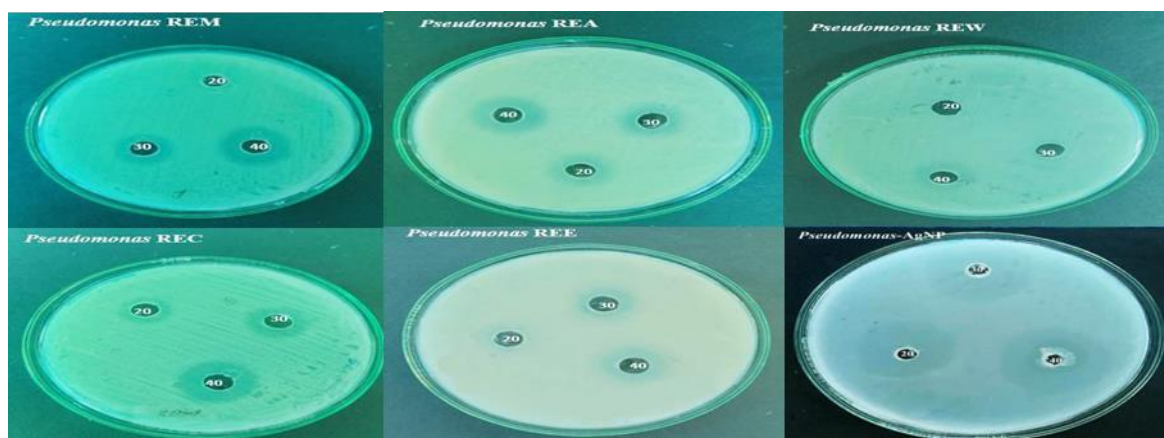


Figure 4: Well diffusion study indicating anti-microbial activity by different extracts in *Pseudomonas aeruginosa*

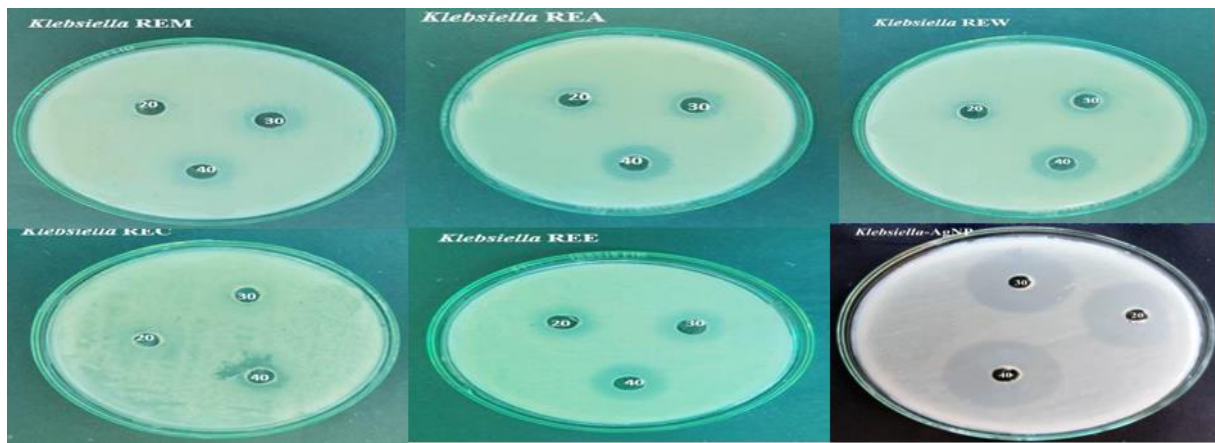


Figure 5: Well diffusion study indicating anti-microbial activity by different extracts in *Klebsiella*

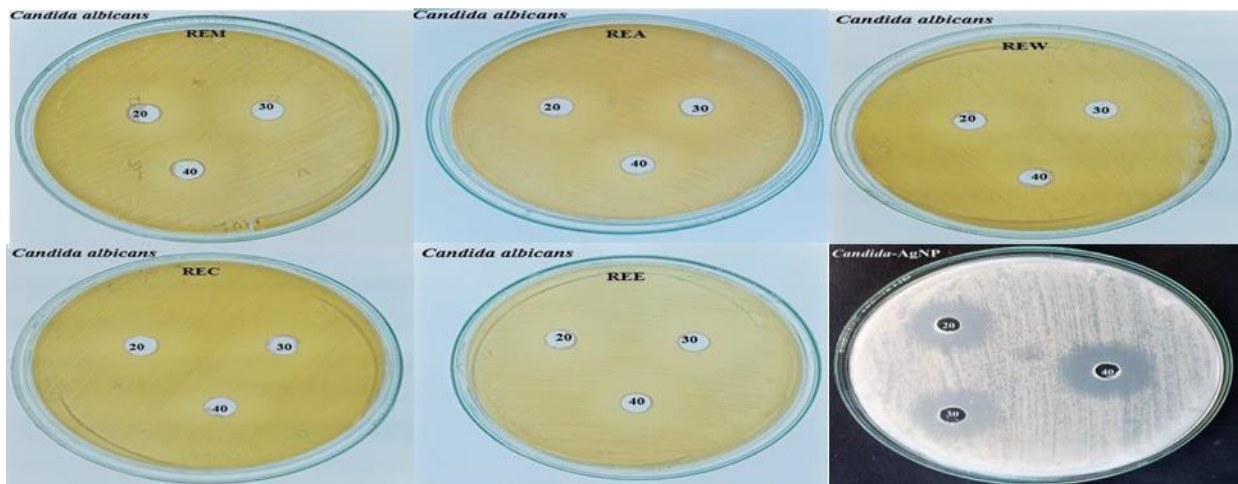


Figure 6: Well diffusion study indicating Anti-fungal activity by different extracts in *Candida albicans*

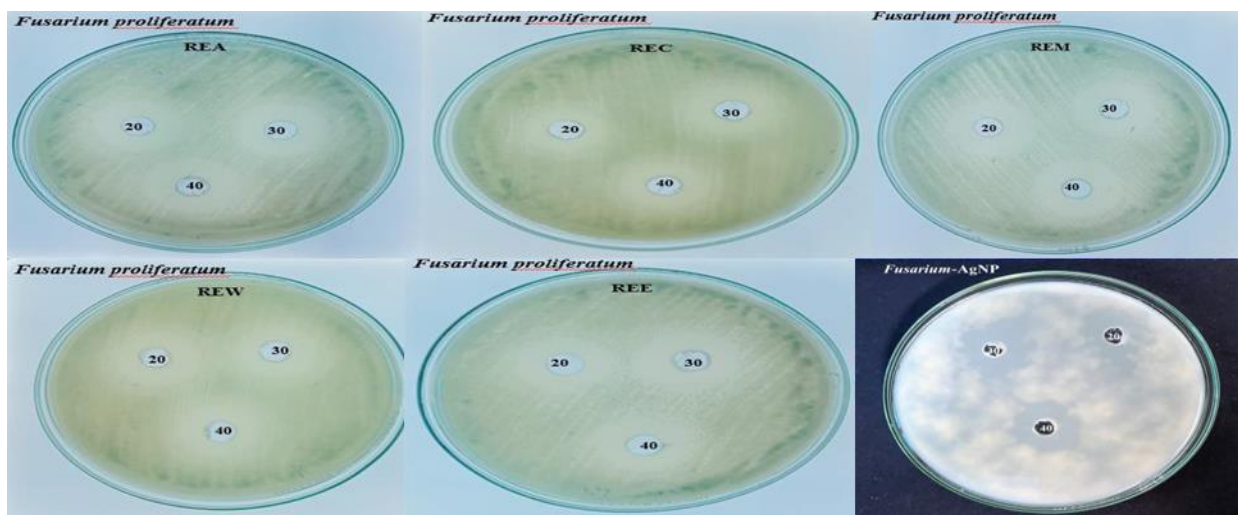


Figure 7: Well diffusion study indicating Anti-fungal activity by different extracts in *Fusarium ploriferatum*

CONCLUSION

The study explored the diverse biological activities of plant extracts and biosynthesized silver nanoparticles (AgNP). The antimicrobial and anti-fungal studies demonstrated the potent activity of the methanol fraction of the plant extract, particularly against bacterial strains. Overall, this research underscores the promising bioactive properties of the plant

extracts and AgNP, encouraging further exploration for potential therapeutic applications.

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