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EXPLORING INHIBITORY EFFECTS OF *Crataevanurvala*-DERIVED SILVER NANOPARTICLES ON HIGH RESISTANCE BACTERIAL AND FUNGAL PATHOGENS

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ABSTRACT

Silver nanoparticles are increasingly being utilized as antibacterial as well as antifungal agentsinseveral applications. Theuse of antibiotics has emerged microbe resistance amongmultidrug-resistantorganisms, which is endangering the global health community. Plants are a good source of phytoconstituents and have been successfully applied for the treatment of microbial diseases. Thus, silver nanoparticles from the ethanol extract of leaves of *Crataeva nurvala* were biosynthesized and aimed to explore the antibacterial and antifungal effects. The antibacterial and antifungal properties of the ethanol extract and its silver nanoparticles were evaluated at concentrations of 200 mg/ml and 50 mg/ml, respectively, using the well diffusion technique. Ethanol extract and its silver nanoparticles from *Crataeva nurvala* showed antibacterial activity against *E. coli, Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger, Rhizopus oryzae, Mucor azygosporus* and *Penicillium chrysogenum*. Antibacterial and antifungal effects of silver nanoparticles were significantly higher than compared of ethanol extract and standard drugs. Hence, findings of the research suggested that the silver nanoparticles from *Crataeva nurvala* leaves exhibited antibacterial and antifungal effects, which might help to develop a new drug for treatment against various diseases.

Keywords: *Crataeva nurvala*, Silver Nanoparticles, Antibacterial, Antifungal, Well Diffusion.
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INTRODUCTION

The overuse of antibiotics led to microbial resistance worldwide and representsa major threat to humanity. [1]It results in an increased demand forthe discovery of novel antibacterial and antifungal agents.[2]WHO has identified antimicrobial resistance as one of the top ten worldwide public health concerns.[3] Nonetheless, plants are frequently utilized in rural regions as a remedy fora variety of illnesses, and because they are abundant in bioactive substances like antimicrobials, which can help to treat difficult bacterial infections.[4]Since plants contain secondary metabolites with a variety of biological activities, like antibacterial, antioxidant, antifungal, and anti-inflammatory properties, they have long been used as prophylactics.[5,6] The use of herbal medicine has increased due to the side effects of synthetic drugs, as well as resistance to drugs in a specific treatment.[7,8]The nanoparticles have gained large interest for the past two decades as their physico-chemical properties, controlled size and shape, and ability to interact with other molecules.[9]Apart from several applications of nanoparticles, antibacterial and antifungal uses of nanoparticles are expected to be fruitful in treating bacterial and fungal illnesses, and also in multidrug resistance.[10] The biosynthesized nanoparticles can enhance the effect of phytoconstituents in the extract against microbial activity.[11] There are various ways to biosynthesize silver nanoparticles, which are simple, fast, non-toxic, effective, and environmentally sustainable. It has broad-spectrum efficacy and low cytotoxicity and has been shown to be very effective against microorganisms. Plant parts are used to

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prepare plant extracts, which are utilized for the biosynthesis of Silver nanoparticles (AgNPs) for their application in numerous sectors, especially in Pharmaceuticals.[12]*Crataeva nurvala* Buch. Ham is found in tropical regions of the world, including India. The plant has been used as a traditional remedy for blood purification, fever, respiratory issues, memory loss, and weakened immune systems. Numerous phytoconstituents, including beta-sitosterol, luteolin, kaempferol, rutin, betulinic acid, and catechin, have been identified in the plant. Various plant parts have been documented to provide therapeutic benefits, including anti-inflammatory, wound-healing, cardioprotective, antipyretic, anti-urolithic, anxiolytic, anti-diabetic, and anti-cancer effects.[13] Therefore, this research has aimed to investigate the antibacterial and antifungal properties of ethanol extract and its silver nanoparticles.

EXPERIMENTAL

Collection of Plant Materials

Crataevanurvala leaves were collected from the area around the FRI, Dehradun, Uttarakhand, India, and verified by Dr. Vidit Tyagi, a botanist with the Department of Botany at DIBNS, Dehradun.

Preparation of Ethanol Extract of Leaves of *Crataeva nurvala*

The leaves were washed with water and dried inthe shade. The air-dried leaves (200 g) were crushed and extracted with ethanol. The extract containing ethanol was evaporated till dryness to obtain the residue and concentrated under reduced pressure.[14]

Biosynthesis of AgNPs

AgNPs were biosynthesised using an ethanol extract of *Crataeva nurvala*. An aqueous solution containing 1 mM silver nitrate (AgNO3) was made. 500 mg of ethanol extract was dissolved with a small amount of ethanol and diluted up to 100 mLwith water. 10 ml of the silver nitrate solution was mixed with 60 ml ethanol extract solution and stirred for 2 hrs and kept at room temperature to react.[15]

Inhibitory Effects of Ethanol Extract and AgNPs on Bacterial and Fungal Pathogens

The inhibitory studies on bacterial and fungal pathogens were done by the well diffusion method as described by Barry Afor ethanol extract and AgNPs.[16] The concentration of ethanol extract was 200 mg/ml, and silver nanoparticles were 50 mg/ml. *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa*and*Staphylococcus aureus* were used for antibacterial study. *Aspergillus niger, Rhizopus oryzae, Mucor azygosporus*and*Penicillium chrysogenum* were used for antifungal study.

RESULTS AND DISCUSSION

Earlier, in continuation with the research, we have reported the characterization of biosynthesized silver nanoparticles and their membrane stabilizing and antioxidant activities.[17]

Inhibitory Effects of Bacterial Pathogens

Ethanol extract showed an inhibition zone of 16.83±0.33 mm against *E. coli*, 12.62±0.75 mm against *Bacillus cereus*, 11.75±0.38 mm against *Pseudomonas aeruginosa*, and 16.92±0.22 mm against Staphylococcus aureus. AgNPs showed a bacterial inhibition zone of 17.73±0.25 mm against *E. coli*, 13.50±0.58 mm against *Bacillus cereus*, 15.33±0.60 mm against *Pseudomonas aeruginosa*, and 26.32±0.44 mm against *Staphylococcus aureus*. The standard drug Chloramphenicol was taken for study for antibacterial activity. Chloramphenicol showed an inhibition zone of 20.67±0.55 mm against *E. coli*, 17.42±0.68 mm against *Bacillus cereus*, 16.58±0.79 mm against *Pseudomonas aeruginosa*, and 27.12±0.39 mm against *Staphylococcus aureus* (Table-1 and Fig.-1).

Table-1: Results of Inhibitory Effects on Bacterial Pathogens

Bacterial Strains	Inhibitory effects on bacterial pathogens(zone of inhibition in mm)		
	Ethanol extract	AgNPs	Standard Drug Chloramphenicol
E. coli	16.83±0.33	17.73±0.25	20.67±0.55
Bacillus cereus	12.62±0.75	13.50±0.58	17.42±0.68
Pseudomonas aeruginosa	11.75±0.38	15.33±0.60	16.58±0.79
Staphylococcus aureus	16.92±0.22	26.32±0.44	27.12±0.39

Results are expressed as mean values ± standard error (n = 3)

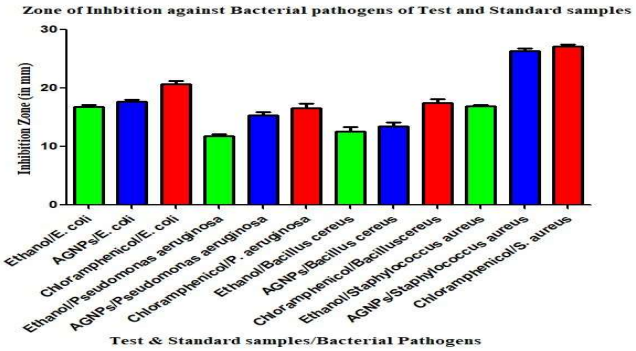

Fig.-1: Results of Zone of Inhibition of Bacterial Pathogens by Ethanol Extract, AgNPs, and Chloramphenicol

Inhibitory Effects of Fungal Pathogens

Ethanol extract showed inhibition zone 15.30±0.39 mm against *Aspergillus niger*, 18.57±0.49 mm against *Rhizopus oryzae*, 11.42±0.57 mm against *Mucor azygosporus* and 9.6±0.43 mm against *Penicillumchrysogenum*. AgNPs showed fungal inhibition zone 17.28±0.21 mm against *Aspergillus niger*, 20.00±0.25 mm against *Rhizopus oryzae*, 19.23±0.52 mm against *Mucor azygosporus* and 12.30±0.22 mm against *Penicillumchrysogenum*. The standard drug Clotrimazole was taken for the study for antifungal activity. Clotrimazole showed inhibition zone 9.27±0.71 mm against *Aspergillus niger*, 11.07±1.08 mm against *Rhizopus oryzae*, 14.70±0.53 against *Mucor azygosporus*, 13.30±0.74 mm against *Penicillium chrysogenum*(Table-2 and Fig.-2).

Table-2: Results of Inhibitory Effects on Fungal Pathogens

Fungal Strains	Inhibitory effects on fungal pathogens(zone of inhibition in mm)		
	Ethanol extract	AgNPs	Standard Drug Clotrimazole
Aspergillus niger	15.30±0.39	17.28±0.21	9.27±0.71
Rhizopus oryzae	18.57±0.49	20.00±0.25	11.07±1.08
Mucor azygosporus	11.42±0.57	19.23±0.52	14.70±0.53
Penicillium chrysogenum	9.6±0.43	12.30±0.22	13.30±0.74

Results are expressed as mean values ± standard error (n = 3)

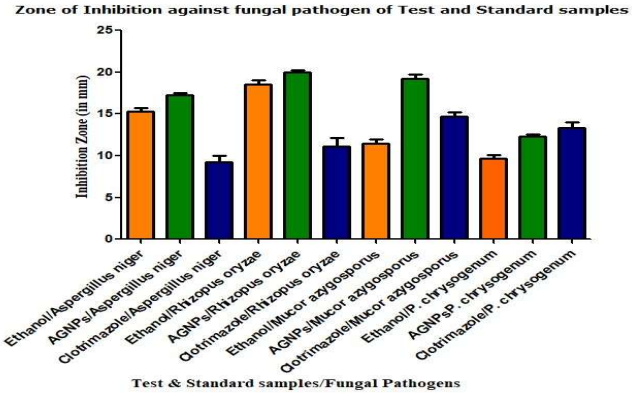

Fig.-2: Results of Zone of Inhibition of Fungal Pathogens by Ethanol Extract, AgNPs, and Clotrimazole

Two very different modes of action should be taken into account when creating antimicrobial materials: first, those that involve direct interactions with bacterial cells, and second, those that facilitate nanomaterial entry into infected host cells to obtain access to intracellular pathogens.[18]While the antimicrobial effect of silver is widely recognized.[19]Regarding AgNPs, the exact mechanism by which nanocrystalline silver causes bacterial cell death is yet unknown, although it probably involves a variety of different ways, mostly due to the release of silver ions (Ag+).[20,21] These include changes to the bacterial cell wall and cell membrane, interactions with DNA, binding or inhibition of membrane proteins and enzymes, and elevated reactive oxygen species (ROS) levels.[21,22] The ability of AgNPs to penetrate

the bacterial cell wall, binding to the cell membrane, changing its permeability and structure, while also modifying transport activity. Furthermore, AgNPs produce silver ions (Ag+), which further damage bacterial cell membranes, accumulating inside the cell, and disrupta number of essential processes.[20,22]

CONCLUSION

We used an ethanol extract of the leaves of the *Crataeva nurvala* plant to prepare a one-step biosynthesis for AgNPs. The process of biosynthesis is quick, easy, repeatable, and safe for the environment. AgNPs and ethanol extract exhibited antibacterial and antifungal activities. These biosynthesized AGNPs can be used in a variety of pharmaceutical products, including coatings, tablets, creams, ointments, gels, and more.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-9292/2025]