Original Article

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Microbiology Section

In-vitro Antimicrobial Activity of Green Synthesised Silver Nanoparticles of Leaf Extract of *Rhinacanthus nasutus* against Bacterial Food Borne Pathogens

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ABSTRACT

Introduction: Green synthesis of nanoparticles plays a major role in the control of virulent pathogens due to their ecofriendly, depreciated cost and naive nature. This peculiarity of plant based nanoparticle synthesis accomplishes them as fleeting development in nanobiotechnology. *Rhinacanthus nasutus* is one such plant, which is generally known as snake jasmine has prodigious medicinal properties to explore.

Aim: The present intent of this study was to harmonise silver nanoparticles (AgNPs) from leaves extract of *Rhinacanthus nasutus* (Snake jasmine) and interrogate its vitality against virulent bacteria secluded from food.

Materials and Methods: The in-vitro study was conducted in Department of Microbiology at Muthayammal College of Arts and Science, Namakkal, Tamil Nadu, India, during November 2018 to November 2020. Nanoparticles were harmonised using 2 mM silver nitrate with leaves extract of *Rhinacanthus nasutus* which was then characterised by ultraviolet-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX) analysis and Transmission Electron Microscopy (TEM) imaging and Energy Dispersive X-ray (EDX) analysis. Followed with this the antibacterial activity of silver nanoparticles was evaluated by agar well diffusion method against virulent bacteria isolated from food samples. Furthermore AgNPs was subjected to antibiofilm activity. Origin tool and Microsoft word 2010 were used for statistical analysis.

Results: In this present study, 13 food samples were used from which 56 different isolates of various pathogens were isolated. The green synthesis of silver nanoparticles possessed antibacterial activity against antibiotic resistant, biofilm and beta-lactamase producing *Escherichia coli, Enterococcus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp, *Staphylococcus* spp, *Salmonella* spp and *Shigella* spp. Together with this the amalgamated AgNPs also exhibited antibiofilm activity in all the isolates. The better results of antibiofilm activity was observed against *Salmonella* species (74.1%).

Conclusion: This study divulges the presence of substantial antibacterial activity of green synthesis of silver nanoparticles of *Rhinacanthus nasutus* leaves extract against virulent bacterial species. Hence, it can be explored and exploited for the formulation of new antimicrobial against biofilm and betalactamase producing bacterial isolates due to its modest and ecofriendly nature.

Keywords: Antibacterial, Biofilm, Fourier transform infrared spectroscopy, Nanotechnology, Scanning electron microscopy, Transmission electron microscopy

INTRODUCTION

One of the extensively used technologies in modern research is the Nanotechnology. The augmentation of nanoparticles using metals confining biological materials through an ecofriendly modus has fascinated momentous thought. Nanotechnology accords with the molecules size spectrum from 1-100 nm, their amalgamation, and their handling. This science domain uniformly intermingle all the environs of essential science together with biological sciences, chemistry, physics, computational sciences and engineering material sciences for the establishment of nanostructures [1]. Discrete methods like biological, chemical and physical methods can be implicated for the amalgamation of nanoparticles.

Nanomaterials have been explored as propitious mechanism for the improvement of diagnostic biosensors, gene and drug delivery and biomedical imaging owing to its nanoscale effects and expanded surface area. Nanotechnology has been proclaimed as the novel industrial revolution, both developing and developed countries are venturing in this technology to reap the market share. The safety and quality of the food can be probably enhanced by the nanotechnology. The detection of pathogens in food systems are improved by the intelligence of nanosensors is been reviewed in few other studies [2,3]. The cell membrane of the pathogenic bacteria can be penetrated by nanoparticles there by interfering with their molecular pathways and formulating exclusive antimicrobial mechanisms. The global crisis or the resistance against the antimicrobials can be limited by the synergy development of nanoparticles and the optimal antibiotics [4]. The drug resistance mechanisms in bacteria is deceived by the nanoparticles that may be metallic/carbon/organic nanotubes and also associated with their antimicrobial potential, biofilm formation inhibition or any other important processes [5].

Antibiotic resistant bacteria are evolved by the excessive improper use of antibiotics which finally leads to Multidrug Resistant (MDR) bacteria. Resistance is achieved by a process either by reducing or eliminating the drug effectiveness by the bacteria that undergo such changes in their gene. Such bacteria remain and multiply to cause more severe infection. This resistance in a bacterium is achieved by many mechanisms like mutations, Deoxyribonucleic Acid (DNA) exchange mechanisms and so on [6]. Such resistant bacteria cause two fold higher infection risks that may either be clinical or economical finally resulting in delay or even failure in treatment. It is seen highly as severe disease, virulent strain and difficulty in treatment procedures. It may also major cause of high morbidity and mortality in patients [7].

Novel approach that indulges the united use of nanoparticles and plant based antimicrobials to swamp the toxicity concern are also being researched. Blending nature based antimicrobials along with nanoparticles for the inhibition of the activity of efflux pumps

in bacteria, quorum sensing intervention and conceivably plasmid curing are few approaches to fight against MDR bacteria. Plants use leaves as their food factories, this is the incentive step for scientists to exploit leaves as nanofactories for manufacturing silver nanoparticles. In spite of easy and uncomplicated design of the leaf extract they have been benefited for the silver nanoparticle production [8].

The ability of antimicrobial activity against fungal and bacterial plant pathogens of the gold and silver nanoparticles produced from a variety of plant extracts were reported in previous research [9]. Another interpretation disclose that nanoparticles can infiltrate the bacterial cell membrane and adhere to Nicotinamide Adenine Dinucleotide dehydrogenases (NADH), proliferating a huge amount of Reactive Oxygen Species (ROS), which cause the reduction of Adenosine Triphosphate (ATP) and hinders the respiratory chain. These radicals have the capacity to interact with proteins, sulfur, phosphorus-containing cell constituents, and DNA, destroying those [10].

In another research, it is stated that the ethyl acetate and methanol extracts of *Rhinacanthus nasutus* leaves were more effective than the standard antibiotics ciprofloxacin and ampicillin discs against *Staphylococcus aureus* and *Klebsiella pneumonia*. The silver nanoparticles produced using *Rhinacanthus nasutus* (Snake jasmine) leaf extract showed the potential antimicrobial activity against both bacteria (*Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli*) and fungi (*Aspergillus niger, Aspergillus flavus*) [11].

The silver nanoparticles produced using the methanolic leaf extracts of *Rhinacanthus nasutus* (Snake jasmine) possessed remarkable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. These silver nanoparticles (AgNps) were highly toxic against different pathogenic bacteria and also to some fungal species there by exhibiting high antibacterial activity and moderate antifungal activity. These nanoparticles are environmentally safe that showed significant medicinal activities hence can be considered or used in medicinal aspects [12]. In this current study the antibacterial activity of silver nanoparticles of leaf extracts from *Rhinacanthus nasutus* were studied against beta-lactamases and biofilm producing bacteria and furthermore antibiofilm activity was also carried out.

MATERIALS AND METHODS

The present in-vitro study was conducted in Department of Microbiology at Muthayammal College of Arts and Science, Namakkal, Tamil Nadu, India, during November 2018 to November 2020. No use of animal models for this work was done, so no ethical clearance certificate was required.

Isolation of Bacteria

Different kinds of fruit juices (apple, orange, pomegranate and papaya) and chicken samples were collected from local shops in Namakkal district. The bacteria were isolated and identified using HiCrome agar [13]. The identified bacteria belong to 8 different genera, which were then subjected to antimicrobial study, biofilm production and betalactamase production [14-16]. From these results, highly potential bacteria from each genera were selected for further study.

Plant collection and extract preparation: In the current study, leaves of *Rhinacanthus nasutus* have been exploited which is possessed from the Salem area, Tamil Nadu, India. The cleaned and air dried leaves were then subjected to grinding for powder formation. A soxhlet extractor was used for the extract preparation using 200 mL of ethanol and acetone as the solvents. The extracts were prepared until a colourless extract was seized from the top of the extractor. Each extract were separately concentrated under reduced pressure. Upon complete evaporation the dry extracts were weighed and used for further studies. Extracts were maintained at a temperature between 2-8°C for further studies [17].

Phytochemical studies: Various phytochemical components i.e, carbohydrates, alkaloids, phenols, quinols, saponins and proteins from the leaves extract of *Rhinacanthus nasutus* was tested as per the procedure given by Solomon CU et al., procedure [18].

Silver Nanoparticles Preparation

Nanoparticle synthesis with silver nitrate was prepared using the ethanol extract based on the qualitative phytochemical and antibacterial activity to study the qualitative phytochemical and antibacterial activity. Silver nitrate ($AgNO_3$) solution of 2 mM concentration was used, to 5 mL of this solution 0.5 mL of extract was added and observed for the change of dark brown to light brown colour because of the silver ion formation and AgNPs [19]. These synthesised nanoparticles were then used for the antibacterial study by agar well diffusion method.

Analysis

Ultraviolet-visible spectrophotometric analysis: The silver nanoparticles synthesised can be better understood by ultraviolet-visible spectrophotometric analysis as it helps to determine the molecular size of the particles. A spectrum scan from 300-800 nm was accomplished out using ultraviolet-visible spectrophotometer to persuade the absorption maxima of synthesised silver nanoparticles [20]. The reduction of silver ions and formation of silver nanoparticles developed within an hour of reaction. Control was maintained by using $AgNO_3$.

Fourier Transforms Infrared Spectroscopy (FTIR): A broad range of materials from liquids, pastes, solids, powders, fibres or even from other forms can be investigated either qualitatively or quantitatively by FTIR with a significant standard [21]. The synthesised silver nanoparticles solution was centrifuged for 30 minutes at 10000 round per minutes (rpm) to perform FTIR measurements. The free proteins or enzymes were get rid by washing the pellet thrice with 5 mL of deionised water later it is dried in a vacuum drier and then analysed by FTIR.

Scanning electron microscopy (SEM) with Energy dispersive X-ray (EDX) analysis: An electronic beam generates X-rays in this EDX analysis that helps to analyse the quality and quantity of a coating and the underlying surface which can be combined with electron microscopes provides elemental analysis of small areas of even in nanometers [22]. The green synthesised AgNPs with ethanol extract of *Rhinacanthus nasutus* morphology was investigated using SEM coupled with EDX. The AgNPs solution was centrifuged at 10,000 rpm for 20 minutes and drop coated on to thin glass film fabricating and allowing water to completely evaporate and analysed using Zeiss EVO 18 at a voltage of 20kV. The elemental identification and quantitative compositional information were obtained using EDX.

Antibacterial Activity of *Rhinacanthus nasutus*

Followed by drying of the extract obtained from soxhlet apparatus using vaccum pump, it was then dissolved using ethanol. The antibacterial activity of *Rhinacanthus nasutus* leaf extract was studied by agar well diffusion method against the isolated 8 bacterial genera [23]. In a sterile Muller-Hinton agar plates were taken in which the 24 hours old nutrient broth cultures of test bacteria were swabbed and to which 8 mm well were made using sterile cork borer. Different concentrations of the leaf extract were filled in the wells and were labeled properly along with this reference antibiotic (chloramphenicol, 1 mg/mL of sterile distilled water) and ethanol was also used as control. All the plates were incubated in upright position or 24 hours at 37°C upon incubation the zones were measured. Same procedure was utilised for the synthesised nanoparticles.

Antibiofilm activity of AgNPs: A 96-well µL plate (flat bottom, polystyrene) was used to determine the anti biofilm activity of the AgNPs as described by Mohanta YK et al., [24]. The percentage of inhibition of biofilm formation was calculated using following equation:

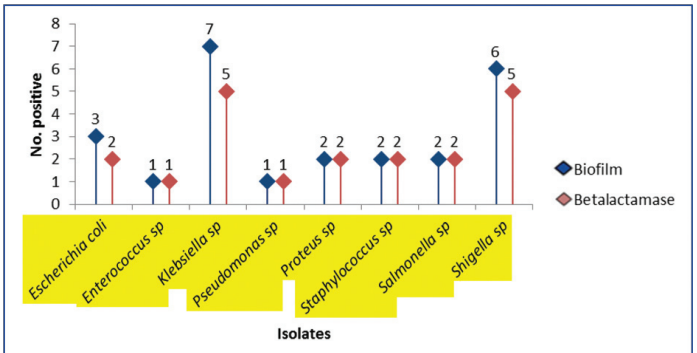
Percentage biofilm inhibition=[1-(OD620 of cells treated with Ag NPs or plant extracts/OD620 of the non treated control)×100].

STATISTICAL ANALYSIS

Origin tool and Microsoft word 2010 were used for statistical analysis.

RESULTS

In this present study, 13 food samples were used from which 56 different isolates of various pathogens (*E. coli*-9, *Enterococcus* spp-9, *Klebsiella* spp-7, *Pseudomonas* spp-4, *Proteus* spp-2, *Staphylococcus* spp-9, *Shigella* spp-12 and *Salmonella* spp-4) were isolated. Out of 56 isolates tested for antibiotic resistance with multiple antibiotics, 32 isolates exhibited resistance above 50% to the tested antibiotics. Out of 56 isolates, 35 isolates were from fruit juice sample and 21 isolates from chicken samples were isolated. Based on the results, it is evident that isolate from fruit juice exhibited more resistance (47.72%) than the isolates from chicken samples (45.89%). Out of this 75% were exhibiting biofilm production property [Table/Fig-1].



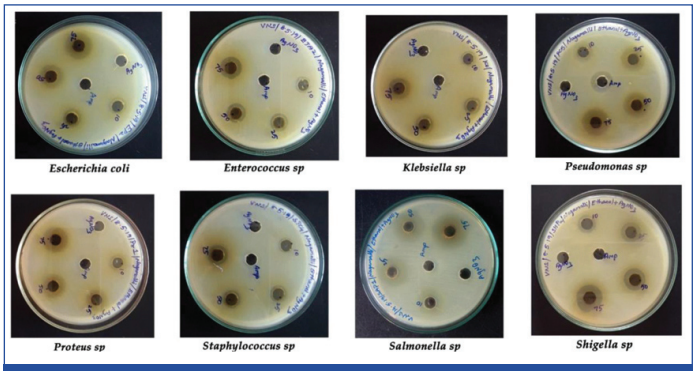
[Table/Fig-1]: Virulence characteristics of the isolates.

By using the soxhlet extraction method, the highest yield was obtained from ethanol extract of 25.4% while the least yield was of acetone (14.5%). Both solvent extracts were subjected to preliminary phytochemical analysis. Alkaloids, flavanoids, phenols, saponins terpenoids and quinols were observed in ethanol extract. In case of acetone extract, following phytochemicals were obtained namely alkaloids, flavanoids, phenols, saponins and terpenoids. The tannins, carbohydrates, quinones and protein were not observed from both solvent extracts. According to phytochemicals results, highest phytochemicals containing ethanol solvent extract was subjected to further analysis [Table/Fig-2].

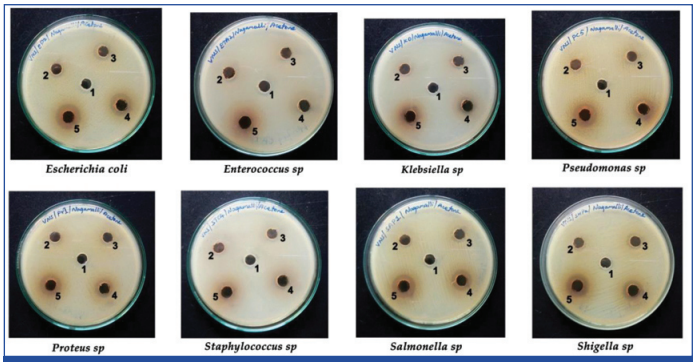
Phytochemicals	Test name	Observation	Ethanol	Acetone
Alkaloids	Wagner's	Reddish brown colour	Positive	Positive
Carbohydrates	Molisch's	Purple ring at the junction	Negative	Negative
Flavonoids	With sodium hydroxide	Yellow colour	Positive	Positive
Phenols	Ferric chloride	Deep chloride	Positive	Positive
Sapiens	Foam test	Frothing	Positive	Positive
Sterols	Braymer's test	Greenish colour	Negative	Negative
Tannins	With hydrochloric acid	Yellow precipitate	Negative	Negative
Terpenoids	Salkowski test	Bluish red	Positive	Positive
Quinoas	Salkowski test	Reddish brown	Positive	Negative
Protein	Million's test	Red colour	Negative	Negative

[Table/Fig-2]: Preliminary phytochemicals screening on leaves extract of *Rhinacanthus nasutus*.

Presently, both extracts have been subjected for the determination of antibacterial activity against biofilm and betalactamase producing isolates. When ethanol extract was used, *Salmonella* spp was highly suppressed, revealing a barrier zone of 10-14 mm. The second most suppressed isolate was *P. aeruginosa*. The 75% of the isolates were suppressed while using 5 mg concentration of extract and 7.5 mg of concentration of extract suppressed all the isolates. While using the control agents of standard antibiotic and ethanol, it did not exhibit any inhibitory activity. The present study revealed that the antibacterial efficacies of ethanol solvent extract of plant was diverse. In this study, when acetone extract was used, the inhibitory zone was obtained from 11 mm to 20 mm; highest inhibitory activity was obtained against to *E. coli, Shigella* spp and *Salmonella* spp. Among the eight isolates, *E. faecalis* and *S. aurues* were not inhibited by the acetone extracts [Table/Fig-3,4]. When compared to ethanol extract, acetone solvent extract have been slightly inhibitory activity. Hence, ethanol extract was subjected to silver nanoparticle synthesis.



[Table/Fig-3]: Antibacterial activity of ethanol extracts of *Rhinacanthus nasutus*.
1-Ethanol, 2-2.5 mg (RNEE), 3-5 mg (RNEE), 4-7.5 mg (RNEE), 5-10 mg (RNEE)
RNEE: *Rhinacanthus nasutus* ethanol extract



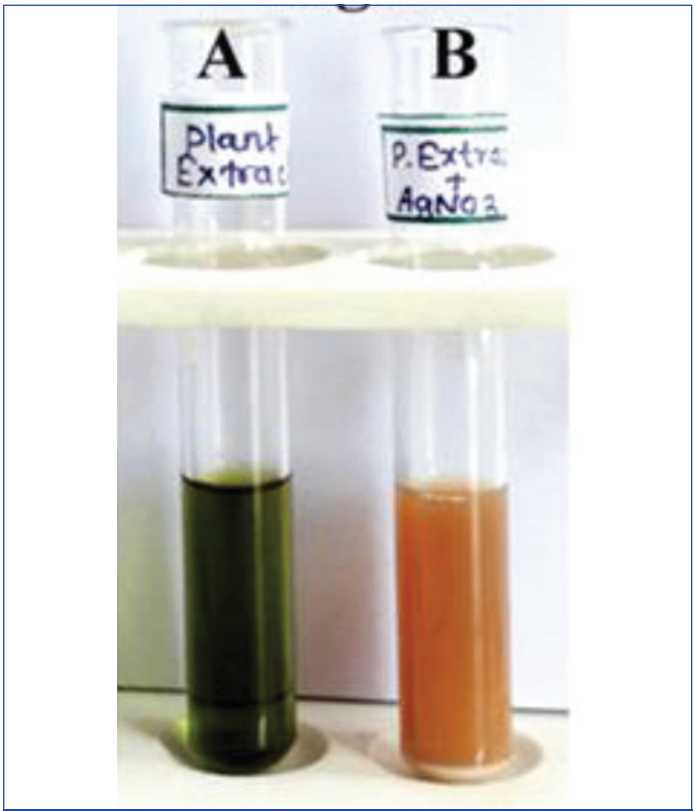
[Table/Fig-4]: Antibacterial activity of acetone extracts of *Rhinacanthus nasutus*.
1-Acetone, 2-2.5 mg (RNAE), 3-5 mg (RNAE), 4-7.5 mg (RNAE), 5-10 mg (RNAE)
RNAE: *Rhinacanthus nasutus* (Snake jasmine) acetone extract

The green synthesis of silver nanoparticles was successfully carried out using *Rhinacanthus nasutus* leaf extract (ethanol solvent extract) because the colour of the solution changes from yellowish brown to dark brown, revealing a reduction of silver nitrate in the aqueous solution due to stimulation of surface plasmon vibrations in silver nanoparticles [Table/Fig-5]. The ultraviolet-visible absorption spectrum of the synthesised nanoparticles showed a broad peak at 426 nm which is a characteristic band for Ag and this peak indicated that the particles are polydispersed.

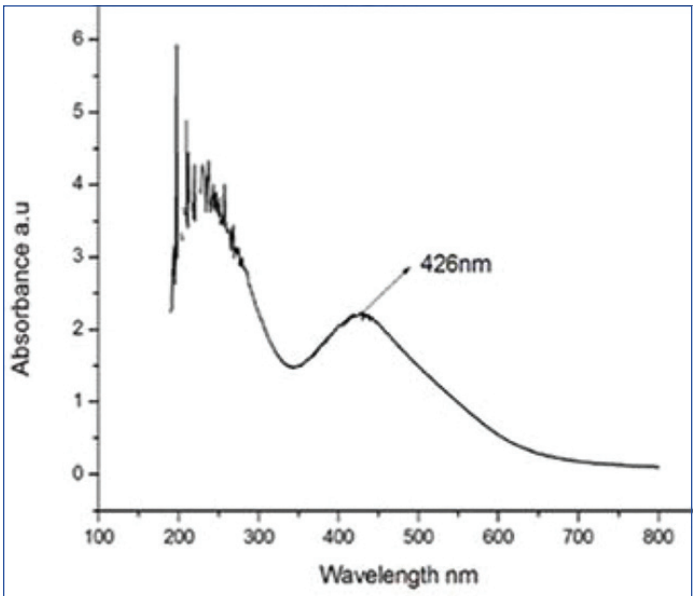
The FTIR spectra of *R. nasutus* leaf extract was shown in [Table/Fig-6]. The samples were analysed with plain KBr pellets as blank. The spectral data were compared with a reference to identify the functional groups existing in the sample. The FTIR spectrum of crude plant extract and green synthesised silver nanoparticle incorporated crude extract was compared with reference FTIR spectrum chart to assign a functional group with respect to the obtained peaks [Table/Fig-7,8].

The biosynthesised silver nanoparticles were characterised by SEM for their morphology and size. The SEM micrograph showed that the synthesised silver nanoparticles have spherical morphology. The sizes of the nanoparticles were ranged from 18.2-66.1 nm. The EDX result shows the presence of silver ions as the ingredient element

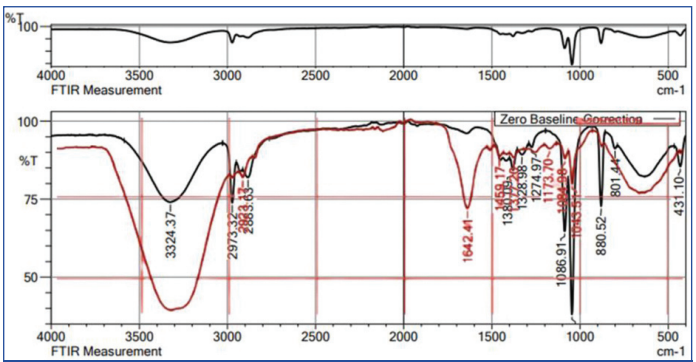
[Table/Fig-9]. The metallic AgNps generally show a typically strong peak at 3 keV, due to surface plasmon resonance.



[Table/Fig-5]: A): leaves extract of *Rhinacanthus nasutus*; B): Change in the colour f the solution indicates as AgNPs synthesis.



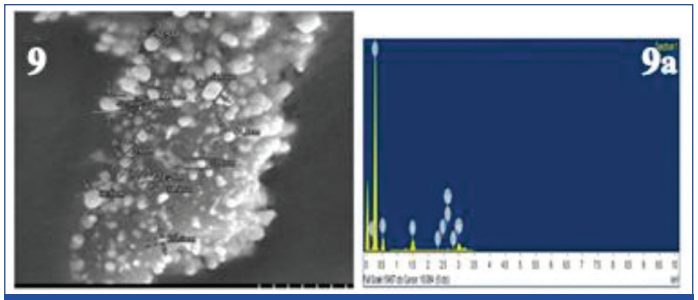
[Table/Fig-6]: Ultraviolet-visible spectra of synthesised AgNPs of *Rhinacanthus nasutus*.



[Table/Fig-7]: Black spectrum: Crude plant extract; Red spectrum: Green synthesised silver nanoparticles with crude plant extract.

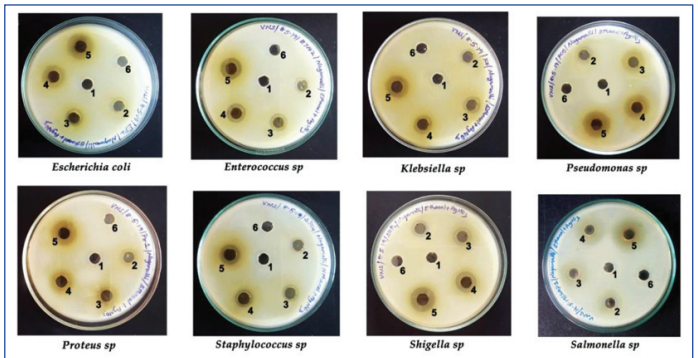
Crude extract FTIR peaks	Functional groups	AgNPs-crude extract FTIR peaks	Functional groups
431.10	Benzene erivative	1043.51	Anhydride,CO-O-CO stretching
801.44	Alkane, C=C bending	1084.98	Primary alcohol, C-O stretching
880.52	Alkane, C=C bending, vinylidene	1173.70	Ester, C-O stretching
1045.44	Anhydride, CO-O-CO stretching	1459.17	Methyl group, C-H bending
1086.91	Fluoro ompound, C-F stretching	1642.41	Alkene, C=C stretching
1274.97	aromatic amine, C-N stretching, strong	2923.17	Amine salt, N-H stretching
1328.98	aromatic amine, C-N stretching, strong	-	-
1380.09	Alkane, C-H bending	-	-
2883.63	Alkane, C-H stretching	-	-
2973.32	Amine salt, N-H stretching	-	-
3324.37	Secondary amine, N-H stretching	-	-

[Table/Fig-8]: The FTIR peaks of crude plant extract and green synthesised silver nanoparticles with crude plant extract.



[Table/Fig-9]: SEM images of silver nanoparticles synthesised by AgNPs of *Rhinacanthus nasutus*. (a) EDAX spectrum of synthesised AgNPs using by *Rhinacanthus nasutus*.

In the present investigation, the antibacterial effect of prepared silver nanoparticles was studied on different types of bacteria such as *E. coli, K. pneumoniae, P. aeruginosa, Salmonella* spp, *Proteus* spp and *Shigella* spp (Gram negative) *E. faecalis, S. aureus* (Gram negative). While using the ethanol extracts of AgNPs, zone of inhibition was ranged from 10-24 mm, among the various bacteria, *Salmonella* spp was highly suppressed and lowest activity was against *S. aureus* [Table/Fig-10].



[Table/Fig-10]: Antibacterial activity of nanoparticles of ethanol solvent of *Rhinacanthus nasutus.*
1-Ampicillin (10 mg), 2-2.5 mg (AgNPs of RNEE), 3-5 mg (AgNPs of RNEE), 4-7.5 mg (AgNPs of RNEE), 5-10 mg (AgNPs of RNEE), 6-Control (AgNO3)
AgNPs-Silver nanoparticles; RNEE-*Rhinacanthus nasutus* Ethanol Extract

As one of the main findings of this study, AgNPs retard the formation of biofilms of bacterial isolates, which is due to the inhibitory effect of AgNPs on the flagella. In the present study, the antibiofilm activity of AgNPs was evaluated in-vitro against various bacterial isolates; *Salmonella* spp was treated for 24 hours with AgNPs (5 mg/mL)

synthesised from *Rhinacanthus nasutus* extract and biofilm formation was reduced by 74.1%, same time while using non AgNPs, 44.1% of biofilm formation was reduced.

DISCUSSION

Microbial resistance to antimicrobial agents has become a serious concern worldwide, leading to an increase in mortality in most cases. A research conducted in India implies that there is an upsurge in the resistance and antibiotic use in tertiary hospitals. Therefore, finding new alternatives to the currently used antibiotics has become a necessity. The present study result for isolation of bacteria is correlated with Nagarajan V et al., who observed the presence of various bacterial genera in different food samples [25]. Similarly, Babiye B, in his study stated that fresh juices harbour many kinds of enteropathogenic bacteria especially *Shigella, Salmonella, Staphylococcus aureus* were predominantly seen [26]. In a recent study done by Kowalska J et al., they have stated that all the 40 isolates collected from the food samples have formed biofilms [27]. Among the 75% of biofilm positive isolates, 83% of isolates were beta-lactamases producing. Sivakumar M et al., revealed the presence of 60.62% beta-lactamase producing bacteria from raw food samples [28].

The results of previous study made on phytochemical components indicate that the ethanolic extract of *R. nasutus* contains secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, and triterpenes. Likewise the study of Nanthakumar et al., reported that highest inhibitory activity of aqueous extract of *Rhinacanthus nasutus* than ethanol extract [29]. In 2017, Antonysamy J, reported that various solvent extracts of *Rhinacanthus nasutus* were active against gram positive and gram negative isolates, which is probably attributed to the presence of secondary metabolites [30]. In line with previous observations, the present study's results of phytochemical properties are corroborated with these results also.

The spectral data were compared with a reference to identify the functional groups existing in the sample [31]. The metallic AgNps generally show a typically strong peak at 3 keV, due to surface plasmon resonance [32]. Similarly, Pasupuleti VR et al., and Giridharan T et al., also characterised the AgNPs of *Rhinacanthus nasutus* with UV spectra, FTIR, TEM, XRD and DLS analysis [11,12]. Nanoparticles have significantly better performance compared to plant extracts; 5 mg of plant extract does not inhibit bacteria, at the same time killing all bacteria when the sharp sword of nanoparticles rotates [33].

This study was consistent with previous studies, which suggested that the number of phytochemicals containing *Rhinacanthus nasutus* showed strong antibacterial activity against *E. coli*, *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Klebsiella pneumonia* [11,26]. Several mechanisms have been reported to explain the lethal effect of AgNPs. This might involve the release of Ag^+ ions from AgNPs that may attach to the positive charged cell wall, thus leading to the deformation of the proteins and resulting in cell death. On the contrary, some studies suggest that the growth inhibition around the well is due to the release of diffusible inhibitory Reactive Oxygen Species (ROS) from AgNPs. The free radicals from metals may damage the bacterial membranes, mitochondria and DNA, which can subsequently cause bursting and death of the cell. Presently MIC was carryout with ethanol extract of AgNPs. The lowest MIC of 0.5 mg was for *Salmonella* spp and second most 0.6 mg was for *P. aeruginosa*.

In order to create biofilms, bacteria must first attach to a surface. Many factors may affect bacterial adherence, including the growing environment and the surface properties of the material. Giridharan T et al., explored the role of AgNPs in bacterial adhesion [12]. AgNPs have been shown in several studies to reduce bacterial swarming capability and biofilm formation, which decreases pathogenicity. Plant derived AgNPs have an advantage over non derived AgNPs due to plant metabolite's ability to act as capping and stabilising agents, improving AgNPs' antibacterial activity.

Nanoparticles can possess antimicrobial activity depending on their size. Smaller particles are more likely to interact with cells and attach to cytoplasm since less space barriers are present. Additionally, small nanoparticles provide a large area to interact with microorganisms or biological components, making them highly effective. The small size (18.2-66.1 nm) of AgNPs in this study was another contributing factor to its antibacterial and antibiofilm effect. Similarly, Singh P et al., used 15 nm size AgNPs to inhibit the biofilm formation. It is also that reported the antibiofilm potential of AgNPs against human pathogens [34].

In view of the results, it can be concluded that *Rhinacanthus nasutus* ethanolic leaf extracts of nanoparticles have potential as antimicrobial components against biofilm and betalactamase producing isolates and could be used to treat infectious diseases caused by resistant microbes. Further research is needed to isolate the pure compounds from the leaf extract studied for testing specific activity.

Limitation(s)

In the augmentation of AgNps either by physical or by chemical methods, it comprises of valuable procedure and sometimes the use of chemicals that may cause some ill-effects. Hence, to overcome this, there is a need of clinical trials in animal models and cell cultures which are required to test their antitoxic nature which will be achieved in the future studies.

CONCLUSION(S)

According to these researchers, nanoparticles with a particle size less than 100 nm have excessive antibiofilm activity. Based on the current findings AgNPs were found to have an effect on biofilm formation. In future these findings can be used in clinical trials in animal models for their efficiency in control of pathogens.

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