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Research Article

ANTIBACTERIAL ACTIVITY OF SILVER-EXTRACT NANOPARTICLES SYNTHESIZED FROM THE COMBINATION OF SILVER NANOPARTICLES AND *M. CHARANTIA* FRUIT EXTRACT

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ABSTRACT

Silver Nanoparticles (AgNPs) constitute a very promising approach for the development of new antibacterial systems. Nanoparticulate objects can bring significant improvements in the antibacterial activity of this element, through specific effect such as an adsorption at bacterial surfaces. In this study, polyaniline coated AgNPs-extract nanoparticles were investigated for their antibacterial activity. Silver nanoparticles were prepared by the reduction of silver nitrate and NaBH₄ was used as reducing agent. Silver nanoparticles and extracts were mixed thoroughly and then coated by polyaniline. Prepared nanoparticles were characterized by Visual inspection, Ultraviolet-visible spectroscopy (UV), Fourier transform infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) techniques. Antibacterial activities of the synthesized silver nanoparticles were tested against *Antibacterial* activities of the synthesized Ag-Extract nanoparticles were tested against *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. UV-Vis spectrum of reaction mixture showed strong absorption peak with centering at 400 nm. The FT-IR results imply that Ag-NPs were successfully synthesized and capped with bio-compounds present in *Momordica charantia*. SEM results revealed that the particle size of these combined nanoparticles was 78.5 to 100 nm in most cases. In most of the cases Ag-Extract NPs showed better antibacterial activity in compared to our control ciprofloxacin. The result revealed that Ag-extract NPs showed 28±0.68, 8±0.30, 35±0.48, 27±0.35mm zone of inhibition against for *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa* respectively, whereas ciprofloxacin (positive control) showed 23±0.40, 21.40±0.40, 26.18±0.40, 25.32±0.40 mm zone of inhibition for these selected strains. Further studies should undertake to elucidate the exact mechanism of action by which AgNPs-extracts exert their antimicrobial effect which can be used in drug development program for safe health care services.

Keywords: Silver Nanoparticles, *Momordica charantia*, Antibacterial activity, Scan Electronic Microscopy (SEM), Zone of inhibition.

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INTRODUCTION

Due to unique properties of nanoparticles, the appliance of nanotechnology is being significant day by day. Various types of nanoparticles are produced by nanotechnology. Thus, they are gaining more and more attention to researches¹.

The contribution of nanoparticles to modern medicine is tremendous. Indeed there are some instances where nanoparticles are used to analyze and in therapies that simply cannot be performed otherwise². Nanoparticles have a relatively large surface which has the ability to bind, adsorb and carry other compounds such as drugs, probes and proteins³. Nanoparticles of silver which have the size range between 1 nm and 100 nm are

known as silver nanoparticle⁴. A common application of silver nanoparticles in antimicrobial coatings is increasing now-a-days. Many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles that provide protection against bacteria by continuously releasing a low level of silver ions⁵.

Momordica charantia (bitter melon) is one of the most important species of the family Cucurbitaceae. Its fruit and other parts of the plant have medicinal value^{7, 8}. The fruit is consumed as part of the diet but they are also reported to possess a wide range of pharmacological activities; for example, hypoglycemic, antidiabetic, antifungal, ability to inhibit p-glycoproteins, antihyperlipidemic and antioxidant effects have all been reported. The fruit has been used traditionally as anthelmintic, antiemetic, carminative, and purgative, as well as for the treatment of anemia, jaundice, malaria, cholera etc⁹. The in vitro and in vivo trial has shown that 3 % aqueous extract of *M. charantia* whole fruit possessed anthelmintic activity against *Ascaridia galli*¹⁰. *M. charantia* contains a number of potential pharmacologically active constituents such as charantine, goyaglycoside, mormodicoside, mormodicoside 3 β , 25-dihydroxy-5 β , 19-epoxycucurbita-6, (23E)-diene, momordicine-I, karavilagenin, karavilagenin C, karaviloside, karaviloside, kuguacin, kuguacin A, kuguacin B, kuguacin E, 3,7,23-trihydroxy-cucurbita-5,24-diene-19-al, 3,7,25-trihydroxy-cucurbita-5, 23-diene-19-al,3,7-dihydroxy-25-methoxycucurbita-5, 23-diene-19-al⁹.

The aim of this study was to synthesize *M. charantia* loaded AgNPs using AgNO₃ as a precursor and NaBH₄ as reducing agent and characterization of AgNPs. Moreover, we also evaluated the antibacterial activity of AgNPs and AgNPs-extract nanoparticles against some Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Materials

Silver nitrate (MERCK, Germany) used as a silver precursor and sodium borohydride (LOBA CHEMIE) used as reducing agent. All other reagents used in this study were analytical grades and collected from the laboratory of the Dept. of Pharmacy and Dept. of Microbiology, Noakhali Science and Technology University.

Microorganisms

To evaluate antibacterial activity, four different ATCC bacterial cultures were used. The ATCC cultures were collected from the department of microbiology, Dhaka Shishu (Children) Hospital and from the department of microbiology, University of Dhaka. The cultures were *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028.

Collection and selection of the plant material

The plant *Momordica charantia* was collected from Noakhali, Bangladesh. The fruits of the plant were collected followed by thorough washing with distilled

water several times. During collection any type of adulteration was strictly prohibited.

Preparation of plant extract

200 gm. fresh bitter melon at first rinsed with distilled water to remove dust. They were cut down into small pieces then boiled at 100 °C in 1000 ml of double distilled water for about 45 minutes, delayed until the preparation was cold. Then the aqueous extract obtained was by filtering through Whatman filter paper.

Preparation of silver nanoparticles

Ag nanoparticles were prepared according to Creighton, 1979 with slight modification¹¹. At first, 100mL aqueous solution of 1.0 X 10⁻³ M silver nitrate was mixed thoroughly with a 300-mL aqueous solution of 2.0 X 10⁻³ M sodium borohydride. Triply distilled water was used for solution making and both solutions were allowed to chill (0 °C) before mixing. After mixing, Ag ions were reduced and formed nanoparticles in aqueous medium. Nanoparticles (Ag) containing solution was transparent and turned yellow color during mixing. The stirring was continuing for an hour until the color of the solution become yellow, which indicates the stabilization of Ag ion. At this point, Ag nanoparticles were stabilizing enough for long run without any stabilizing agent. As particle concentration of the solution was very low, it was concentrated 10 times using a rotary vacuum evaporator. Synthesis of AgNPs-Extracts nanoparticles Silver nanoparticles were mixed by magnetic stirrer for half an hour with *M. charantia* aqueous extracts which was prepared previously. These uncoated combined nanoparticles needed to coat.

Coating of Ag-extract nanoparticles by polyaniline

Coating on the AgNPs-extract was done according to the method of K. Gopalakrishnan et al., 2012 with slight modification¹. At first, 2.7 g aniline which was dissolved previously at 300 ml deionized water and 68ml of 6 % H₂O₂ were added slowly with the uncoated Ag-Extract NPs solution within 30 minutes at room temperature. This addition was continuing for 23 hours as the coating substances finely coat the nanoparticles. Produced coated nanoparticles were filtered and dried at room temperature for concentrating and further analysis.

Characterization studies

Silver nanoparticles were characterized by visual inspection, UV spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopic (SEM) analysis.

Antimicrobial assay

In-vitro antibacterial activity of the samples was determined by deploying the disc diffusion method using Mueller–Hinton Agar (MHA) with determination of inhibition zones in millimeter (mm), which conform recommended standards of the National Committee for Clinical Laboratory Standards. In order to do this, firstly we made Mueller–Hinton Agar plates aseptically. After that, we prepared bacterial lawn on MHA plates using the reference bacterial species, which were growing fresh culture in Luria Bertani broth. Next, previously

prepared paper discs of each samples including aqueous extract of *Areca catechu*, silver nanoparticle, coated silver nanoparticle were placed on each bacterial lawn on MHA plates. The paper discs were prepared by impregnating the sterile paper discs of 4 mm in diameter into the three different test samples each of which was at a concentration of 20 mg/ml. The impregnated paper discs were dried out at 37°C for 24 h in a sterile condition. Standard antibiotic discs (Ciprofloxacin) were used as positive respectively. Finally, the agar plates were incubated at 37°C for 24 hours and the zone of inhibition were observed.

RESULTS

Assessment of AgNPs through visual inspection

The formations of silver nanoparticles were confirmed visually. Within a short period of the reaction, mixer color turned into dark brown from brownish yellow color. This color changing indicated the synthesis of silver nanoparticles. (Figure 1).

Evaluation of Ag-NPs by UV spectrum

UV-Vis spectrum of reaction mixture at different wavelengths ranging from 300 to 700 nm showed strong absorption peak with centering at approx. 400 nm indicated the formation of Ag-NPs. (Figure: 2)

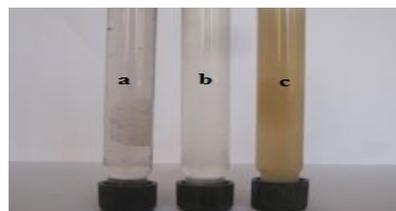


Figure 1. (a): Ag NPs (b): Extract (c): Coated Ag NPs-extract

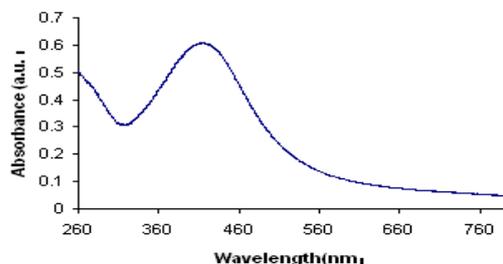


Figure 2: UV-Visible spectrum of AgNPs

Evaluation of Ag-NPs through FT-IR spectra

The FT-IR spectra were used to identify the possible biomolecules (including functional group) responsible for the reduction of the Ag^+ ions and capping of the *M. charantia* formed Ag-NPs. Figure 3, showed the FTIR spectra of *M. charantia* aqueous extract and synthesized Ag-NPs.

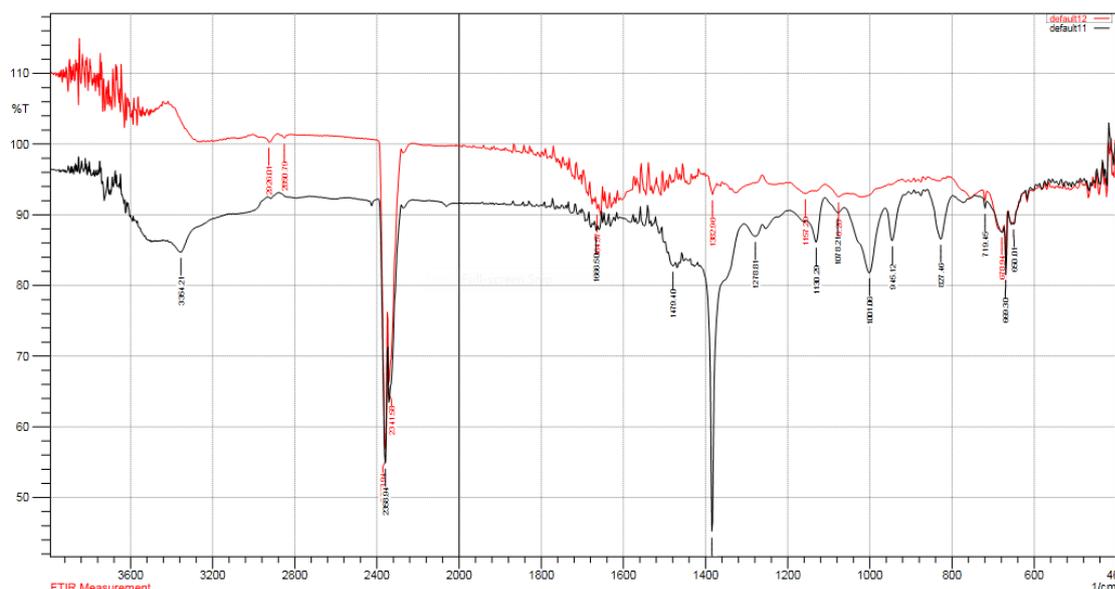


Figure 3: FT-IR spectrum for curve (a) red color- *M. charantia* aqueous extract; and curve (b) Ag-Extract nanoparticles

The possible functional groups of fruit extract involved in coating nanoparticle are identified by FTIR analysis. The intense absorption peaks at 3400.50 cm (curve-a, red color) and 3364.21 cm (curve-b, black color) correspond to N-H stretching of primary amine. The band observed at 2900 cm (curve-b) represents =C-H stretching. The weak band observed at 2926.01 cm and 2880.79 cm (both in curve-a) indicates the H-C-H asymmetric and symmetric stretching of alkanes. The band observed at 2341.58 cm (curve-a) and 2368.94 cm (curve-b), which are very nearer band, denotes the

presence of hydrogen bonded OH stretching of carboxylic acids in leaf extract which may be a reducing/coating agent for silver nanoparticles.

The band at 1650.58 cm (curve-a) and 1666.50 cm (curve-b) represents N-H bending vibration of primary amine. The peak occurs at 1479.40 cm (curve-b) for N-H bending vibration of sec. amine. C-N stretch occurs at 1400.02cm. The band observed at 1332.96 cm (curve-a) represent N=O stretching of nitro groups of leaf extract coated on nanoparticles. The arising of functional groups

in FTIR spectrum indicates proper coating of fruit extract on silver nanoparticles. The bands at 1157.33 cm (curve-a), which are nearly the same in curve-b, 1130.29 cm denotes C-H stretching vibration of ester. Beside, C-O stretch occurs at 100.08, 1072.47, 1026.17 cm (curve-b) where first is stronger and broader than the second. The band at 915.12, 843.89, 829.43, 602.78, 668.36 cm shows C-H bending vibration of alkynes. The band at 751.31 cm and 719.45 cm represents the ortho substituted and mono substituted aromatic stretching respectively.

The FT-IR results imply that the Ag-NPs were successfully synthesized and capped with bio-compounds present in the *M. charantia* extract by using a green method.

Evaluation of Ag-NPs by SEM

SEM analysis shows AgNPs synthesized. It was shown that irregular AgNPs were formed with diameter of 78.5 to 220 nm. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements (Figure 4).

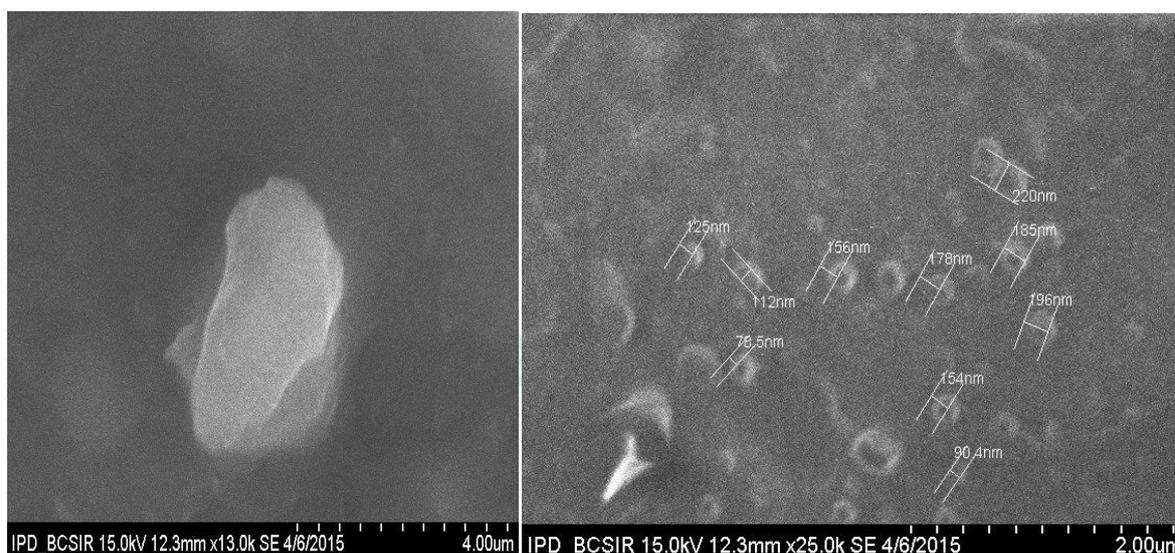


Figure 4: SEM image of AgNPs

In vitro antibacterial activity investigation

The aqueous extract and AgNPs-extract showed strong and moderate antibacterial activity against several test

organisms. The result of antibacterial activity, measured in term of diameter of zone of inhibition in mm is shown in Table.

Table: Antibacterial activity of *M. charantia* coated Ag-NPs

Items	Zone of inhibition (mm)			
	<i>S. aureus</i> ATCC 25923	<i>S. typhi</i> ATCC 14028	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
<i>M. charantia</i> fruit extract	18±0.54	8±0.22	30±0.45	22±0.48
AgNPs	16±0.36	7±0.28	21±0.57	19±0.34
AgNPs-extract (coated)	28±0.68	8±0.30	35±0.48	27±0.35
Ciprofloxacin (control)	23±0.40	21.40±0.40	26.18±0.40	25.32±0.40

The antibacterial activity of the aqueous extracts of *M. charantia* fruit were analyzed against both Gram-positive and Gram-negative bacteria. After analyzing the above results, it is clear that gram negative bacteria *E. coli* is more susceptible than experimental species of gram positive bacteria *S. aureus*. In most of the cases Ag-Extract NPs showed better antibacterial activity in compared to our control ciprofloxacin. The result revealed that Ag-extract NPs showed 28±0.68, 8±0.30, 35±0.48, 27±0.35mm zone of inhibition against for *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa* respectively,

whereas ciprofloxacin (positive control) showed 23±0.40, 21.40±0.40, 26.18±0.40, 25.32±0.40 mm zone of inhibition for these selected strains. From the above information it is clear that *S. typhi* is more resistant to AgNPs-extract than other gram negative bacteria used.

Similar result were obtained by Rashid et al., (2015) and Alam et al., (2017) showed resistance profile of *S. typhi*^{12, 13}.

DISCUSSION

There are various reports which have been providing the evidences that silver nanoparticles can be used as powerful tool against multidrug-resistant bacteria^{14, 15}. The mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. It is reported that the positive charge on the silver ion is the reason for antimicrobial activity as it can attract the negatively charged cell membrane of microorganisms through the electrostatic interaction¹⁶. Prema *et al.*, tested antimicrobial activity of nanoparticles against human bacterial pathogens (*Bacillus cereus*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* & *Vibrio vulnificus*). They found bacterial growth was highly inhibited by the nanoparticles¹⁷. Muthupandi Kasithevar and his team investigated antibacterial activity of the synthesized Ag-NPs against multi drug resistant (MDR) *P. aeruginosa*, *Staphylococcus aureus* and CoNS isolates from post-surgical wound infections. Ag-NPs synthesized from aqueous leaf extract of CRCP shows significant antibacterial potential against MDR isolates from post-surgical wound infections¹⁸. Sundeep *et al.*, described Green synthesis and characterization of Ag nanoparticles from *Mangifera indica* leaves for dental restoration and antibacterial applications. They found promising antibacterial activity of the synthesized nanoparticles¹⁹. In our experiment, when we compared the antibacterial activity of AgNPs and

coated AgNPs-extract, it was found that AgNPs-Extract have shown more antibacterial activity than either AgNPs or *M. charantia* aqueous fruit extract alone against these bacterial strains. The antibacterial efficacy of synthesized coated AgNPs enhances because the use of silver and *M. charantia* extract, as silver reduced in nano form which increases its surface area, thus make AgNPs more reactive and *M. charantia* extract enhances the therapeutic efficacy of AgNPs due to its good antibacterial efficacy. Our experiment value revealed that coated AgNPs-extract showed excellent antibacterial activity against all types of test bacteria (Figure 5) with highest zone of inhibition was 35 ± 0.48 mm, found against *E. coli*.

CONCLUSION

In our study we found that gram negative bacteria are more susceptible on AgNPs-extract rather than Gram positive bacteria. The synthesized combination may enhance the therapeutic efficacy and strengthen the medicinal values. Hence, our results are promising and prove to be an important step in this direction as it decreases the burden of multidrug resistance of patients. Further investigation is needed to establish the antibacterial efficacy of AgNPs-extracts (*M. charantia*) in drug development program for safe health care services.

CONFLICT OF INTEREST

The authors claim no conflict of interest for this research work.

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