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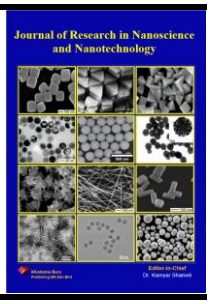

Biosynthesis and Characterization of Silver Nanoparticles from Bitter Melon (*Momordica charantia)* Fruit and Seed Extract and their Antimicrobial Activity

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ABSTRACT

Momordica charantia is a phenolic rich vegetable. In this study, the fruits and seeds extract of *M. charantia* were used to synthesize silver nanoparticles (Ag NPs) using biotechnological approach. Structural, morphological, and antimicrobial properties of the synthesized Ag-NPs were characterized using UV/Vis Spectrophotometry, Dynamic Light Scattering (DLS), High Resolution Transmission Electronics Microscopy (HRTEM), Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray diffraction (XRD). In DLS, the average particle size of Ag-NPs was found 17.5 ± 2.1 nm and 18.3 ± 1.9 nm using seed and fruit extract, respectively. HRTEM has revealed their spherical structure for both seed and fruit extract of *M. charantia*. FESEM images found Ag-NPs with the size between ~20 and ~35 nm. The Ag NPs exhibited Surface Plasmon Resonance (SPR) centered at 405 nm for seed extract and 402 nm for fruit extract using a UV–visible spectrophotometer. FT-IR results showed phenolic and carbohydrate compounds involved in the synthesis of the Ag NPs. Furthermore, the synthesized Ag NPs has found highly rich in antibacterial properties against *Escherichia coli* and *Pseudomonas aeruginosa* bacterium. Thus, bioconversion of Ag NPs by *M. charantia* could be employed as a potential antibacterial source to eliminate pathogenic microorganisms from agricultural and food preservation industry.

Keywords:

Green synthesis, Silver nanoparticles,
Antimicrobial, *Momordica charantia*,.

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1. Introduction

Nanotechnology is one of the upcoming fields of research in modern science. The word "Nano" is derived from the Greek word "dwarf "which means one billionth. A nanometer is one billionth of a meter (10−9) and it might be represented by the length of ten hydrogen atoms lined up in a row [1, 2]. In nanotechnology, various nanoparticles have been showing potential in many areas such as agricultural, medical, electronics, and environmental fields. Nanoparticle is the most unique discovery in nanotechnology. Nanoparticles with new or improved properties, such as size, distribution and morphology and their excellent physicochemical and biological properties have exhibited more effective in various application compared to bulk chemical composition, due to their high surface-to-volume ratio, penetration capability, and low side effects [3, 4].

Among other nanoparticles metallic nanoparticles have different physical and chemical properties from bulk metals such as size, orientation, lower melting points, higher specific surface areas, specific optical properties, mechanical strengths, and specific magnetizations. All these properties are useful in various industrial applications and these properties can potentially change the performance of any material [5, 6]. For many applications of nanoparticles, the scientific community has extensively effort to develop suitable synthetic techniques for producing nanoparticles. However, various physiochemical approaches are limited by the environmental pollution caused by chemical compounds during the synthesis of metal nanoparticles. Due to the advantages of nontoxicity, reproducibility in production, easy scaling-up, and well-defined morphology, biological method such as biosynthesis or green synthesizing nanoparticle has become a new trend in nanoparticle production [7, 8]. The biosynthesis over chemical and physical synthesis are environment friendly, cost effective and easily scaled up for large scale synthesis, besides there is no need to use high temperature, pressure, energy, and toxic chemicals [9, 10]. For all these reasons, green synthesis of nanoparticles from plant extract comes over the chemical and physical synthesis methods.

Among several noble metal nanoparticles, Ag-NPs have potentially utilized in many applications. Silver is a well-known antimicrobial agent against a wide range of over 650 microorganisms from different classes such as gram-negative and gram-positive bacteria, fungi, and viruses. In 1884, during childbirth it became a common practice to administer drops of aqueous silver nitrate to new-born baby's eyes to prevent the transmission of Neisseria gonorrhea from infected mothers. Silver has the most effective antibacterial action and is least toxic to animal cells comparatively than other metals. Silver is generally used in the nitrate form to induce antimicrobial action. However, due to high amount requirement, the substitute of silver nitrate to Ag-NPs are more beneficial to the application. Nanoparticles have a huge increase in the surface area for the microbes to be exposed to which makes silver nanoparticle more effective antimicrobial agent compare to chemical form of silver nitrate [11, 12].

Momordica charantia is a vegetable from *Momordica cymbalaria* climber plants belonging to the family of *Cucurbitaceae*. It is commonly known as bitter gourd or bitter melon in English. It is initially has found in Southern India and South East Asian countries and currently found all over the world. The vegetable includes the fruit and seed containing high levels of antioxidant properties, phenolic compounds, calcium, potassium, vitamin C and fiber content [13,14].

For metal nanoparticles synthesis, many scientists have been used plant extract from different sources. In this study, the fruit and seed aqueous extracts of bitter melon (*M. charantia*) were used as reducing and capping agents to synthesize Ag-NPs through a completely biological process.

Additionally, the antimicrobial potential of biosynthesized nanoparticles was assessed against some pathogenic microorganisms of *E. coli, P. aeruginosa. M. charantia* has found four main constituents of the phenolic acids-catechin, gallic acid, gentisic acid, chlorogenic acid. The most phenolic acids contained in bitter melon were catechin and gallic acid [15-17]. The phenolic acid works as both reducing and capping agents which converts silver ions (Ag^{+}) to Ag-NPs and covers the surfaces of formed nanoparticles and prevents from the agglomerations [18]. Silver in nanoparticle form is more efficient than silver ions in terms of its antimicrobial activity. It is also known to exert lower toxicity to mammalian cells as well [19].

2. Materials and Methods

2.1 Materials

Materials: $AgNO_3$ (99.98%) as silver precursor was supplied from Merck (Darmstadt, Germany), Bitter melon was purchased from a local market. All the solutions were prepared with deionized water.

2.2 Preparation of Bitter Melon (M.charantia) Extract

M. charantia fruit and seed were used to make the aqueous extract to synthesize silver nanoparticles. Bitter melon (M. charantia) was carefully washed with distilled water, cut into fine pieces, dried in oven drier at 1200 C, 24 h. After drying the seed and fruit parts were separated and crushed into powders separately. The powders of *M. charantia* were boiled in distilled water about 30-45 min and filtered through Whatman No.1 filter paper (pore size 125 mm) and then, the extracts were collected and stored at 4 °C before use.

2.3 Synthesis of Silver Nanoparticles

The Erlenmeyer flask containing 50 mL of *M. charantia* extracts of seed and fruit were mixed separately with 0.05 m/moL of $AgNO_3$ under continuous stirring at 30 °C. The color of solutions was changed from light brown into dark brown color after several min which indicates formation of silver nanoparticle (Figure 1). The dark brown solid products were collected through centrifugation at 4000 rpm for 15 min. After washing several times with distilled water, the obtained solid products were dried in an oven at 60°C. The final powders were stored in airtight bottles for further analysis.

2.4 Physicochemical Characterization

The UV spectrophotometry name PerkinElmer precisely Lambda 25 UV /Vis spectrophotometer was used in order to analyses UV at room temperature operated at a resolution of 1 nm between 200 and 1000 nm ranges. The characterizations of Ag NPS were done using the dynamic light scattering (Nanosizer, Malvern, NanoS, Malvern Panalytical Ltd., United Kingdom) for particle size distribution and polydispersity index (PDI). The X-ray diffraction (Shimadzu XRD 6000, Kyoto, Japan) was used for crystalline phase analysis at 2–60° range using CuKα radiation (λ =1.54060 Å) at 40 kV and 30 mA. High Performance Transmission electron microscope (Hitachi H-7100, Tokyo, Japan) was used for morphological characteristics, at 100 kV accelerating voltage using carbon film, 300 mesh copper grids. The Field emission scanning electron microscope (FESEM, Nova™ NanoSEM 230 - FEI Company, California, and USA) was used for surface morphology and shape analysis. The Fourier transform infrared spectroscopy (FTIR) for functional group analysis was done using FT-IR spectrometer (Perkin Elmer, SPECTRUM 1000, NJ, USA) with a resolution of 4 cm^{-1}.

2.5 Antibacterial Activity Test

The antibacterial activities of Ag-NPs were carried out by disc diffusion method. Muller Hinton Ager plates were prepared, sterilized, and solidified. After solidification E. coli and Pseudomonas aeruginosa bacterial cultures were swabbed on these plates. The sterile blank discs were dipped in Ag-NPs solution (10mg/ml) and placed in the Muller Hinton Agar and kept for incubation at 37°C for 24 hours.

3. Results and Discussion

3.1 Synthesis of Ag-NPs

Since reducing agents for silver nanoparticle synthesis are often considered toxic or hazardous, the use of green synthesis methods is becoming a priority [13]. Bitter melon has the polyphenolic compounds which are useful to synthesize nanoparticles. Polyphenols are typically act as reducing and capping agents to synthesize silver nanoparticle [20]. It is well known that Ag-NPs exhibit yellowish brown color in aqueous solution due to excitation of Surface Plasmon Resonance (SPR) in Ag-NPs [21]. The reduction of silver ion (Ag+) to Ag-NPs by using of *M. charantia* extracts containing of many polyphenols and flavones as both reducing and stabilizing agent could be followed by the color change from yellow to dark brown during the incubation period of 35-45 min at 30°C (Figure 1).

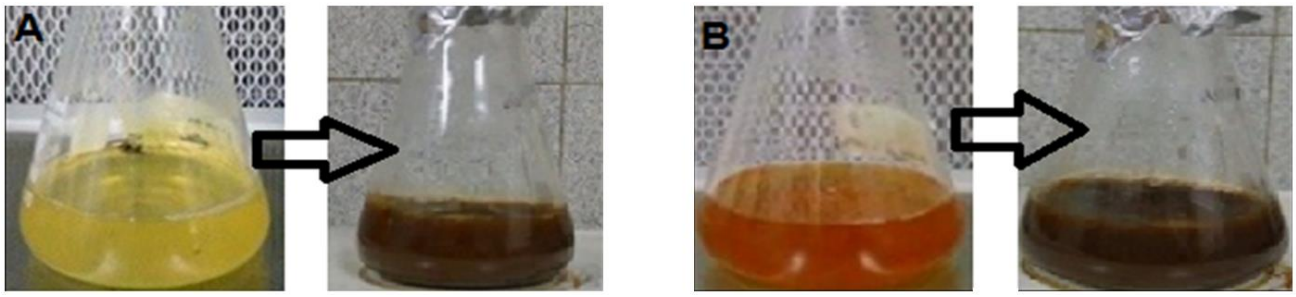

Figure 1: Colour change of (A) seed extract and (B) fruit extract before and after synthesis of Ag-NPs

3.2 pH analysis

The initial pH of solution was changed in the end of reaction. Table 1has shown the pH of seeds and fruits extract NPs in solution. As the pH value did not found considerable difference, so it can be not a factor in synthesis parameter.

Table 1. pH conditions for synthesis of Ag- NPs.

Extract	Temperature (˚C)	Initial PH	Final PH	Approximate time
Seed	30	7.52	6.51	35 min
Fruit	30	7.29	6.30	45 min

3.3 Dynamic Light Scattering analysis

Particle size found by the DLS machine with PDI > 0.7 index considering the broad particle size distribution of seed extract and fruit extract synthesis of Ag-NPs are shown in Figure 2. Figure 2A shown the seed extract Ag-NPs with size of 18.3 ± 1.9 nm and fruit extract synthesis of Ag-NPs are

shown in figure 2B with size of 17.5 ± 2.1 nm. Both fruit and seed extract-based Ag-NPs has shown similar nanometre size in DLS confirming the nano diameter of particles formation. Due to hydrodynamic size, the DLS size is showing slightly bigger than then HRTEM size.

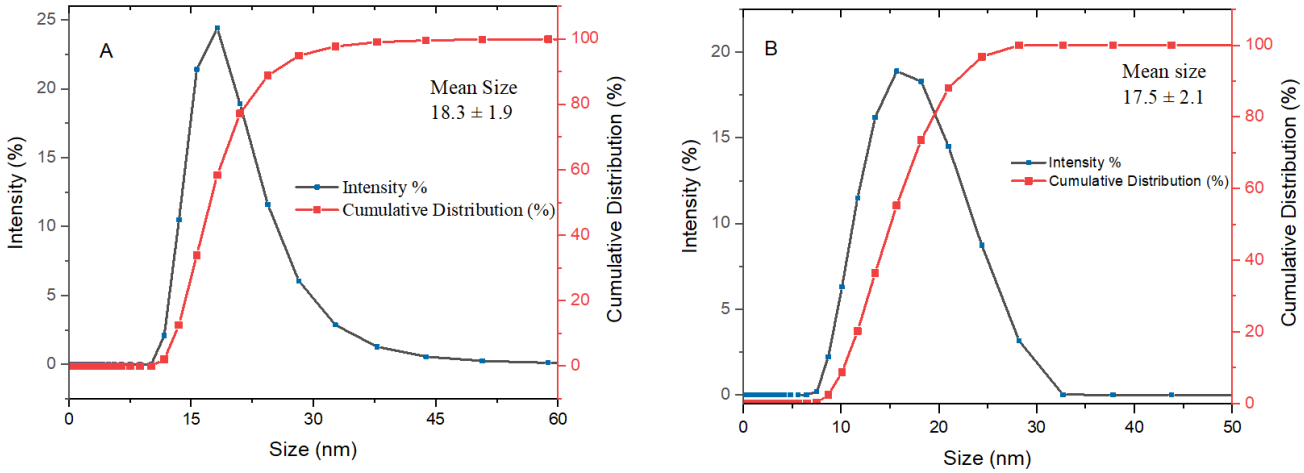

Figure 2. Particles size distribution by the intensity and cumulation of (A) seed extract and (B) fruit extract synthesis of Ag-NPs

3.4 UV/Vis Spectrophotometry analysis

UV–Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions [22]. The peaks were found at 401 and 402 nm for silver nanoparticle synthesized by fruit and seed extracts respectively in Figure 3A and 3B confirming the formation of Ag-NPs. The spectra result from the excitation of SPR due to the reduction of silver ions to Ag-NPs in the extract [23]. The spherical Ag-NPs display the characteristic SPR at the wavelength between the ranges of 400–450 nm [24].

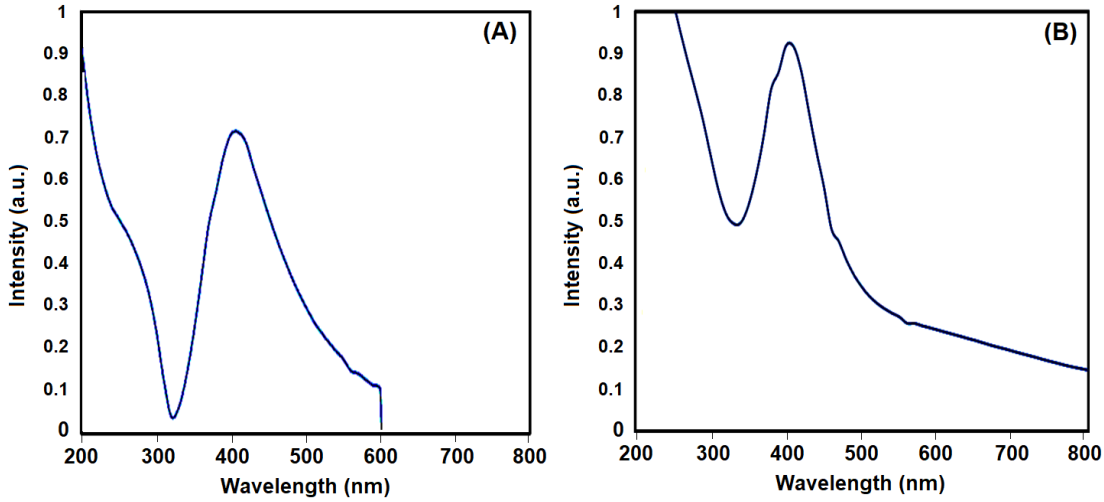

Figure 3. FTIR spectra of Ag-NPs synthesized by (A) Fruit and (B) seed extract.

3.5 X-Ray Diffraction analysis

The X-ray diffraction patterns of seed extract and fruit extract synthesis of Ag-NPs are shown in Figure 4. The XRD patterns of Ag-NPs shows that the biosynthesized particles are crystalline with

small sizes. The lattice planes (111), (200), (220), and (311) were established with the corresponding Bragg's angles of 38.45°, 45.21°, 64.87°, and 76.36°, respectively, which confirm the face-centered cubic structure of the bio-formed Ag-NPs. The sizes of formed Ag-NPs were calculated by using Scherer's equation [25] by determining the width of the (111) Bragg reflection and were estimated to be around 14 and 9 nm for Ag-NPs synthesized via fruit and seed extract, respectively.

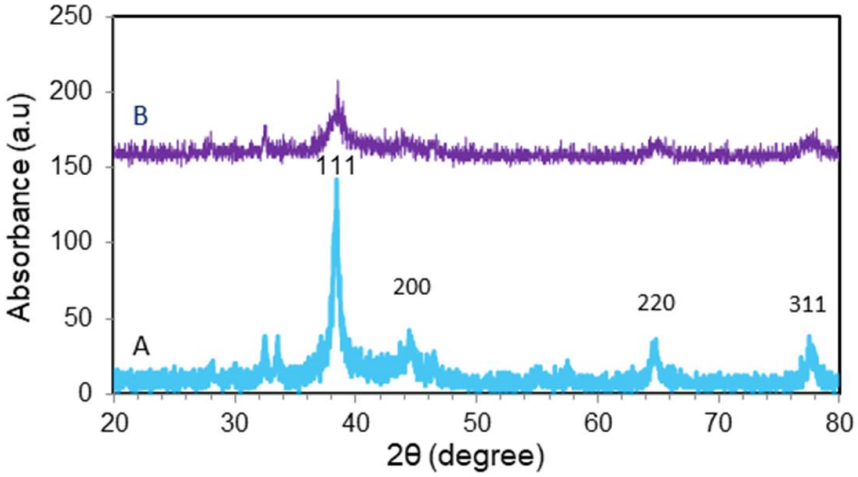

Figure 4. XRD diffraction pattern of Ag-NPs synthesized by (A) Fruit and (B) seed extract.

3.6 High Resolution Transmission Electronics Microscopy (HRTEM)

The morphology and size of Ag-NPs were determined by TEM images. Based on HRTEM micrographs (Figure 5A and 5B), it was observed that Ag-NPs were mostly spherical in shape with uniform structure. The average particle sizes of Ag-NPs were 7.5±2.1 nm and 10.3±1.9 nm synthesized using seed and fruit extracts, respectively. The observation of HRTEM image analysis has confirmed the nanoparticles synthesis.

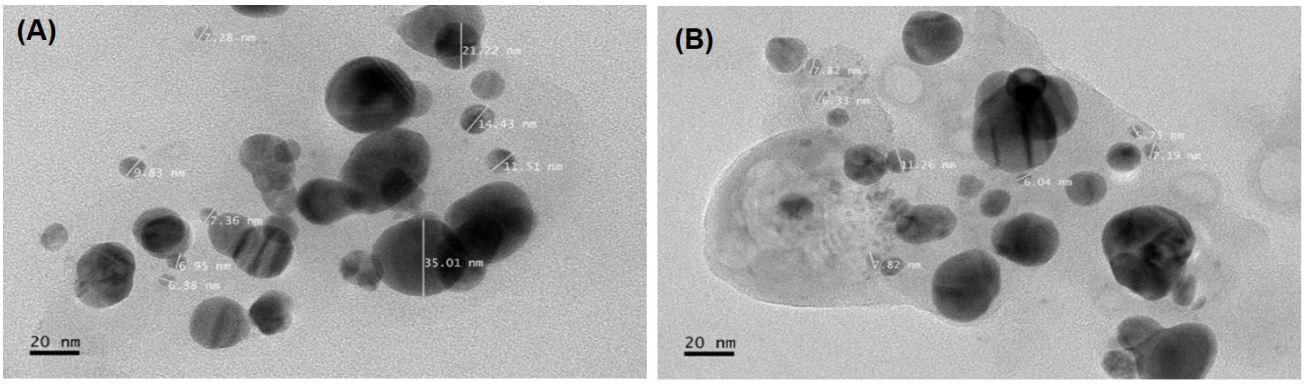

Figure 5. TEM images of (A) fruit and (B) seed extracts synthesized Ag-NPs.

3.7 Field Emission Scanning Electron Microscopy

FESEM observations further confirm the formation of the Ag-NPs using of *Momordica charantia* extracts. The morphology of green synthesized Ag-NPs was viewed by FESEM as were shown in Figure 6A and 6B. FESEM images show agglomerated Ag-NPs with the averages size between ~10 and ~30 nm was synthesized by seed and fruit extract, respectively. The particles are clearly identified by their spherical shapes. The FESEM results are well match with the HRTEM observations. The surface morphology was observed by FESEM images of both Ag-NPs. The results were conducted

with the powder form of the nanoparticles, as a result, the agglomeration was observed in the Figure 6.

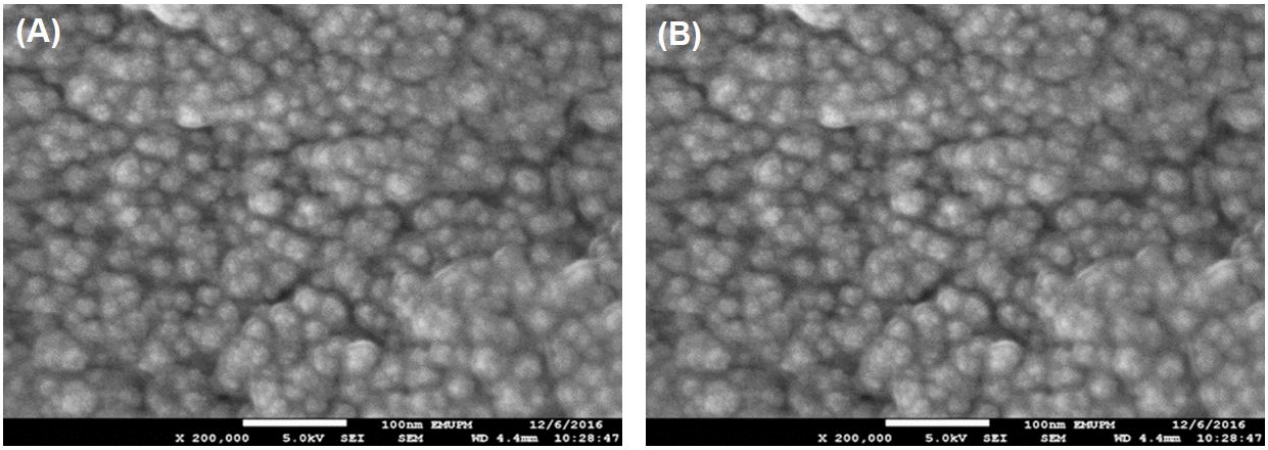

Figure 6. FESEM images of (A) fruit and (B) seed extracts synthesized Ag-NPs.

3.8 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR measurements were carried out to identify the biomolecules involved in the reduction process Ag° →Ag-NPs and the efficient stabilization of the synthesized silver nanoparticles. In Figure 6, it was found that in silver nanoparticle fruit extract, the band found in 1697.36 cm^{-1} and 1604.77 cm^{-1} which corresponds to carbonyl (C=O) Stretch and Amide NHC=O Stretch and in Ag-NPs seed extract, the bond found in 1705.07 cm^{-1} which represents C=O Stretch. Carbonyl groups confirm the phenolic compound in the extract. The carbonyl stretching absorption is one of the strongest IR absorptions.

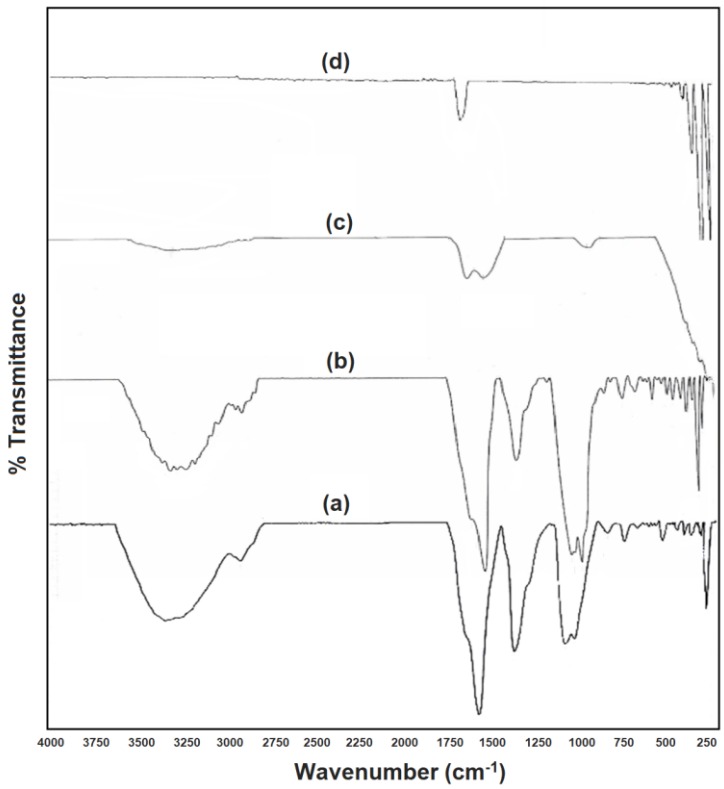

Figure 7. FTIR Spectra of pure fruits extract (a) pure seed extract (b), silver nanoparticle from fruit extract (c), silver nanoparticle from seed extract (d).

Therefore, in both seed and fruit extract silver Ag-NPs were found around the range of 290 to 490 cm^{-1}. In pure extract fruits and seed there are several band around 2900 to 3500 cm^{-1} which indicates the Carboxylic acid O-H Stretch, alcohol/phenol O-H Stretch. However, there is also band found around the range of 300 to 1650 cm^{-1} which indicates the Amide C=O Stretch, aromatic C-H Bending, aromatic C=C bending. It is proven that the phenolic compounds are presence in the extract which work as capping and reducing agent to synthesis Ag-NPs from silver nitrates.

3.9 Antimicrobial Activity

Antibacterial test was performed against the Gram-negative bacterium *E. coli* strain and *P. aeruginosa* by a disk diffusion method. Research shows that biosynthesized Ag-NPs exhibit more antimicrobial activity on gram-negative microorganism than gram-positive ones [26,28]. Figure 7 shows the antimicrobial effect of *E. coli* bacteria in pure silver nitrate (A), fruit extract silver nanoparticle (B), seed extract silver nanoparticle (C). Figure 8 shows the antimicrobial effect of P. aeruginosa in fruit extract Ag-NPs (A), seed extract Ag-NPs (B). Both fruit and seed extracts show the similar effect of antimicrobial activity on both bacteria [28].

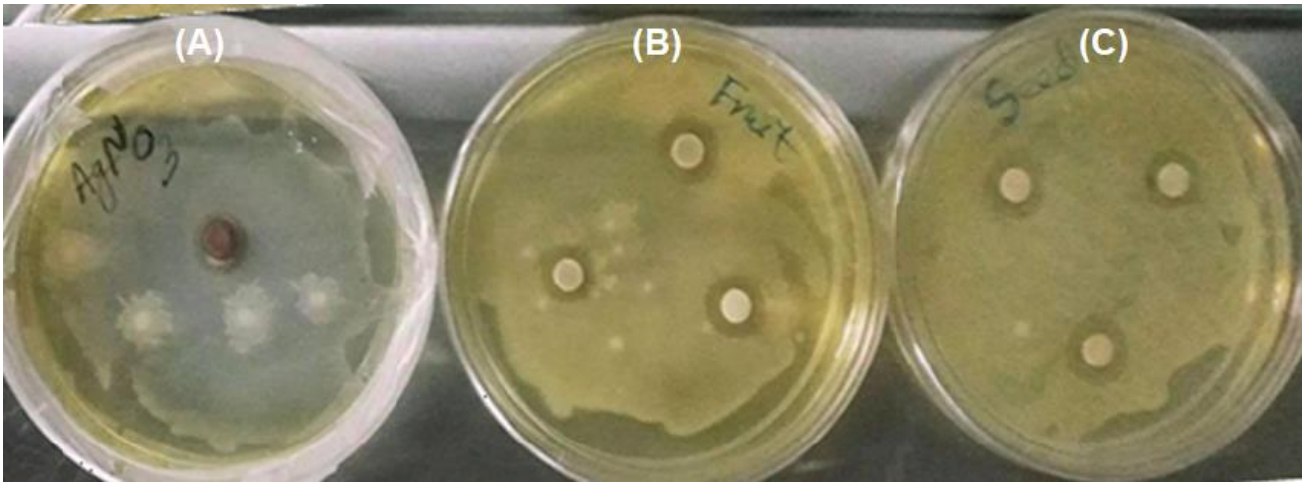

Figure 8. Antimicrobial test of *E. coli* bacteria for $AgNO_3$ (A), Ag-NPs synthesis in fruit extract (B), Ag-NPs synthesis in seed extract (C).

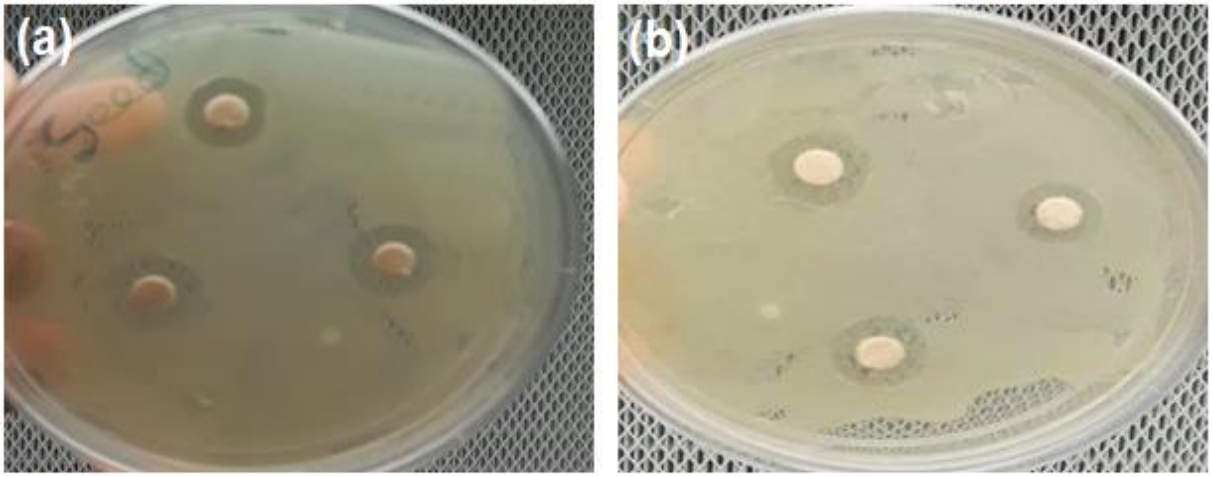

Figure 8. Antimicrobial test of *P. aeruginosa* fruit extract of Ag-NPs (a), Seed extract of Ag-NPs (b).

4. Conclusions

The green synthesis process of producing nanoparticles has potentially beneficial to produce different structure of nanomaterial. The experiment was done to synthesize Ag-NPs by using *Momordica charantia* extract which acts as a capping agent. By this method of producing Ag-NPs by the phenolic compounds existing in *M. charantia* extract, the problems of chemical synthesis can be solving which help to reduce the chemical treatment of antimicrobial action in various application. Ag-NPs were successfully characterized by UV-Vis, DLS, FTIR, FESEM, HRTEM and the average size of Ag-NPs is 10-30 nm. These nanoparticles showed antibacterial activity against E. coli bacteria and P. aeruginosa. Due to their small sizes, the nanostructures exhibit unique physicochemical and biological properties (e.g., an enhanced reactive area as well as an ability to cross cell and tissue barriers) that make them a favourable material for biomedical applications as well as other applications.

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