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Green Synthesis of Silver Nanoparticles Using Fruits Extract of Momordica dioica and studied Its Antioxidant and Antimicrobial Activity

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Abstract

In the present study, the AgNPs were Biosynthesized by green method using *Momordica dioica* plant fruit extract. The synthesis of AgNPs from *M. dioica* fruits has more advantages such as its eco-friendly; high yield and capability for pharmaceutical and other biomedical applications. For the reduction of $AgNO_3$, fruit extract of *M. dioica* is used as a reducing agent. The AgNPs were confirmed by biophysical methods such as UV-visible spectroscopy, FTIR, X-ray diffraction analysis, and scanning electron microscopy. Biosynthesized AgNPs showed good antioxidant Activity and also showed good antimicrobial activity against some selected microorganisms. Bioconversion of AgNPs by *M. dioica* plant extract could be employed as a potential antibacterial source to eliminate pathogenic microorganisms from the agricultural and food preservation industry.

1. Introduction

Nanotechnology is an innovative and revolutionary area of research in the search for more environmentally friendly processes. It is an interface between chemical engineering, material science, and biotechnology [1]. Nanotechnology is the approaching field of learning in modern science. The word “Nano” is consequent from the Greek word “dwarf” which means 1 billionth. An nm is 1 billionth of a meter (10^{-9}) and it might be represented by the length of 10 hydrogen atoms lined up in a row [2]. Using plant extracts for the green synthesis of metal NPs is a recently growing area of interest due to its benefit in relationship to traditional physicochemical methods. NPs are a small particle that ranges between 1 to 100 nanometers in size [3]. Plant-derived extracts, including those from leaves, roots, fruit, bark, seeds, peels, pods, stems, and flowers have been used as sources for the reduction in metallic precursor to nanoparticles. This is mainly owing to biomolecules, such as phenolic, alkaloids, flavonoids, steroids, and terpenoids present in plants that can act as stabilizing and capping agents [4][5]. The silver NPs have more biological and clinical impact, it's Non-toxic to humans but most effective against bacteria, viruses, and other microorganisms at small concentrations. The AgNPs obtained from green synthesis have potential applications in agriculture and agro-industry. The AgNPs show an essential role in diagnostics, imaging, sensing, gene delivery, artificial implants, drug delivery, and tissue engineering as it has unique electrical, optical, and thermal properties [6]. It has been effectively proven antimicrobial agent for certain microorganisms.

Green methods of nanoparticle synthesis are significantly attractive because of their probability of reducing the toxicity of NPs. Traditional methods are used by our ancestors but researchers have proved that the green methods are more effective for the fabrications of NPs with the advantage of lower chances of failure, low cost, and easy to characterization. Therefore, the use of vitamins, amino acids, and plant extracts is being greatly promoted nowadays [7]. The present research work is on the synthesis of AgNPs using $AgNO_3$ and plant extract of *M. dioica* without the aid of external precipitation agents, solvents and surfactants. The *M. dioica is* commonly called a spine gourd. It’s Also known as Kantoli, in the Maharashtra region of an India. The *Momordica dioica plants belong* to the family *Cucurbitaceae* [8]. It is a perennial climbers. It has two different types of varieties male and female well as fruited variety

and fruitless variety. *M. dioica* plant (chromosome number 28) is dioecious and propagated vegetatively through a tuberous root. Green fruit is extensively used as a vegetable by cooking or frying by local people. Leaves 1.5-4 inches long, cordate, acute more or less 3–4 lobed, flowers large, dioecious, and yellow, fruit 1-3-inch-long, shortly beaked, densely covered with soft spine [9]. The flowering occurs during June, July and fruiting during September, and November. The Male flower is oblong and lanceolate. Calyx is five lobed, linear lanceolate. The corolla is five partite and stamen three. Female flower is solitary small bract below the middle of the peduncle, calyx and corolla in male without staminodes or in the form of gland three united, ovary clothed with long soft papillae and many ovulated, ellipsoid [10].Its seeds are rounded broadly ellipsoid, slightly and irregularly corrugated enclosed in red pulp. Its stem is slender, branched, furrowed, glabrous and shining. Tendrils are elongated, simple and glabrous [11].

Identification and Classification of Selected Plant

Classification:

Division: Magnoliophyta

Class: Magnoliopsida

Order: violales

Family: *Cucurbitaceae*

Genus: *Momordica*

Species: *dioica*

Medicinal use: *Momordica dioica is* traditionally used as an antiseptic, astringent, febrifuge, spermicidal, and anthelmintic. Also, it is used as medicine for bleeding a pile, urinary infection, and as a sedative. The literature shows that it possesses antidiabetic, antioxidant, Antiallergic, anticancer, antimicrobial, nephroprotective, anti-inflammatory, antiulcer, antitumor, anti-fertility and analgesic properties [12].

2. Materials and methods

2.1 Preparation of plant sample

The fresh and mature fruits of *M. dioica* were collected during the season. Fresh fruit of *M. dioica* is thoroughly washed with distilled water to remove dust particles and air dried at room temperature to prevent the decomposition of phytochemicals. Then the fruits were finely crushed into small pieces and powder was obtained in a grinder machine.

2.2 Extraction of plant sample

The powder of plant sample extract was obtained by the Soxhlet method. This method is used when the phytochemicals are soluble in the solvent and the impurities are less soluble in the solvent. If the desired compound is highly soluble in the solvent the impurities can be removed by simple filtration. The advantage is that the solvent is recycled in this method and hence there is less wastage of the solvent. Also, thermo bile compounds cannot be extracted in this method [13] For extraction10 gm of plant fruit powder was placed into the thimble of Soxhlet in a filter paper roll. The 250 ml ethanol was used as a solvent. The ethanol was placed in the round bottom and kept at heating metal. The temperature of the heating metal was adjusted to the boiling point of ethanol and water. After 5 cycles the solvent present in the thimble appears to be transparent. The extract was evaporated to get a solid plant extract. After that plant extract Stover at 4 ^{o}C for further experimental use.

3. Qualitative tests for phytochemical analysis

Phytochemicals are chemical compound naturally found in all plants attributing to health effects. Identification of phytochemicals in the plant sample helps to predict the prospective pharmacological activity of plants [14]. The present study aimed to find the phytochemical constituents present in the ethanol and water extract of *M. dioica* Phytochemical screening is mostly important for identifying essential compounds such as Alkaloids, Flavonoids, Phenolic Compounds, Saponins, Steroids, Tannins, Terpenoids by using the standard methods[15]. Test for alkaloids using Mayer's reagent, for tannins using $FeCl_3$, flavonoids using NH_3, for glycosides with $CHCl_3$and 10% ammonium solution. The Saponins test done by distilled water with vigorous shaking, the presence of terpenoid confirmed with $CHCl_3$ and H_2SO_4 [16-18].

Table 1: Qualitative test for phytochemical Screening

Sr. No	Constituents	Detection test	Observation	Indicates
1	Flavonoid	5 ml Plant extract + few drops of acetate Solution	Form yellow color ppt	Present
2	Alkaloids	Mayer's test: plant extract + Mayer's reagent.	Formation yellow color ppt	Present
3	Saponin	Plant extract + dil. Water- shake for 15 minutes	Formation of layer	Present
4	Tannins	Plant extract + 10% $FeCl_3$	Change in color to bluish-black	Present
5	Phenols	Plant extract treated with 3 drop of 1% $FeCl_3$ & potassium Ferro cyanide	Formation of bluish green color	Present
6	Cardiac Glycosides	5ml plant extract + 2ml of glacial acetic acid + drop of $FeCl_3$ + 1ml conc. H_2SO_4	Formation of brown ring	Present
7	Terpenoids	Extract dissolve in ethanol + add 1ml acetic anhydride + Conc. H_2SO_4.	Change color from pink to violet	Present
8	Anthraquinone	10ml of 10% NH_3 + few ml Filtrate and shake for 2 minutes	Form pink, Violet or red colored Solution	Present
9	Carbohydrates	Fehling's test: plant extract + 5ml of Fehling's reagent kept in boiling water bath for 5 min.	Formation yellow or red color	Present

4. Green Synthesis of silver nanoparticles

Two different Extracts of *M. dioica* fruits were used for the AgNP Synthesis.1mM Silver Nitrate ($AgNO_3$) solution was prepared by adding 0.1699 g of $AgNO_3$ to 1L distilled water [19]. A 10 ml suspension of fruit extract of *M. dioica* was added to 90 ml aqueous solution of $AgNO_3$ (1mM) solution separately for the reduction into Ag^+ ions and incubated at room temperature for 24 hours [20].

5. Biophysical Characterization of Synthesized Nanoparticles

The primary detection of synthesized AgNPs was carried out in the reaction mixture by observing the color change. The synthesized AgNPs were confirmed by various techniques such as ultraviolet-visible (UV) Spectra showing $AgNO_3$ was confirmed by a UV-visible spectrophotometer. Then X X-ray diffraction study was performed for the investigation of crystal structure. The surface morphology and shape of AgNPs were investigated by scanning electron microscopy.

5.1 UV-visible spectroscopy analysis

The optical property of synthesized AgNPs was determined by using a UV-visible spectrophotometer [21]. UV-Vis absorption spectroscopic Analysis confirmed the formation of AgNPs and the stability in aqueous solution [22]. The UV absorption spectra were studied to find the energy band gap of synthesized AgNPs. As AgNPs are obtained from metal salts ($AgNO_{3)}$, the conduction band and valence band are positioned very close to each other in which electrons travel freely. These free electrons are responsible for SPR surface Plasmon resonance) absorption band [23]. The absorption of AgNPs depends on the particle size, dielectric medium, and chemical surroundings [24].

5.2 X-ray diffraction analysis

For structural analysis of the synthesized silver nanoparticles X-ray powder diffractometer was used. The data were collected in 2θ range [25]. The average size of AgNPs was calculated using the Debye-Scherrer equation by determining the width of the Bragg relation [26]. The average crystalline size of AgNPs was calculated using the Debye–Scherrer equation [27, 28]. The equation is as follows :($D = k\lambda/\beta\cos\theta$).

D = average crystalline size of particles,

K = 0.9, λ = wavelength, β indicates Full Width Half Maximum (FWHM); and θ is the Brag's angle).

5.3 Scanning Electron Microscopy Analysis

The Scanning electron microscopy (SEM) analysis was carried out to understand the surface morphology, shape, and size of AgNPs. The researcher used this technique to study not only the morphology of their nanomaterial but also the scattering of NPs in the bulk or matrix [29]. Due to the smaller size obtained AgNPs have a large surface-to-volume ratio. Such NPs are very useful for drug delivery, antimicrobial activities, Anti-oxidant and anti-cancer activity. From this observation, it was confirmed that plant extract might act both as a reducing and stabilizing agent in synthesizing AgNPs. The SEM analysis confirmed that the metal particles present in nano-size.

5.4 FTIR Analysis

To study the AgNPs and their associating molecules, FTIR measurements were carried out for both plant extract and the as-prepared Ag nanoparticles. The AgNPs were centrifuged at 9000 rpm for 30 min. The AgNPs were dried and ground with potassium bromide to form a pellet and analyzed on Jusco 5300 FTIR Instrument with a range of 400–4000 cm^{-1}.

6. Antioxidant activity by DPPH Method

The free radical-scavenging activity of all the extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. The DPPH and L-Ascorbic acid standard antioxidants were soluble in methanol [30]. A DPPH (0.1 mM) solution in ethanol was prepared and 1.0mL of this solution was added to 3.0mL of all the extracts solution in water at different concentrations. 30 minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Ascorbic acid was used as a standard

[31]. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following equation:

Effect of scavenging (%) = [control reading -sample reading/ control reading] ×100

7. Antimicrobial activity

Silver and its compound are well known as some of the most universal antimicrobial substances against a wide range of microorganisms from different classes [32]. Silver metal has been used as an antiseptic for wound curing, which encouraged researchers to find the role of Ag NPs as antimicrobial agents against microbes. Ag NPs have excessive potential against multi-drug-resistant fungi and bacteria [33]. Analysis of antimicrobial activity was investigated by using the agar well diffusion method. The antimicrobial ability was tested against *S. aureus* and *E. coli* bacteria. Bacteria sample inoculums were prepared in peptone broth and incubated for 4 hrs. MH agar medium (20ml) was aseptically transferred to pre-sterilize Petri dishes. Then 0.1 ml of inoculums was homogenously inoculated and spread by a sterile glass spreader on a petri plate containing MH agar media. Then wells of 6mm diameter were punctured on it by using a sterile cork-borer and 50ul of chemically synthesized AgNPs were poured into the wells using sterilized micropipettes. Silver has been said to be a universal antimicrobial substance for centuries [34]. The determinations were done in triplicate and all plate was incubated for 24 hrs. at 37 C, aerobically. The diameter of inhibition zones was determined in (mm) as compared to standard.

8. RESULT AND DISCUSSION

8.1 Qualitative phytochemical analysis

The phytochemical screening is shown in Table No. 2. It confirms that the *M. dioica* plant extract contains a phytochemical as Alkaloid, saponin, terpenoids, steroids, and cardiac glycoside are present in both Extracts of fruit. Flavonoids are absent in the water extract of fruit. However, Anthraquinone is absent in the ethanol extract of fruit.

Table 2
Qualitative test for phytochemical Analysis

Sr. No.	Photochemical	MDWE OF FRUIT	MDEE OF FRUIT
1	Alkaloids	+	+
2	Flavonoids		+
3	Saponins	+	+
4	Steroids	+	+
5	Terpenoids	+	+
6	Tannins		
7	Phenol		
8	Anthraquinones	+	
9	Cardiac glycosides	+	+

Where, (+) indicate present and (-) indicates absent'

MDWE- *M. dioica* water Extract, MDEE- *M. dioica* ethanol extract

8.2 Mechanism of AgNP Formation

In the present work, we have used the aqueous and ethanol extract of *M. dioica* fruit as the stabilizing as well as the reducing agent for AgNP formation. The phytochemicals responsible for reducing silver ions into AgNPs are alkaloids, terpenoids, steroids, and others. This organic group interacts with $AgNO_3$. Since $AgNO_3$ breaks into two ions, one is Ag + and the other $NO3^-$ ions when dissolved in solution. The (-ve) functional groups, such as coo- in acid and o- present in plant extract produce electrostatic linkage with Ag + ions. During forming linkage Ag + ions get reduced. The other ion NO_3^-linked with H + from the acid of phytochemicals to produce HNO_3. Since HNO_3^- is water soluble, whereas Ag^+ survives in a free metallic state to form AgNPs. The formation of AgNPs was visually confirmed by the color change during the synthesis of AgNPs using different extracts of *M. dioica* fruits.

8.3 Biophysical Characterization of Synthesized Nanoparticles

8.3.1 Ultraviolet-Visible Spectroscopy Analysis

The bio-reduction of silver nitrate into silver nanoparticles during contact with the fruit extracts was observed as a result of the color change. The color change of the mixture is due to the Surface Plasmon Resonance phenomenon. From the spectral analysis, it is observed that the AgNPs from *M. dioica* fruit ethanol extract, peak was obtained at 320 nm and the energy band gap is 2.62 eV. As shown in Fig-1 (a)

and (c). In the spectral analysis of AgNPs synthesized from M dioica fruit water extract, a peak was obtained at 338 nm and the energy band gap is 0.85 eV. As shown in Fig-1 (b) and (d).

Fig-1: UV-Visible spectra of synthesized AgNPs- (a) spectra of AgNPs synthesized from *M. dioica* fruits ethanol extract and (c) its energy gap. (b) Is spectra of AgNPs synthesized from *M. dioica* water extract and (d) is its energy gap.

8.3.2 X-ray Diffraction Analysis:

The crystalline nature of biosynthesis silver nanoparticles was confirmed by X-ray diffraction analysis. The XRD pattern of the synthesized AgNPs from *M. dioica* Fruit water and ethanol extract (Fig. 2), The Fig. 2(A) shows XRD pattern of the synthesized AgNPs from *M. dioica* Fruit ethanol peaks, where the main peaks located at 32.14°, 38.11°, 44.37° 54.87°,57.87^{o},64.44^{o} and 77.35^{o}, corresponding to the (110), (111), (200), (211), (211), (220)and (311) planes (JCPDS, No.00-076-1393). The Fig. 2(B) shows XRD pattern of 0the synthesized AgNPs from *M. dioica* Fruit water several peaks, where the four main peaks located at 38.10°, 44.20°, 64.41° and 77.39°, corresponding to the (111), (200), (220) and (311) planes, respectively, its means that facets of face-centered cubic (fcc) crystal structure of silver (JCPDS, No. 89-3722). Diana Garibo et al show the similar results of synthesized silver nanoparticles [35]. The unspecified peaks were noted due to formation phases due to organic phytochemicals responsible for the reduction of Ag. The Average grain size of biosynthesized AgNPs was calculated using the Debye-Scherrer formula for a crystalline cube system. This equation is represented as:

$D = K\lambda/\beta\cos\theta$, where D is crystalline size, K is Scherer constant, λ is x-ray wavelength used (0.154 nm), and β is full-width half maxima of diffraction peak. The average crystal size of AgNPs synthesized from *M. dioica* fruit ethanol extract was obtained at 48.2 nm. The average crystal size of AgNPs synthesized from *M. dioica* fruit water extract was obtained at 29.2 nm.

Fig – 2: XRD analysis of synthesized AgNPs from (A) *M. dioica* fruit extract water (B) *M. dioica* fruit extract Ethanol

8.3.3 Scanning electron microscopy

The Surface and structural morphology of bio-synthesized AgNPs were investigated by scanning electron microscopy (SEM). Fig-3(A) at low magnification formation of irregularly shaped AgNPs of *M. dioica* fruit ethanol extract and Fig. 3(B) shows a higher magnification formation of NPs in agglomerated form and size of NPs are seen in the range of 100–500 nm. Figure 3 (C, D) shows the lower and higher magnification images of AgNPs of *M. dioica* fruit water extract. It is observed all NPs are formed and merged in the range of 100–500 nm.

Fig – 3: SEM analysis of synthesized AgNPs from *M. dioica* fruits extracts

Fig-4: EDS analysis of synthesized AgNPs from *M. dioica* fruit extracts.

8.3.4 FTIR Analysis

The FTIR analysis was investigated for the functional group and nature of bonding NPs. Figure 4 presents the FTIR spectra of the nanoparticles synthesized using *M. dioica* fruit ethanol and water extract. Several stretching vibration bands related to organic functional groups were significantly observed in all the spectra we recorded. The 3132 cm^{-1} is attributed to the O-H group of water molecules adsorbed on the surface of AgNPs [36].The lower 1655 cm^{-1} absorption peak of C = C stretching residue of plant extract. The lower 1214 cm^{-1} absorption peak of C-O. The peaks 612 cm^{-1} and 430 cm^{-1} are Ag-O bonding.

Fig-5: (A) FTIR Analysis of synthesized AgNPs from *M. dioica* fruit ethanol extract and (B) synthesized AgNPs from *M. dioica* water extract.

8.4 Antioxidant activity

Table No 3: % free radical scavenging (DPPH Method) of biosynthesized AgNPs from *M. dioica* Fruit extract.

Sr. no	sample	200ul	400ul	600ul	800ul	1ml
1	AgNPs of MDFWE	47.23	48.94	51.11	53.02	54.95
2	AgNPs of MDFEE	46.42	47.96	50.29	52.38	52.97
3	L-Ascorbic acid	49.40	52.38	55.35	58.33	61.30

Fig-6: DPPH free radical scavenging activity of synthesized AgNPs.

The antioxidant activity of synthesized silver nanoparticles extract of *M. dioica* fruit was evaluated by DPPH radical scavenging assay. Ascorbic acid was used as a positive control. The antioxidant property of AgNPs could be attributed to functional groups present in the plant extract. Table No. 3 shows the % DPPH radical scavenging activity of biosynthesized silver nanoparticles. An IC50 value refers to the various concentrations of the sample required to scavenge 50% of the free radicals present. IC 50 value obtained for silver nanoparticles was found to be 652µg/ml for AgNPs of fruit ethanol extract and 490.40 ug/ml for AgNPs of Fruit water extract.

8.5 Antimicrobial Activity of AgNPs

Table 4
Result showing measurement of the zone of inhibition formed by AgNPs against *S. aureus* and *E. coli*

Sr. no.	Sample name	Zone of Inhibition (mm)		streptomycin	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
1	AgNPs of MDFEE	18	21	35	25
2	AgNPs of MDFWE	15	17	33	24

The antibacterial effect of Green synthesized AgNPs was investigated against *Staphylococcus aureus (*gram-positive) and *Escherichia coli* bacteria (gram-negative) has been studied and shown in Figure (A) ethanol extract of fruits and (B) water extract of fruits. *M. dioica* fruit ethanol extract synthesized AgNPs show a zone of inhibition of 18 mm for *E. coli* and 21mm for *S. aureus*. *M. dioica* fruit water extract synthesized AgNPs show a zone of inhibition of 15 mm for *E. coli* and 17mm for *S. aureus* respectively.

Fig-7: Antimicrobial activity of synthesized AgNPs from *M. dioica* fruit extract.

9. Conclusion

In the present study, the phytochemical screening confirms that the *M. dioica* plant extract contains a photochemical as flavonoids, alkaloids, saponin, tannin, terpenoids, cardio glycoside, and carbohydrates are present. We also describe a simple green method for AgNP synthesis. The extract of *M. dioica* leaves in contact with silver ions is capable of producing AgNPs. The color change of the $AgNO_3$ solution confirmed the synthesis of nanoparticles. The UV-Vis the absorption spectra of the AgNPs dispersed in water. The absorption peak is in the range of 320 and 338 nm for synthesized Ag NPs from *M. dioica* fruit ethanol and water extract. The XRD peaks were very intense and showed that the AgNPs were synthesized under a nano regime, having a crystalline nature. The average crystal size of AgNPs synthesized from *M. dioica* fruit ethanol extract was obtained at 48.2 nm and 29.2 nm for water extract. The morphological feature of synthesized AgNPs from *M. dioica* fruit extract was studied by Scanning Electron Microscope. The figure shows the high density of AgNPs synthesized using the extract of *M. dioica* has confirmed the presence of AgNPs. The electrostatic interactions between bonds such as hydrogen bonds, bio-organic bonds, and capping molecules are the reason for the biosynthesis of silver nanoparticles. Free radical scavenging activity of synthesized AgNPs shown IC 50 value obtained was found to be 652µg/ml for AgNPs of fruit ethanol extract and 490.40 ug/ml for AgNPs of Fruit water extract. The antibacterial activity of biosynthesized AgNPs from *M. dioica* fruit ethanol and water extract against *E. coli* and *S. aureus* bacteria has been studied and shown good antibacterial activity.

Declarations

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Author Contribution

V. K. D. has performed experimental results and wrote a draft of a research article. V. S. Edited and reviewed the manuscript.M. K. Z. has made technical and data analysis. The B. D. G. has the role of Investigator of the research all the results are verified.All authors read and approved final manuscript.

Data availability:

All the data available in manuscript are generated from the experimental results. The data of XRD is matched with standard data of JCDPS cards synthesized AgNPs.Similarly the data of FTIR and EDAX are matched with standard results that are available in software.

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Figures

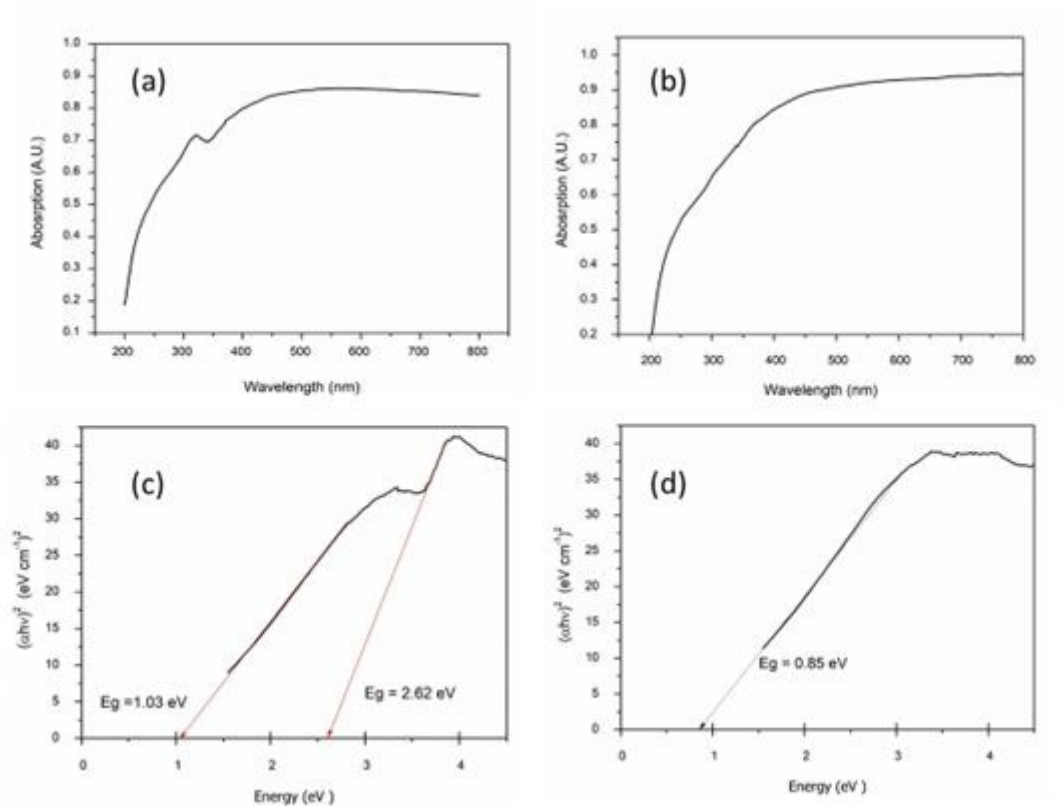


Figure 1

UV-Visible spectra of synthesized AgNPs- (a) spectra of AgNPs synthesized from *M. dioica* fruits ethanol extract and (c) its energy gap. (b) Is spectra of AgNPs synthesized from *M. dioica* water extract and (d) is its energy gap.

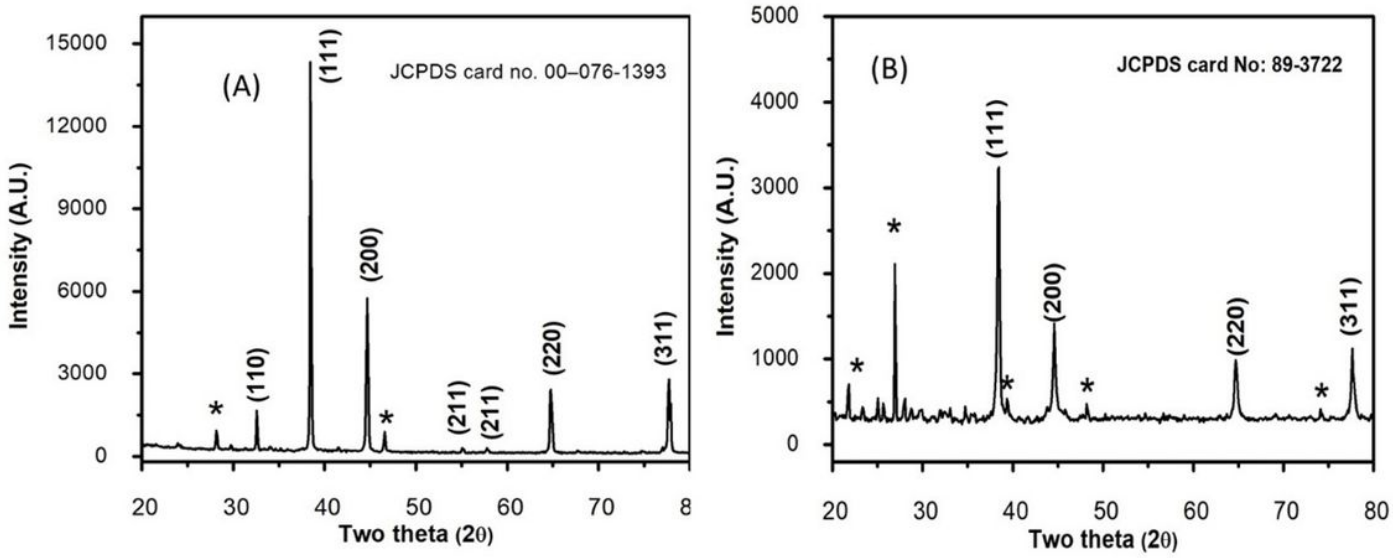


Figure 2

XRD analysis of synthesized AgNPs from (A) *M. dioica* fruit extract water (B)*M. dioica* fruit extract Ethanol

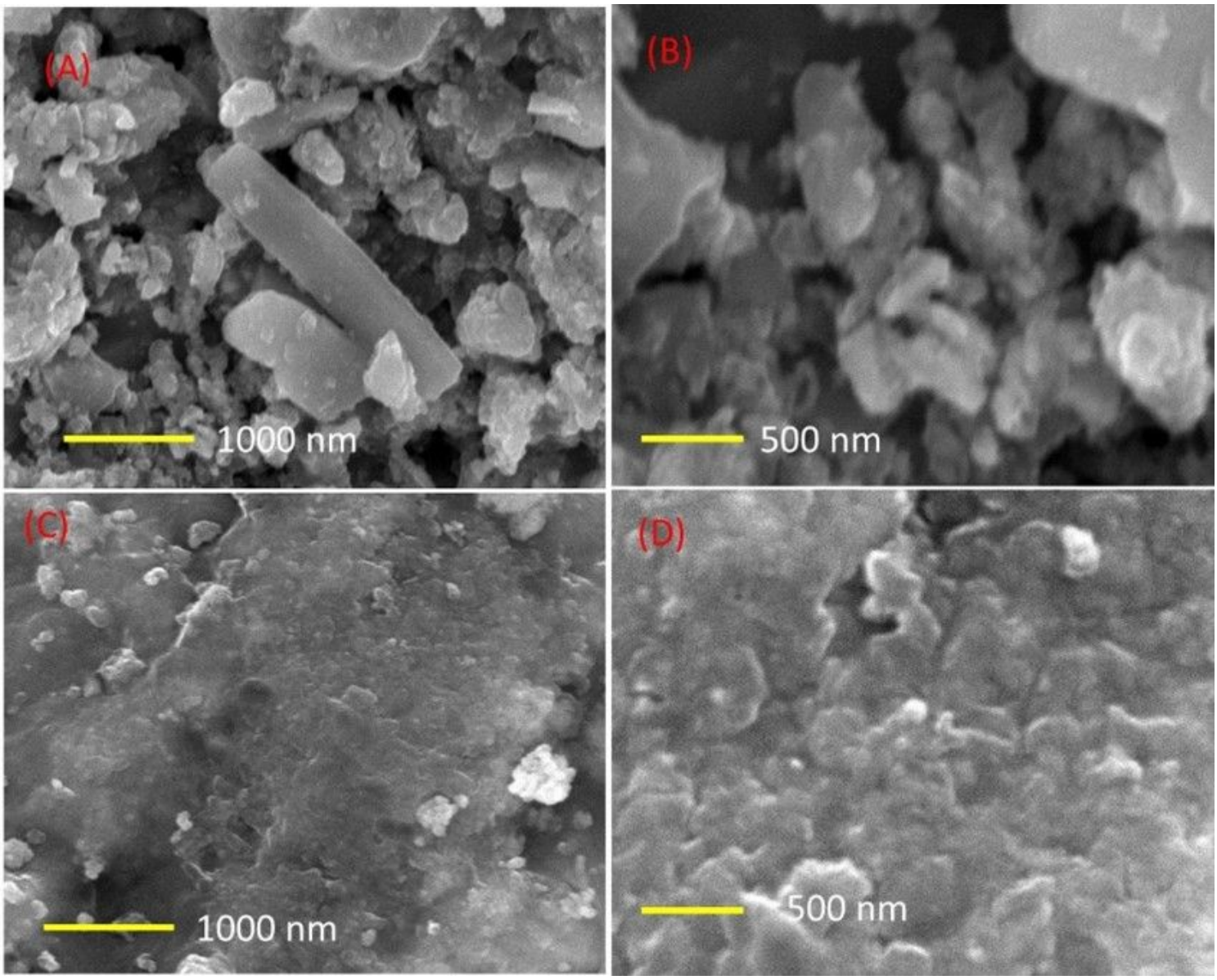


Figure 3

SEM analysis of synthesized AgNPs from *M. dioica*fruits extracts

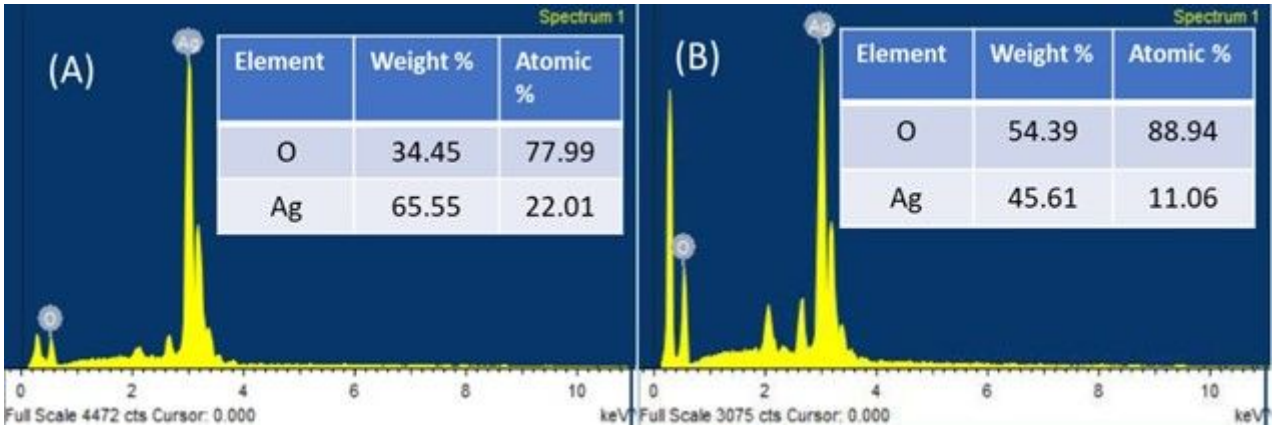


Figure 4

EDS analysis of synthesized AgNPs from *M. dioica* fruit extracts.

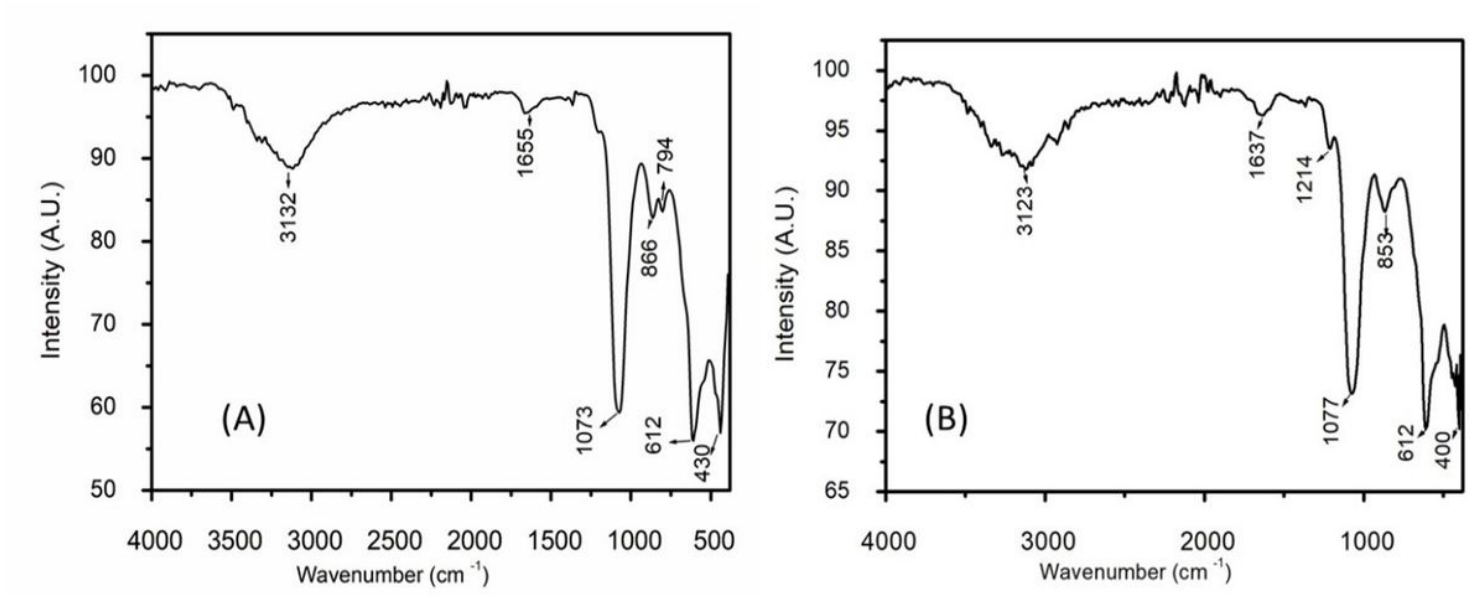


Figure 5

(A) FTIR Analysis of synthesized AgNPs from *M. dioica*fruit ethanol extract and (B) synthesized AgNPs from *M. dioica* water extract.

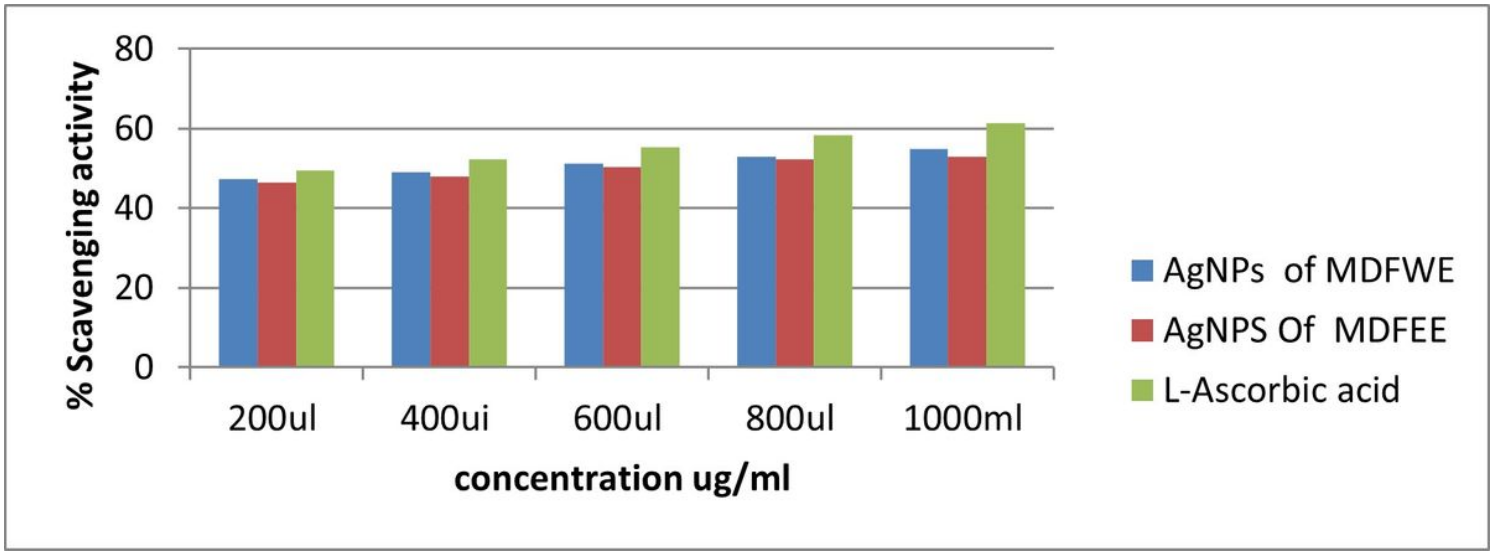


Figure 6

DPPH free radical scavenging activity of synthesized AgNPs.



Figure 7

Antimicrobial activity of synthesized AgNPs from *M. dioica* fruit extract.