Rapid Synthesis, Characterization and Antibacterial Activities of Ag-NPs Derived from Marsilea quadrifolia

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Abstract

The present study focuses on a green approach method for the synthesis of silver nanoparticles using aqueous extract M.quadrifolia. These silver nanoparticles were characterized by using Ultraviolet-visible (UV-Vis) spectrophotometer, X-ray diffractometer (XRD), Scanning electron microscope (SEM), Transmission electron microscope (TEM), Dynamic light scattering (DLS), and Zeta, Fourier transform infrared (FTIR) and evaluated its growth inhibitory efficacy against different microorganism. These silver nanoparticles (Ag-NPs) have a colour surface Plasmon resonance (SPR) single absorption peak at 436nm, when analyzed by UV-Vis spectrum. An XRD study showed a peak at 38.3° of 2θ value and confirmed that the nanoparticles were amorphous in nature with 27 nm average size of particles. Higher magnification studies with SEM and TEM analysis revealed that the particles were poly-dispersed, spherical in shape and have the size range from 19 to 30 nm without agglomeration among the particles. Energy dispersive X-ray analysis showed a 52.84 weight percentage of silver content in the sample, which indicates the towering purity of the sample. DLS and zeta potential studies revealed the average size of 62.1 nm and -18.3 mV zeta potential value of nanoparticles. FTIR analysis revealed the presence of silver content and the appearance of phytochemicals such as primary amines of proteins, carbohydrates and nitrogenous compound, phenols were mainly responsible for capping and stabilization of silver nanoparticles. The obtained nanoparticles were tested for growth inhibitory activity on different microbial pathogens, resulting in potential inhibitory activity. This study concluded that the plant M.quadrifolia was an excellent and reliable green source for production of potential bio antimicrobial silver nanoparticles.

Keywords

Green synthesis, Silver nanoparticles, UV, TEM, MIC

1. Introduction

Nanobiotechnology deals with biotic and abiotic materials with at least one dimension sized from 1nm to 100nm. [1] Nanoparticles have novel properties which depend on specific characteristics such as size, topography, and distribution [2].Now a day’s inorganic nanoparticles and their nanocomposite are extensive, used in various industries, biomedicines, and catalysis reaction and biotechnology application [3]. Among the noble metal nanoparticles such as gold, silver, titanium and platinum are a wide range of application in nano medicinal [4-6]. In particular, silver nanoparticles have a various important application from the biotechnology field are as anticancer [7-9], antibacterial and antifungal agents [10, 11], in textile engineering, waste water treatment and silver - based consumer products [12, 13].

The benefits of the green route are that it offered cost-effective, environment friendly and scalable options as compared to the established chemical and physical methods [14]. Furthermore, the biological synthesis of nanoparticles route was advantageous as it does not involve the use of toxic chemicals, high temperature, high pressure and energy. Presently however, chemical and physical methods are mainly employed. Despite that, there is a persistent requirement for a commercially viable, economic and environment friendly route to silver nanoparticles synthesis [15]. The biological methods of AgNPs synthesis can be done by the using of biological entities like microorganism, yeast, fungi, algae and plants mediate[16-18]. Also, plants extract mediated synthesis of nanoparticles cheap.
Several reports are available in the literature on the biological synthesis of silver nanoparticles those Ocimum basilicum [19,20], Dodonaea viscose [21], Andrographis echioides [22], Syzygium aromaticum [23] Beta vulgaris [24], etc. M.quadrifolia is an aquatic fern belongs to the family (Marsileaceae) is an important medicinal plant. M.quadrifolia is beneficial for the nutrient mitigation from the fresh water of the lake significant progress has been made for wetland restoration. It is commonly known as four leaf clover, water clover, pepperwort and water shamrock and it is also commonly called as sushni in parts of India and Aaraikeerai in Tamil Nadu, India. The plant M.quadrifolia has diverse medicinal properties and also it is in use for more than 3000years as part of food [25, 26]. It is used in the Ayurvedic system of medicine for curing several ailments. M.quadrifolia is used to treat cough, eye diseases, diarrhea and skin diseases, psychiatric disease, diabetes [26, 27]. M.quadrifolia also has a few pharmacological effects such as anti microbial, anti-oxidant, Larvicidal activity, anticancer has been reported in the plant [25, 28, 29]. In this study, we have investigated the biogenic synthesis of silver nanoparticles through leaf extract from M.quadrifolia. Varied analytical studies such as UV, XRD, SEM – EDAX, TEM, DLS and FTIR spectrophotometers were used to studies biogenic silver nanoparticles. Also we have investigated the antibacterial activity of silver nanoparticles (Ag-NPs) and determination of minimum inhibitory concentration (MIC) E.coli and S.aures.

2. Materials and methods

2.1. Collection of plant materials and chemicals

Fresh leaves of Marsilea quadrifolia were collected from the fields of Cheyyar village, Tiruvannamalai District, Tamil Nadu, and India. Silver nitrate (AgNO₃) was purchased from alrich, India with ≥99.5% purity, and used without further purification.

2.2. Preparation of plant extract and silver nitrate solution

Freshly collected leaves of M.quadrifolia were thoroughly washed with running tap water followed by ultra pure deionized water to remove the filth. M.quadrifolia broth solution was prepared by taking 10g of finely harvested leaves in a 250 ml Erlenmeyer flask along with 100ml of distilled water and then the mixture was boiled at 80°C for 20mins. The extract was filtered through whatman’s No.1 filter paper. Initially, 1mM of AgNO₃ (Sigma Aldrich, India) has been weighted and dissolved in 100 ml of distilled water for the stock solution of AgNO₃.

2.3. Synthesis of Bio-inspired silver nanoparticles

20ml of M.quadrifolia leaves extract was added to the 80ml of 1mM AgNO₃ aqueous solution and kept in 80°C temperature within 25 minutes under vigorous stirring, the bioreduction of the Ag⁺ was the solution turned yellow into dark brown indicating the formation of silver nanoparticles in Fig. 1.

2.4. Phytochemical screening analysis

2.4.1. Test for tannins

Ferric chloride test: To 2ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.4.2. Test for flavonoids

Sulphuric acid test: A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of yellow colour.

2.4.3 Test for alkaloids

Mayer’s test: to 2 ml of plant extract, 2ml of concentrated hydrochloric acid was added, and then few drops of mayer’s reagent were added. Formations of green color or white precipitate indicate the presence of alkaloids.

2.4.4. Test for carbohydrates

Molisch’s Test: To 2ml of plant extract, 1ml of Molisch’s reagent and few drops of concentrated sulphuric acid were added. Formation of a purple or reddish ring indicates the presence of carbohydrates.
Ninhydrin test: To 1ml of plant extract, an equal volume of chloroform and few drops of concentrated sulphuric acid were added. Formation of a brown ring indicates the presence of steroids and the formation of bluish green colour indicates the presence of phytosterols.

2.4.6. Test for saponins

Foam Test: To 1ml of plant extract, 5ml of distilled water was added and shaken in a graduated cylinder for 15 mins lengthwise. Formation of a 1cm layer of foam indicates the presence of saponins.

2.4.7. Test for coumarins

Sodium hydroxide test; To 1ml of the extract 1ml of 10% NaoH was added. Formation of yellow colour indicates the presence of coumarins.

2.4.8. Test for proteins

Ninhydrin test; To 2ml of the extract, a few drops of 0.2% ninhydrin was added and heated for 5 min. formation of a blue colour indicates the presence of proteins.

2.4.9. Test for quinones

Sulphuric acid test: To 1ml of the extract, 1ml of conc.H2SO4 was added. Formation of red colour indicates the presence of quinines.

2.4.10. Test for glycosides

Sulphuric acid test: To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added to which few drops of concentrated sulphuric acid were added. Presence of greenish blue colour indicates the presence of glycosides.

2.4.11. Test for terpenoids

Sulphuric acid test: To 0.5 ml of extract, 2ml of CHCl3 and conc.H2SO4 was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids.

2.4.12. Test for triterpenoids

Libermann-Buchard test: To 1.5 ml of extract, 1ml of Linermann-Buchard reagent (acetic anhydride and conc.H2SO4) was added. Formation of bluish green colour indicates the presence of triterpenoids.

2.4.13. Test for phenols

Ferric Chloride test: To 1ml of the extract, 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

2.4.14. Test for anthocyanin and betacyanina

Sodium hydroxide test: 2ml of plant extract, 1ml of 2N sodium hydroxide was added and heated for 5min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin and the formation of yellow colour indicates the presence of betacyanin.

2.4.15. Test for cardiac glycosides

Ferric chloride test: To 0.5ml of extract, 2ml of glacial acetic acid and a few drops of 5% ferric chloride was added. This was under layered with 1ml of conc. H2SO4. Formation of the brown ring at the interface indicates the presence of cardiac glycosides.

2.5. Characterization of silver nanoparticles

The biogenic of Ag-NPs formation in colloidal solution was analyzed in UV - Vis spectroscopy and the spectrum were in a Shimadzu (model UV-2480) spectrophotometer and then measured in the wavelength range of 200 to 800 nm. The FTIR
measurements were carried out using an (Affinity-1, Schimadzu) spectrometer and KBr pellet 4000 to 400 cm⁻¹. The spectrum of synthesized silver nanoparticles and the spectrum of leaves extract were studied. In order to ascertain the presence of various functional groups in the samples, several modes of vibrations were recognized and assigned. The XRD was analyzed for the formation of the structure and composition of synthesized Ag-NPs by Bruker D8 Advance X-ray diffractometer (cook), the diffraction peaks were obtained in 2θ and the scanning range was done between 10° to 90°. The colloidal solution of the synthesized Ag-NPs was calculated by using a dynamic light scattering technique (HORIBA, SZ-100). The size and distribution of the nanoparticles were also determined. Scanning electron microscopy (FEI Quanta FEG 200 high-resolution electron microscope) equipped with an X-ray energy dispersive spectrometer was used to study the size and morphological characterization of the synthesized AgNPs. The natural history of the element was also analyzed. The size and shape determination of synthesized Ag-NPs were obtained by using TEM images (Libra 200 TEM) operated at an accelerating voltage of 115kv and 200kv, the samples for TEM analysis were prepared by drop-coating the silver nanoparticles solution onto carbon coated copper TEM grids.

2.6. Application of silver nanoparticles

2.6.1. Antibacterial activity

Test pathogens were spread on Mueller-Hinton agar (MHA) plates. A well of diameter 6 mm was made using a sterile cork borer and loaded with the required concentration of the drug over the agar. The test plates were incubated for 24h at 37°C. The zone of inhibition (mm in diameter) was read and considered as the action against the test pathogen. The tested strains *S.aureus* and *E.coli* Zone of inhibition are expressed in mm.

2.6.2. Minimum Inhibitory Concentration

The test material was dissolved in 10% DMSO. The initial test concentration was serially placed (something missing) in a 96 well plate and inoculated with 5 µl of a suspension containing 10⁸ CFU ml⁻¹ of bacteria. The 96 well plates were incubated for 24 h at 37 °C for bacterial growth. The culture intensity of each well was read at 600 nm in comparison with the untreated control. The MIC of the extract was determined as the lowest concentration of the extract inhibiting the visual growth of the test cultures.

3. Results and discussion

3.1. Photochemicals

Preliminary photochemical screening analysis of *M.quadrifolia* leaves extract revealed the presence of active phytochemicals were compound as flavonoids, steroids, proteins, tannins, saponins, quinines, triterpenoids, cyanin. These results were presented in Table 1. Several photochemical extracted from varied botanical sources have been reported to have detrimental effects on antibacterial activities [25].

3.2. Formation of silver nanoparticles using *M.quadrifolia*

The yellowish extract of *M.quadrifolia* leaves were shown in Fig 1(a), and the colorless silver nitrate (AgNO₃) solution was shown in Fig. 1(b). The effective color change for *M.quadrifolia* leaves extract was observed by heating at 60°C for 20mins under vigorous stirring for mixture of *M.quadrifolia* extracts and AgNO₃ solution. The bio-reduction of the silver ions was facile as the solution turned to grayish red as shown in Fig. 1(c), which confirms the formation of nanoparticles.

![Fig 1. Formation of Ag-NPs synthesis using *M.quadrifolia* extracts](image)
Table 1
Phytochemical screening of aqueous leave extracts of *M.quadrifolia*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Secondary metabolites</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Alkaloids</td>
<td>_</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Coumarins</td>
<td>_</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>+++</td>
</tr>
<tr>
<td>9</td>
<td>Quinones</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>Glycosiders</td>
<td>_</td>
</tr>
<tr>
<td>11</td>
<td>Terpenoids</td>
<td>_</td>
</tr>
<tr>
<td>12</td>
<td>Triterpenoids</td>
<td>++</td>
</tr>
<tr>
<td>13</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Acids</td>
<td>_</td>
</tr>
<tr>
<td>15</td>
<td>Cyanin</td>
<td>++</td>
</tr>
<tr>
<td>16</td>
<td>Cardiac glycosides</td>
<td>_</td>
</tr>
</tbody>
</table>

(+++) - Strongly Positive; (+++) – Positive; (+) - Trace; (-) - Not detected

3.3. UV – Visible spectroscopy

Facile biosynthesis of Ag-NPs was synthesized from the aqueous silver ions using the biomass reducing and capping agents of leaves *M.quadrifolia* extracts. This indicates the synthesis of Ag-NPs, which was further confirmed by preliminary study UV-vis spectroscopy. The synthesized Ag-NPs were analyzed in SPR range at around 400-480nm and the spectra of curve bell shaped with a single. Generally, the SPR single bands have influenced the size, shape, morphology, and spherical and uniform of the formed Ag-NPs [30-32]. From this research, the red shift in the $\lambda$ max of the single SPR peak around at 426nm has confirmed the product of Ag-NPs (Fig. 2) shows, which strongly suggests that the silver nanoparticles were spherical in shape and have been confirmed by the TEM results of the study[33].

![UV – Vis spectrophotometer of Ag-NPs using *M.quadrifolia* extracts](image)

**Fig. 2.** UV – Vis spectrophotometer of Ag-NPs using *M.quadrifolia* extracts
3.4. X-ray diffraction (XRD) Analysis

The study of crystalline nature and particle size analysis of as synthesized silver nanoparticles using of M.quattrifolia leaves extract was carried out by XRD. XRD pattern of the silver nanoparticles (Fig. 3) indicated five diffraction bands $2\theta$ (30° < $2\theta$ <85°) ascertained that the values at 38.13, 44.27, 64.39, 77.32, and 81.70 which can be attributed to (111), (200), (220), (311) and (331) Bregg’s reflections of face centered cubic (fcc) structure of metallic silver nano crystal respectively. Matching with the database of the joint committee on powder diffraction standards (JCPDS) (Ag XRD Ref no: 04-0783) [33]. This clearly indicated the crystalline nature of the present study reports on a green approach method for synthesis of silver nanoparticles from M.quadrifolia. This study concluded that the plant M.quadrifolia was an excellent and reliable green source for production of potential bio antimicrobial silver nanoparticles biosynthesized Ag-NPs [34].These results indicated that the average grain size of the as prepared silver nanoparticles was determined from FWHM of the most intense reflection (111) peak from the XRD data using Debye- Scherer’s equation [35].

$$D = \frac{k\lambda}{\beta \cos \theta}$$

Where $k = 0.9$ is the shape factor, $\lambda$ is X-ray wave length(0.154056 Å), $D$ is the standard crystalline size in nanometer, $\beta$ full–width at half–maximum of diffraction line in radians and $\theta$ half Bragg angles radians. The average crystalline size calculated using Scherer’s equation was 15.47nm.which are also in line with the observation of the TEM results discussed later.

![Fig 3. The x-ray diffraction pattern of prepared Ag-NPs using M.quadrifolia leaves extracts](image)

3.5. SEM and EDAX analysis of silver nanoparticles

Morphology and size of the biosynthesized Ag-NPs were observed from SEM image (Fig. 4). The average size of particles was estimated 22 to 45nm by SEM images and them that were spherical in shape in nature and they rarely combined each other to form a cluster of particles. The presence of the biomolecular coating is also distinctly observed from the photograph. The elemental composition of green synthesized nanoparticles was determined by EDAX detector, (Fig. 4). The obtained results revealed that the presence of pure Ag metals in the form of nanoparticles, the presence of oxygen and carbon indicates the presence of bioactive compounds of Marsilea quadrifolia extracts.
3.6. TEM analysis of synthesized silver nanoparticles

The TEM image of the biosynthesized silver nanoparticles using by the Marsilea quadrifolia leaves extract showed shape, size and morphology. The synthesized nanoparticles were polydispered and predominantly spherical in shape, which we can conclude that the average size of silver nanoparticles was 19-30nm as shown in Fig. 5, it showed the SAED pattern also confirms the crystalline nature of the compound with the plane (111), (200), (220) and (311) for the silver nano crystal respectively, which is evident from the XRD pattern. In addition the fringe spacing measured from the image in Fig. 5 is found to be 0.28 nm which corresponds to the (111) plane of the face centered cubic structure of silver nanoparticles. The TEM images clearly confirm that the present method can be effectively used for the synthesis of well dispersed and evenly shaped AgNPs impregnated in a cellulose network [36].
3.7. Dynamic light scattering analysis

The DLS size distribution image of biosynthesized silver nanoparticles using M. quadrifolia of nanoparticles is shown in Fig. 6(a). It is observed that the size distribution of silver nanoparticles ranges from 20 to 112nm. The calculated average particle size distribution of silver nanoparticles is 54.61nm. The zeta potential of the biosynthesized Ag-NPs was found as a sharp peak at -18.0 mV in Fig. 6(b). Which was confirmed the reasonable stability of the nanoparticles. The negative possible value shown by biosynthesized silver nanoparticles may possibly be due to the existence of bio natural components in the extract. The Dynamic Light Scattering measured size in marginally bigger as compared to the particle size measured from TEM and SEM micrographs. So that, the hydrodynamic radius was measured using the DLS method.

![Size Distribution by Intensity](image_url)

**Fig. 6(a).** The DLS size distribution of Ag-NPs synthesized using M. quadrifolia extracts

<table>
<thead>
<tr>
<th>Z-Average (d.nm)</th>
<th>Size (d.nm)</th>
<th>% Intensity</th>
<th>St Dev (d.nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>112.3</td>
<td>141.3</td>
<td>100.0</td>
<td>67.11</td>
</tr>
<tr>
<td>Pdi: 0.192</td>
<td>Peak 2: 0.00</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Intercept: 0.920</td>
<td>Peak 3: 0.00</td>
<td>0.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Result quality:** Good

![Zeta Potential Distribution](image_url)

**Fig. 6(b).** Zeta potential distribution of Ag-NPs synthesized using M. quadrifolia extracts
3.8. FTIR spectral analysis

FTIR spectroscopy was carried out to identify the possible potential biomolecules responsible for the reduction of the silver nanoparticles ions and capping of the bio-reduced Ag-NPs [37]. FTIR spectrum of the obtained AgNPs (Fig. 7) manifests 7 absorption peaks 3444 cm$^{-1}$, 2923 cm$^{-1}$, 1634 cm$^{-1}$, 1550 cm$^{-1}$, 1390 cm$^{-1}$, 1268 cm$^{-1}$, 556 cm$^{-1}$. The broad band at 3444 cm$^{-1}$ could be assigned to OH stretching vibration of phenol and alcohol. The band at 2923 cm$^{-1}$ C=C stretching found in Alkenes and 2545 cm$^{-1}$ attributed to CH stretch carboxylic acid. The FTIR band at 1634 cm$^{-1}$ assigned to C=O stretch with N-H deformation found in primary amides II, C=O stretch in the binding of biomolecules such as protein, carbohydrates and nitrogenous compound on the surface of the nanoparticles. The peak at 1550 cm$^{-1}$ assigned to the presence of N-O asymmetric stretching vibrations of the nitro compound. The presence of bands at 1390 cm$^{-1}$ assigned to CH3 deformation in the binding of biomolecules such as protein, carbohydrates and nitrogenous compounds such as flavonoids, terpenoids[38-40]. It showed a sharp intense peak at 1268 cm$^{-1}$ C-N stretching vibration of aromatics. The peaks at 1062 cm$^{-1}$ corresponding to C-N stretching vibration of Aliphatic amines, the peaks at 556 cm$^{-1}$ assigned C-C-CN nitrites, C-C=O bend ketones, respectively. These results suggest that the protein, carbohydrate, flavonoids, and terpenoids have been confirmed by the photochemical screening analysis of the study. Thus these biomolecules, confirmed responsible for capping and efficient stabilization can bind to silver nanoparticles reported that proteins can bind to silver nanoparticles which are compatible with our results.

![FTIR spectrum of biosynthesized Ag-NPs using M.quadrifolia extracts](image)

**Fig. 7.** FTIR spectrum of biosynthesized Ag-NPs using M.quadrifolia extracts

3.9. Antibacterial activity

3.9(a). Disc diffusion method

In vitro susceptibilities of the selected Gram-positivity bacterial (Staphylococcus aureus & Escherichia coli) and Gram Negative bacterial toward the biosynthesized silver nanoparticles by extract M.quadrifolia were determined by disc diffusion method at different concentrations of 20µl, 40µl, 60µl and 80µL, such as the diameter of the inhibition zone (mm) around discs with silver nanoparticles was shown in Fig. 8(a) and Table 2(a). The antibacterial effect of inhibition was found to be more than 16mm for S.aureus and E. coil at 60 µl concentrations (Fig. 8(b)). The enhanced antibacterial effects of silver nanoparticles would interfere with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of peptide substrate critical for cell viability and cell division [41]. The tests were done in replicates of three and the results were given as mean±SD.
Table 2(a)
Antibacterial activity of synthesized AgNPs of *M. quadrifolia* by well diffusion method

<table>
<thead>
<tr>
<th>Pathogenic micorganisms</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 μl</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12</td>
</tr>
</tbody>
</table>

Fig: 8(a). Antibacterial activity of bio-reduced Ag-NPs synthesized using *M. quadrifolia* extracts

Fig. 8(b). Antibacterial activities of Ag-NPs synthesized using *M. quadrifolia* against *S.aureus* and *E.coli* at different concentrations
3.9(b). Minimum inhibitory concentration

The MIC of the Ag-NPs against pathogenic bacteria was shown in Table 2(b). It was found that the MIC value of *Escherichia coli* showed lesser activity followed by *Staphylococcus aureus*. The percentages of inhibition in the growth of biosynthesized Ag-NPs are shown in Table 2(b). The IC 50μg concentration of *Escherichia coli* and *Staphylococcus aureus* were 17.27±2.66 and 46.90 ± 4.23 μg, respectively. In this research work observed results reveal that bioreduced Ag-NPs shows significant antibacterial property compared with positive drug control, it could be explained by their large surface area, which gives better contact with microorganisms thus alter the microbial metabolism, the nanoparticles attached to the cell membrane and penetrated inside the microorganisms. The sulphur-containing proteins were present in bacterial membrane and phosphorus-containing compound present in DNA that was interacted with Ag-NPs and mainly affect the respiratory chain, cell division finally leading to death. The silver ions from nanoparticles released into the bacterial cells which improved their bactericidal activity.

Table 2(b)

MIC of synthesized Ag-NPs of *M.quadrifolia* against various microorganisms (IC 50μg) triplicate values

<table>
<thead>
<tr>
<th>Pathogenic microorganisms</th>
<th>Synthesized Ag-NPs by <em>M.quadrifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>17.27±2.66</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>46.90 ± 4.23</td>
</tr>
</tbody>
</table>

4. Conclusions

Leaf extract of the flavonoids, steroids, proteins in the *M.quadrifolia* plant has been reported for the bioreduction and stabilization of aqueous Ag⁺ ions to Ag⁰ metal ions which lead the formation of well defined Ag⁰ nanoparticles. Size control of nanoparticles has been done by analyzing of silver solution and leaf extract from *M.quadrifolia*. UV-Vis spectroscopy, XRD, SEM and TEM – EDAX, DLS and FTIR spectrum analysis was used to investigate shape and size control of nanoparticles. Analysis of particles size of nanoparticles around 19-35 nm was confirmed by DLS, XRD and Zeta potential analysis as well as TEM image. FTIR spectra show that the protein of polyols presents in leaf extract is reasonable for reduction of Ag⁺ ions to Ag nanoparticles. Biogenic synthesized Ag-NPs show excelled antibacterial activity gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) fairly low concentration.

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References


