

Short communication

Lycium shawii mediated green synthesis of silver nanoparticles, characterization and assessments of their phytochemical, antioxidant, antimicrobial properties

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ABSTRACT

BACKGROUND: The shrub *Lycium shawii* belongs to the Solanaceae family, formerly used by ancestors to cure different illnesses. Dried leaves of *Lycium shawii* were used for extraction by maceration method in distilled water and methanol. Qualitative and quantitative estimation of phytochemicals were carried out in crude extracts. Plant-based silver nanoparticles have been praised over past decades as it is economical, environment-friendly, non-pathogenic, and undemanding. This study includes the synthesis of silver nanoparticles using crude leaf extract of *Lycium shawii*, their characterization, and exploited for different biological activities.

FINDINGS: In phytochemical analysis, the total phenolic content in the methanolic extract was 11.11 mg Gallic acid/g, and the total flavonoid content was 7.76 mg Quercetin/g. The average size of aqueous-silver-nanoparticles and methanolic-silver-nanoparticles was 64 nm and 79 nm, respectively. Crude extracts and nanoparticles showed remarkable antimicrobial potential against the gram-negative bacteria *Salmonella enterica* and fungi *Rhizopus oryzae*. The antioxidant potential of methanolic extract and MeOH-AgNPs manifests more significant activity than aqueous extract and Aq-AgNPs. Minimum inhibitory concentration was observed in a range of 1 mg/ml to 15 mg/ml against different microbes. The observed MIC clearly indicated that the synthesized nanoparticles have strong antimicrobial potential. Hence can be used as a good source for the development of new drugs against resistant microbes.

CONCLUSION: In the era of increased antibiotic resistance, the *Lycium shawii*-mediated silver nanoparticle shows remarkable antimicrobial and significant antioxidant potential. These nanoparticles can be used alone or in combination with antibiotics to combat the effect of multidrug-resistant pathogens. Regarding the novelty, probably this is the first study highlighting the different biological activity of *Lycium shawii*-mediated silver nanoparticles.

1. Introduction

Medicinal plants are the most valuable sources of novel medicines throughout the world. Approximately 25% of recommended

medications in developed countries come from wild plant species [1,2]. The tribal people continue to rely extensively on the locally accessible medicinal flora to treat their illnesses [3,4]. Plants play a significant role in maintaining health and developing novel therapies in traditional

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medicine [5,6,7,8]. The World Health Organization (WHO) estimates that 80% of developing countries' populations rely on herbal medicines for survival [9]. Plants produce primary as well as secondary metabolites that may be valuable for therapeutics [10]. Tannins, alkaloids, sugars, terpenoids, steroids, and flavonoids are essential phytochemical constituents in medicinal plants that contributed to the medicinal properties of the plants [11,12].

According to estimates, approximately 300,000 species of higher plants are present on earth. Biodiversity is the variety and variability of living things and the biological communities in which they coexist [5,13,14]. More than 70,000 medicinal plants are used by billions of people worldwide for medicare [15]. India is rich in plant diversity and has over 8000 critical medicinal plants. Among these, *Lycium*, a genus with over a hundred species, belongs to the Solanaceae nightshade family. *Lycium* is found in tropical climates throughout the world. The genus *Lycium* has seen a sharp rise in importance due to potent phytochemical constituents [16]. *Lycium* species are frequently utilized as a source of food and medicine. Due to the nutritional benefits of *Lycium* fruits, often known as goji or wolfberries, have recently gained popularity in the west. Gojiberry is promoted as a "superfood" in America and European countries [17]. A species of the genus *Lycium*, *Lycium shawii* (Arabian boxthorn or desert thorn) is a perennial plant found on ridges of sandstone and in arid regions. Typically, it can be found in floodplains and depression areas. In its natural habitat, flowering occurs between March and April. Traditional healers use the aerial parts (shoots and flowers) of *L. shawii* as dry powder to treat mouth sores, coughs, stomach ulcers, and jaundice [18]. The *Lycium* species exhibits a wide range of pharmacological properties, such as antimicrobial, antioxidant [19], anti-diabetic [20], anti-inflammatory, anticancer [21], anti-trypanosomal, hepatoprotective, antiplasmodial and cytotoxic properties [15]. A high surface-to-volume ratio is required to get the maximum benefits of important metabolites of medicinal plants. This can be fulfilled by using the emerging field of nanotechnology.

Nanotechnology has recently been suggested as a significant interdisciplinary field dealing with research and development in different areas [9] Nanomaterials, which have at least one dimension between 1 and 100 nm, include nanoparticles, nanocomposites, nanofibers, and nanotubes. They exhibit unique physicochemical, optical, electrical and magnetic properties based on their shape, size, and distribution compared to their bulk counterparts [22] Nanoscale structures have high surface-to-volume and aspect ratios and are appropriate for application in various materials. Numerous disciplines could benefit from nanomaterials, including information technology and photonics, energy, electronics, magnetic separation, food business, chemical sensing and imaging, diagnostics, environmental science, drug delivery and biological labeling [23].

For the synthesis of metal nanoparticles, top-down and bottom-up approaches are available. Top-down is a destructive process in which bulk material is segmented into tiny portions (i.e., micro size to nanoparticles). Bottom-up is a constructive process; in which nanoparticles can be synthesized by combining individual atoms (ions) or molecules [24]. Physical, chemical and biological methods are available to synthesize metal NPs. However, physical and chemical methods synthesize well-defined and pure nanoparticles, but these processes are more expensive, energy-intensive and perhaps hazardous to the environment [25]. Green synthesis of nanoparticles involving enzymes, microbes, fungi and plant extracts is eco-friendly and preferred over chemical and physical methods (Hasan, 2015). Phytoconstituents in plant extracts significantly help to reduce the metallic ions and subsequent stabilization of those ions. The quality, size and morphology of the generated nanoparticles are greatly affected by the properties of the plant extract, including metal salt concentration, temperature, phytochemical content, pH of the reaction solution, and reaction time [26].

Silver nanoparticles are the most substantial NPs used globally because they have a broad range of uses in various industries. It includes clothing, dentistry, photography, coating surgical instruments, the food

industry, catalysis, optics, electronics, mirrors, prosthetics, textiles, agriculture, cosmetics, wound care, etc. [27]. As we know, the antibiotics are the lifesaving drugs that are the most important things for human health. But due to the overuse or misuse of antibiotics there is a tremendous increase in the multidrug resistant human pathogens cause high level health threat. The pathogens have developed different abilities to neutralize the antibiotics [28]. The plant extract based AgNPs can be a good choice to deal with multidrug resistant pathogens. The nanoparticle can be used alone or in the combinations with antibiotics to increase their potential.

To the best of our knowledge, no effort or minimal effort have been laid down by researchers to synthesize silver nanoparticles from crude extracts of *Lycium shawii*. In the present study, the aerial part crude extract of *Lycium shawii* was utilized for qualitative and quantitative estimation of phytochemicals as well as for the synthesis of silver nanoparticles. The synthesized nanoparticles were characterized using various characterization techniques (UV Spectroscopy, Particle size analyzer, Zeta potential, XRD, FTIR, FESEM, etc.). Antibacterial, antifungal and antioxidant, properties of the green synthesized silver nanoparticles have been investigated.

2. Experimental

2.1. Plant sample collection

The sample of *Lycium shawii* was collected from the rural area of district Fatehabad, Haryana, India. The collected samples were washed with tap water, followed by distilled water and dried at room temperature for 4–5 days to remove the moisture. Dry samples were ground in a lab scale grinder to obtain the fine powder and stored in an airtight container at -20°C for further use.

2.2. Chemicals used

Dimethyl sulfoxide, DPPH (2,2-Diphenyl-1-picrylhydrazyl), Hydrogen peroxide, Methanol, Folin-Ciocalteu reagent, Gallic acid, Sodium carbonate, Quercetin, Sodium hydroxide, Sodium nitrate, Aluminium chloride, iodine, Potassium iodide, Phosphate buffer, Hydrogen peroxide, Ferric chloride, Chloroform, Sulphuric acid, Glacial acetic acid, Ascorbic acid, Mueller Hinton agar, Potato dextrose agar, Ciprofloxacin and Ketoconazole. Silver nitrate (AgNO_3) was purchased from SRL, Sigma and Himedia, India. All the chemicals were of analytical grade and used as received. All the solutions used in the present study was freshly prepared in double-distilled water and autoclaved as required.

2.3. Microorganisms

The bacterial and fungal strains used in this study were purchased from MTCC (Microbial Type Culture Collection) Chandigarh, India. The bacterial strains *Escherichia coli* (MTCC-16521), *Pseudomonas fluorescens* (MTCC- 664), *Salmonella enterica* (MTCC- 1164) are gram-negative and *Bacillus subtilis* (MTCC- 441), *Staphylococcus aureus* (MTCC- 3160) are gram-positive used in the antibacterial assay. *Candida albicans* (MTCC-183), *Rhizopus oryzae* (MTCC-262) and *Aspergillus niger* (MTCC-281) are the fungal strains used for antifungal activity.

2.4. Preparation of plant extract

The maceration method of extraction was utilized to obtain the crude extract. A total of 10 g of dried leaf powder was suspended in 100 ml autoclaved double distilled water and 80% methanol (1:10w/v) in the conical flask. The mixture was placed in a shaking incubator for 72 h at 40°C . The extract was filtered with Whatman no.1 filter paper and the filtrate was stored at 4°C [29]. The collected filtrate was concentrated in the rotatory evaporator under low pressure. The residue was put

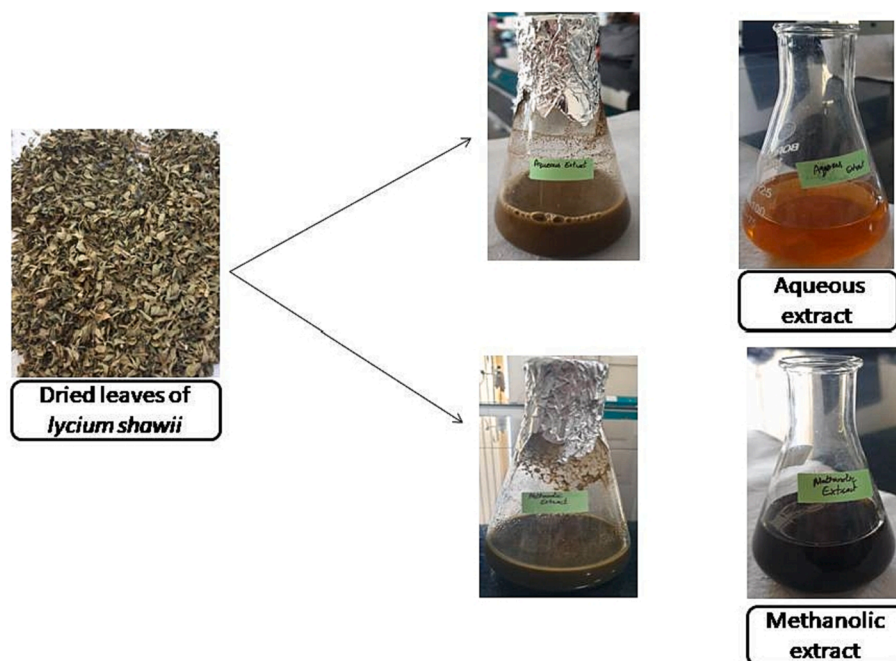


Fig. 1. The preparation of aqueous extract and methanolic extract using leaves of *Lyciumshawii*.

through two further extractions using the same solvent for the complete extraction and subsequently concentrated. Aqueous and methanolic extracts are shown in Fig. 1.

2.5. Quantitative analysis

Total phenolics and total flavonoids content were analyzed in the quantitative estimation of phytochemicals;

2.5.1. Total phenolics estimation

The total phenolics estimation was determined by the Folin-Ciocalteu reagent. A standard of gallic acid 10 mg/ml was prepared in DMSO (Dimethyl sulfoxide). A standard calibration curve of gallic acid was plotted with a dilution of 0.5 to 2 mg/ml. From each dilution, 100 μ l was taken in a separate tube and diluted with 500 μ l of distilled water. Then, add 100 μ l of Folin-Ciocalteu reagent into each tube and incubated for 6 min. Subsequently, 1000 μ l of 7% Na_2CO_3 was added and diluted with 500 μ l of distilled water. After 90 min, the absorbance was measured at 760 nm. The same procedure was repeated for the plant's aqueous and methanolic crude extract [30].

2.5.2. Total flavonoid estimation

Aluminium chloride complex forming assay evaluated the total flavonoid content. A standard of quercetin 10 mg/ml was prepared in DMSO (Dimethyl sulfoxide). A standard quercetin calibration curve was plotted with 0.5 to 2 mg/ml dilution. From each dilution, 100 μ l was taken in a separate tube and diluted with 500 μ l of distilled water. Add 100 μ l of 5% NaNO_3 and incubate for 6 min. Afterwards, 150 μ l of 10% AlCl_3 was added and after 5 min, 200 μ l of 1 M NaOH solution was consecutively added. Using a UV spectrophotometer, the OD was observed at 510 nm. The same procedure was repeated for aqueous and methanolic extracts. Triplicate readings of each experiment were undertaken [30].

2.6. Qualitative analysis of phytoconstituents

The preliminary analysis of bioactive compounds in crude extracts was conducted to depict phytoconstituents' potential. The crude extracts (aqueous and methanol extract) were used to examine the bioactive

component's presence by performing different tests. The qualitative results are represented by (+) for the presence of phytochemicals and (-) for the absence [31].

2.6.1. Test for alkaloids (Wagner test)

A total of 1.27 g of iodine and 2 g potassium iodide were suspended in 5 ml of distilled water and then diluted to 100 ml using distilled H_2O . The dark brown precipitate formed after adding a few drops of this solution to the filtrate, indicating the presence of alkaloids [31].

2.6.2. Test for glycosides (Keller-kilani test)

A 2% solution of FeCl_3 and 1–2 drops of glacial acetic acid were added to the crude extract. Then, the mixture was added to a different test tube with 2 ml of concentrated H_2SO_4 . Cardiac glycosides were detected as a brown ring at the interphase [11].

2.6.3. Test for terpenoid (Salkowski test)

Separately shaking the crude extract (approximately 100 mg) with chloroform (2 ml) and concentrated H_2SO_4 (2 ml) along the side wall of the test tube revealed the presence of terpenoid by the interface's red-dish brown coloring [31].

2.6.4. Test for tannins

A total of 0.5 g of extract in 10 ml of distilled water was mixed before filtration. Then, a few drops of 5% ferric chloride were added. The occurrence of black or blue-green precipitation was regarded as a sign that tannins were present [31].

2.6.5. Test for saponins

A total of 0.2 g of plant extract was blended with 2 ml of distilled water. Upon heating in a water bath, tiny bubbles and static froth after agitation indicated the presence of saponins in the crude extract [10].

2.6.6. Test for flavonoids

2 N sodium hydroxide mixed with plant extract in equal volume. The occurrence of yellow color indicates the presence of flavonoids [32].

2.6.7. Test for phenol

Few drops (20 μ l) of plant extract mixed with Folin and Ciocalteu's

reagent incubate for 5 min. After the addition of sodium carbonate reaction mixture was incubated for half an hour, the occurrence of blue color represents the presence of phenol in the sample [32].

2.7. Synthesis of silver nanoparticles

Aqueous AgNPs and MeOHAgNPs were prepared and used for further study. *Lycium shawii* aqueous and methanolic crude extract dissolve in DMSO at 50 mg/ml concentration. In a volume of 90 ml of 0.1 M AgNO₃ solutions mixed with 10 ml of crude aqueous extract in a flask kept in the dark at 50 °C under magnetic stirring [33]. The color change observed from transparent yellow to brown confirmed the synthesis of AgNPs. Similarly, the same procedure was followed for the methanolic extract to make AgNPs. The suspension was centrifuged at 8500 rpm for 15 min. The supernatant was discarded and the observed pellet was washed twice with double distilled water, once with 70% ethanol, to remove impurities.

2.7.1. Characterization of silver nanoparticles

Synthesized AgNPs were characterized to determine their morphology, stability, size distribution, functional moieties, and crystalline nature. Ultraviolet, visible spectrum analyzed the biosynthesis of Aq-AgNPs and MeOH-AgNPs was recorded at 200–800 nm wavelengths using a UV–vis spectrophotometer (UV-2450, Shimadzu Corporation, Tokyo, Japan). It is used to characterize the optical properties of nanoparticles. A Zetasizer Nanoseries ZS90 (Nano-ZS90, Malvern instrument Ltd, UK) was used to measure particle size distribution via dynamic light scattering and particle stability via zeta potential. Dynamic light scattering (DLS) aims to determine the size distribution and Polydispersive index. The polydispersity index revealed the hydrodynamic monodispersive nature of Aq-AgNPs and MeOH-AgNPs (PDI). Zeta potential is the charge particles acquire and helps predict the nanoparticles' long-term stability. FTIR spectrometer (Perkin Elmer Spectrum, BX II) examines the presence of different functional groups that act as capping and stabilization agents and help in the bioreduction of silver ions to Ag nanoparticles. FTIR spectrum was recorded within the 4000–400 cm⁻¹ wave number range using a potassium bromide pellet. X-ray powder diffractometer (LX-57B, Rigaku, Japan) analyzed the diffraction pattern of synthesized Aq-AgNPs and MeOH-AgNPs. XRD gives information about phase composition, crystal size, crystal structure and orientation, interatomic distances, bond angles, and lattice parameters. The diffracted intensity scan range was recorded at 2θ from 5° to 90°. Field emission scanning electron microscope (JSM-7610FPlus, JEOL, Japan) generates high-resolution images that provide morphological information of Aq-AgNPs and MeOH-AgNPs. A high energy beam of electron 15 kV was used to investigate the size and shape of NPs,

2.8. Antimicrobial activity of crude extracts and AgNPs

The crude extracts and AgNPs were exploited for antimicrobial activity by using the agar well diffusion method. Revived microorganisms, i.e., *Escherichia coli* (MTCC-16521), *Pseudomonas fluorescens* (MTCC-664), *Salmonella enterica* (MTCC-1164), *Bacillus subtilis* (MTCC-442), *Staphylococcus aureus* (MTCC-3160), *Candida albicans* (MTCC-183), *Rhizopus oryzae* (MTCC-262) and *Aspergillus niger* (MTCC-281) were used for the antimicrobial assay. A total of 100 µl of the revived culture of each strain was spreaded on Muller Hinton agar for bacteria and potato dextrose agar petri plates for fungi. Four wells were punctured in each plate using a sterile cork borer. The first well contains negative control DMSO, the second well positive control ciprofloxacin for bacteria and ketoconazole for fungi (100 mg/ml), the third well is loaded with a crude aqueous extract (100 mg/ml), while the fourth well loaded with aqueous extract AgNPs (100 mg/ml). The same procedure was adopted for methanolic extract. Petri plates were incubated at 37 °C for 24 h in an incubator for antibacterial and at 28 °C for antifungal activity. The minimum inhibitory concentration (MIC) of the nanoparticles was also

observed against different pathogens and significant results were obtained. MIC is the minimum amount of the nanoparticles that inhibit the growth of the microorganisms.

2.9. Antioxidant activity of crude extract and AgNPs

2.9.1. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH scavenging activity)

DPPH free radical scavenging method was performed according to the protocol of Sytu and Camacho [34] with minor modifications. The crude extract (aqueous and methanol extract) and AgNPs (Aq-AgNPs and MeOH-AgNPs) were suspended in distilled water at a concentration of 0.2 to 1 mg/ml and ascorbic acid was used as standard. The sample was taken in separate vials at different concentrations (0.2 to 1.0 mg/ml), and 2 ml of DPPH (0.2 mM in methanol) was added to each vial. The reaction mixture was vortexed and incubated for 30 min in the dark. The distilled water was set as blank, while DPPH served as a control. The absorbance of the reaction mixture was taken at 517 nm using a spectrophotometer (Eppendorf AG 22331 Hamburg, Germany). This formula calculated the percentage scavenging activity:

$$\% \text{ scavenging activity} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where, A_{control} = absorbance of DPPH, A_{sample} = absorbance of sample.

2.9.2. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging method was performed according to Keshari et al. [35] with minor modifications. 2 mM H₂O₂ solution was prepared using a 50 mM phosphate buffer of pH 7.4. The crude extract (aqueous and methanol extract) and AgNPs (Aq-AgNPs and MeOH-AgNPs) were suspended in distilled water at a concentration of 0.2 to 1 mg/ml and ascorbic acid was used as standard. From different dilutions of 0.2 to 1 mg/ml, take 100 µl of each. 300 µl of phosphate buffer (50 mM, pH 7.4) was added to each vial, followed by 600 µl of 2 mM H₂O₂ solution. The reaction mixture was thoroughly vortexed and incubated for 10 min at room temperature. The phosphate buffer was used as blank, while hydrogen peroxide and phosphate buffer were set as control. The absorbance of the reaction mixture was taken at 230 nm using a spectrophotometer (Eppendorf AG 22331 Hamburg, Germany). This formula calculated the percentage scavenging activity:

$$\% \text{ scavenging activity} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where, A_{control} = absorbance of phosphate buffer and hydrogen peroxide,

A_{sample} = absorbance of sample.

3. Results and discussion

3.1. Quantitative analysis

3.1.1. Total phenolic content estimation

The estimation of total phenolic content was evaluated by using the Folin-Ciocalteu method. Standard gallic acid equivalents (mgGAE/g) were used to calculate the total amount of polyphenols. Phenolic content varied in both extracts; in the aqueous extract was 6.76 mg/g and in the methanolic extract 11.11 mg/g. The methanolic extract had reported comparatively higher phenolics content than the aqueous extract. The standard curve used to determine total phenolics content was calculated using regression equation $y = 0.198x + 0.058$, R^2 0.99. The reported results were similar to prior research reports for the total phenolics content of dried fruits of *Lycium shawii* used to estimate. The content of phenolics in dichloromethane and ethyl acetate extracts in Gallic acid equivalent (GAE)/g was 377 ± 5.00 mg/g and 260 ± 4.10 mg/g, respectively [18]. Fruit extract of *Lycium barbarum* extracted using water and butanol exhibited 14.13 mg GAE/g and 47.98 mg GAE/g, respectively [36]. Mocan et al. [37] studied the functional components of leaves and reported 11.98mgGAE/g in cultivated and 2.49 mgGAE/g in

Table 1

Represents the result of the phytochemical analysis.

S.No.	Phytochemicals	Aq. Extract	Methanolic extract
1.	Alkaloids	+	–
2.	Glycosides	+	+
3.	Terpenoids	+	+
4.	Tannins	–	–
5.	Saponins	–	+
6.	Flavonoid	+	+
7.	Phenol	+	+



Fig. 2. (A) Represents the preliminary confirmation of the synthesis of Aq-AgNPs (change in color from yellow-orange to black) (B) represents the preliminary confirmation of synthesis of MeOH-AgNPs (color variation from light yellow to black).

wild *Lycium barbarum*. Mocan et al. [38] reported that the leaves of *Lycium chinense* possess 80.64 mg GAE/g. The variation in total phenolics content was observed due to different geographical areas, soil composition, rainfall, altitude, extraction solvent, and method used.

3.1.2. Total flavonoid estimation

The estimation of flavonoid content was determined by an aluminium chloride colorimetric assay. Quercetin equivalents (mg QE/g) were used to calculate total flavonoid content. The total content of flavonoids was 7.51 mg/g in the aqueous extract and 7.76 mg/g in the methanolic extract. The methanolic extract exhibited a higher amount of flavonoid. Observed results are in agreement with existing research, total flavonoid content of dried fruits of *Lycium shawii* was used for estimation. The content of flavonoids in dichloromethane and ethyl acetate extracts quercetin equivalent (mg QE/g) 2 ± 0.05 mg/g and

11.06 ± 0.01 mg/g, respectively [18]. Leaves extract of *Lycium chinense* contains 65.61 mg/g, while *Lycium barbarum* carries 43.73 mg/g [38]. Tayel et al. [39] analyzed 8.62 ± 0.72 mg/g flavonoid content in the seed extract of *Lycium shawii*. The present results show concurrence with prior reports might be due to the same plant genus, similar extraction protocols, and different content due to the use of other extraction solvents.

3.2. Qualitative analysis

Plants have traditionally been a valuable source of versatile phyto-components with medicinal benefits. This study examined the presence of different secondary metabolites, glycosides, terpenoids, flavonoids, and phenols in both crude extracts. Tannins were absent in both aqueous and methanolic extract, alkaloids in methanolic extract, and saponins in aqueous extract. The results of this report favor existing research that leaves of *Lycium ruthenicum* contain alkaloids significantly less in amount; therefore, saponins were absent [40]. The qualitative results of phytochemicals are summarized in Table 1.

3.3. Green synthesis and characterization of AgNPs

Green synthesis of AgNPs was carried out using plant extract (crude aqueous and methanolic extract) helps in the bioreduction of Ag^+ in a solution of AgNO_3 . Primarily, the change in color from light yellow to blackish-brown confirmed the synthesis of AgNPs (Fig. 2). The color variation was due to the excitation of electron surface plasmon resonance. The synthesized Aq-AgNPs and MeOH-AgNPs exhibit bell-shaped absorption peaks of 409 nm with an absorbance of 0.204 and 447 nm with 2.129, respectively (Fig. 3). The absorption peak's appearance at this range confirms the synthesis of silver nanoparticles. The phyto-constituents in the leaf extract of *Lycium shawii* may be responsible for the bioreduction since they participate in electrochemical reactions and facilitate the synthesis and stability of AgNPs through various plant metabolites [22]. Earlier, *Lycium barbarum* dried fruits were used to synthesize silver nanoparticles, showing an absorption peak at 438 nm [41]. The previous study suggested that the absorption peak range from 390 nm to 420 nm had small size nanoparticles, whereas particles showing absorption peak near 450 nm and above exhibit variable size nanoparticles [42].

The polydispersive index and average hydrodynamic diameter of synthesized AgNPs were determined by dynamic light scattering. The

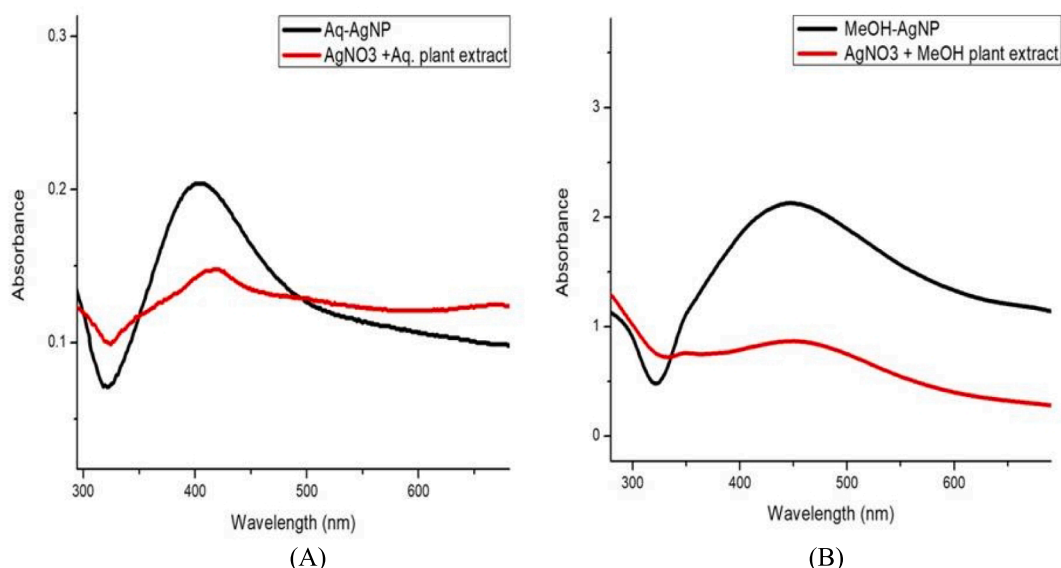


Fig. 3. Representing the Uv-vis spectrum (A) Absorption peak of Aq-AgNPs at 409 nm (B) Absorption peak of MeOH-AgNPs at 447 nm.

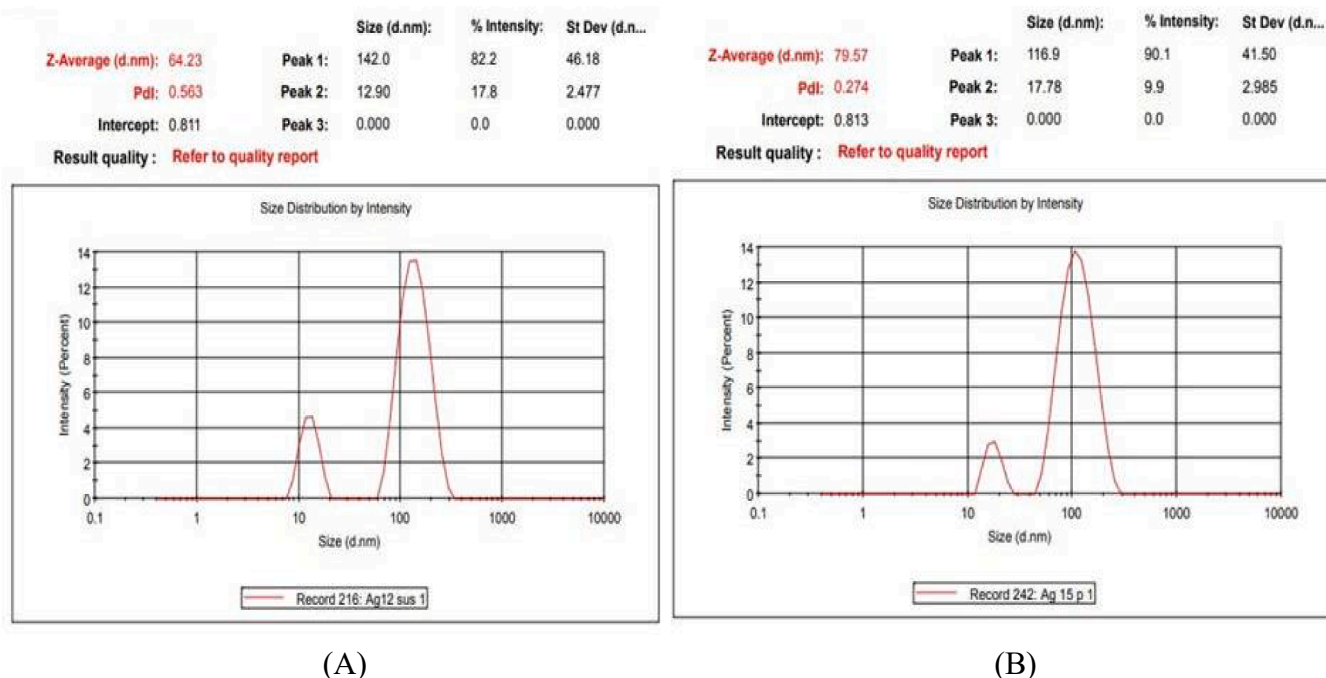


Fig. 4. Showing the size distribution of Aq-AgNP (A) and MeOH-AgNP (B) examined by particle size analyzer.

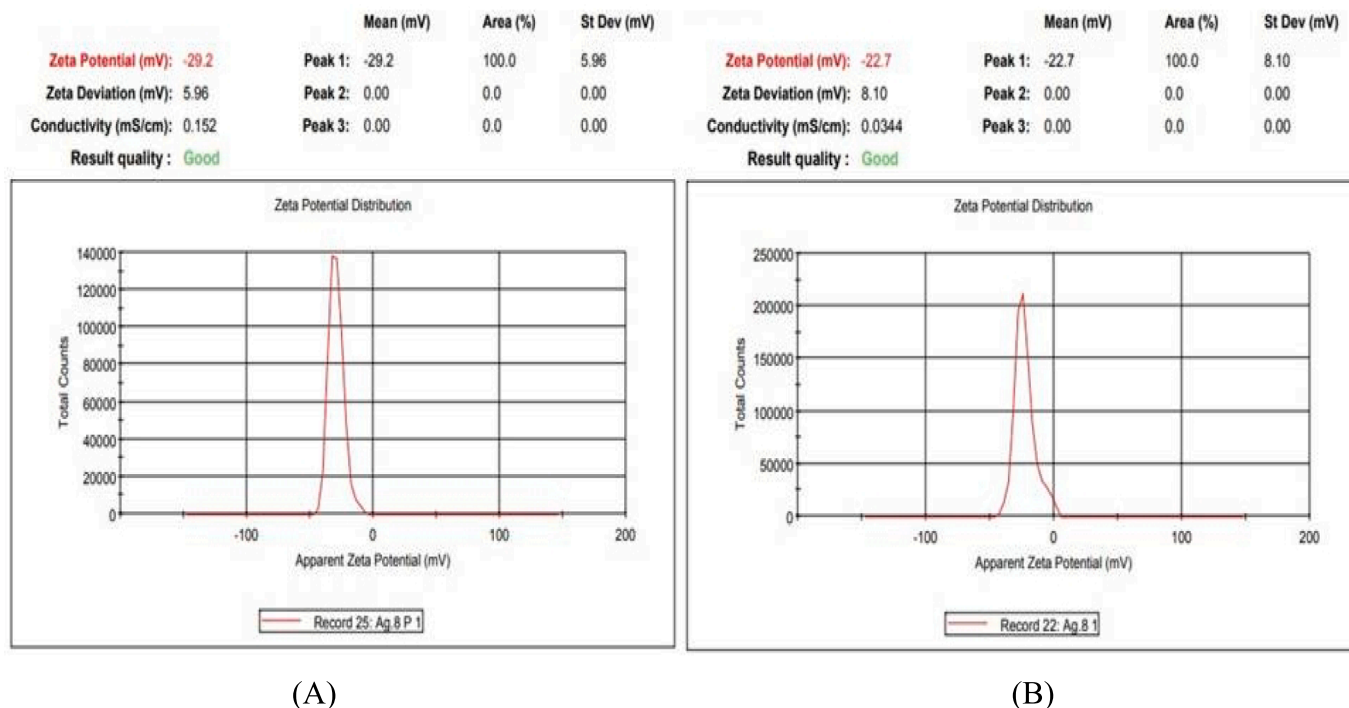


Fig. 5. Representing the zeta potential of Aq-AgNPs (A) and MeOH-AgNPs (B) examined by dynamic light scattering.

DLS revealed the size of Aq-AgNPs lies between 12 nm and 142 nm while the z-average was 64 nm, whereas MeOH-AgNP's size ranged from 17 nm to 116 nm and the z-average was 79 nm (Fig. 4). The resulting size depends on the size of the AgNPs metal core, the biomaterial absorbed onto their surface, and the electric double layer (solvent wall) that flows between particles [43]. The PDI value of synthesized Aq-AgNPs and MeOH-AgNPs was 0.563 and 0.274, respectively, implying the nanoparticles' monodispersive nature. As the PDI value exceeds 0.7, it indicates an unfavorable broad particle size distribution. The usual range

of PDI ranges between 0.05 and 0.7, while PDI values near 0 reveal a monodispersive nature, and 1 shows the polydispersive nature of nanoparticles [44,45]. The zeta potential value was recorded as -29.2 mV for Aq-AgNPs and -22.7 mV for MeOH-AgNPs (Fig. 5). The particles with a negative charge tend to be more stable as they are restricted from aggregation. The observed results are similar to the previous information reported by experts, the negative zeta potential of -29.1 mV [46] and -22.75 mV [47] for biofabrication of silver nanoparticles using different plant extract.

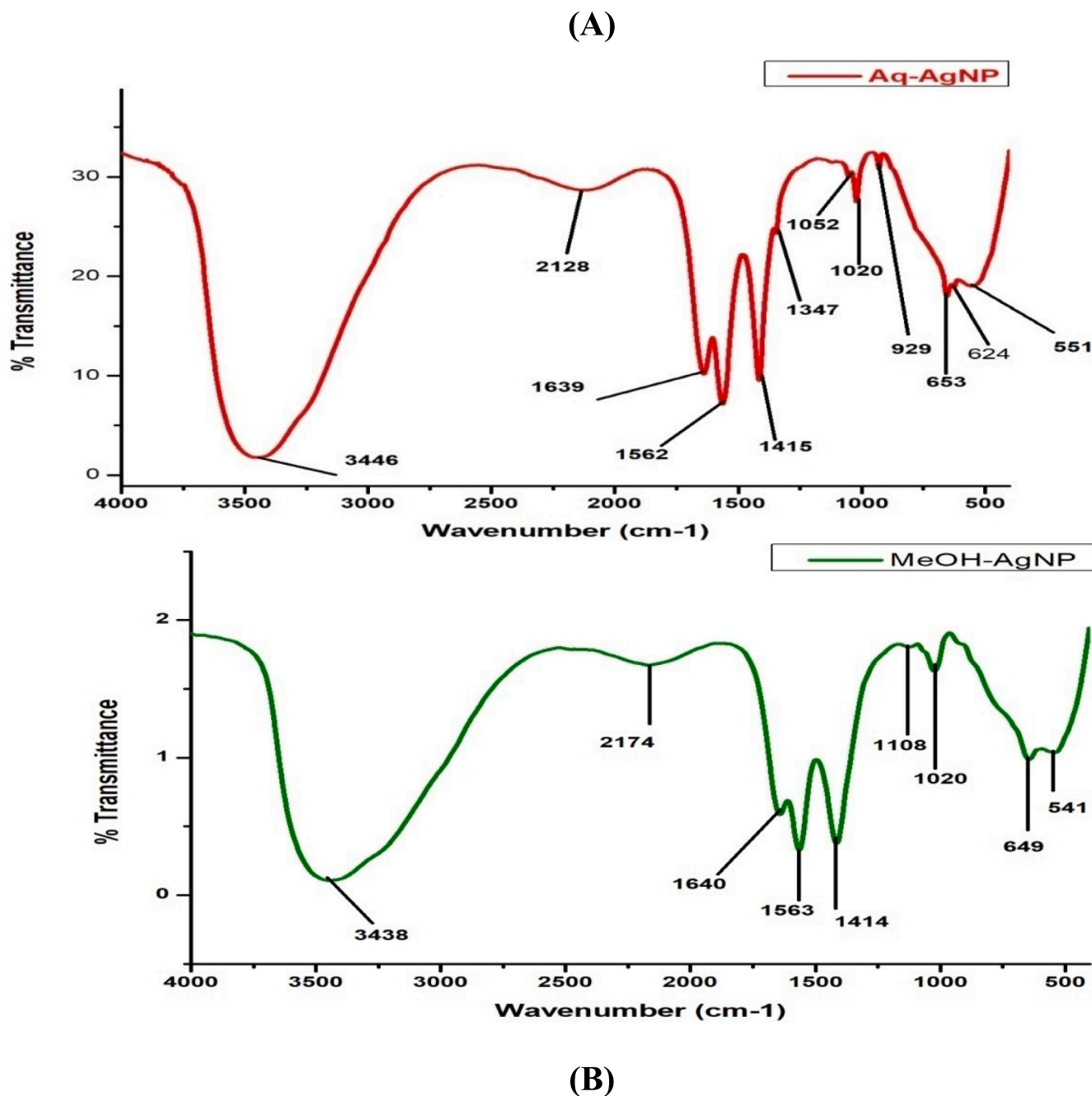


Fig. 6. Showing the spectral analysis of the FTIR (A) spectrum of the Aq-AgNPs (B) spectrum of MeOH-AgNPs.

Furthermore, FTIR analysis demonstrates the presence of biomolecules in leaf extract of *Lycium shawii* interaction of functional group with synthesized AgNPs cause shifting of different peaks. The spectral peaks of Aq-AgNPs were 3446, 2128, 1639, 1562, 1415, 1347, 1052, 1020, 929, 653, 624 and 551 cm^{-1} . Similarly, MeOH-AgNPs exhibit spectral peaks at 3438, 2174, 1640, 1563, 1414, 1108, 1020, 649 and 541 cm^{-1} . The observed graphs of FTIR for nanoparticles for both aqueous and methanolic extracts are shown in Fig. 6. The FTIR absorption band of Aq-AgNPs and MeOH-AgNPs in the range of 3600–3200 cm^{-1} indicates the H-bonding confirmed the presence of ammonium, amino, hydrate and hydroxyl compound. The spectral peaks at 2128 and 2174 cm^{-1} identify triple bonds between carbons ($\text{C}\equiv\text{C}$). The -C=N- stretching of open-chain imino compounds and the -N=N- stretching of open-chain azo compounds may be responsible for the peak in a range of 1690–1575 cm^{-1} . The fingerprint region's peaks lie

between 1420 and 1410 cm^{-1} / 1490–1410 cm^{-1} , revealing carbonate ion and vinyl C–H in-plane bend. The absorbance band at 1140–1070 cm^{-1} indicated cyclic ethers and large rings, 1150–1000 cm^{-1} identified C–O stretch, and 1360–1310 cm^{-1} suggested the presence of aromatic tertiary amine and CN stretch. Another intense peak is 995–850 cm^{-1} , 700–600 cm^{-1} and 600–500 cm^{-1} representing aromatic phosphates, C–Br stretch and C–I stretch, respectively. The FTIR spectrum analysis identified the biomolecules involved in the bioreduction of Ag^+ to Ag^0 , which act as capping and stabilizing agents [48].

X-ray diffraction was conducted to know the crystalline structure of silver nanoparticles. Pictorial representations of X-Ray diffraction spectra for Aq-AgNPs and MeOH-AgNPs are mentioned in Fig. 7. The diffraction spectra of Aq-AgNPs exhibit distinct peaks 38.10°, 44.23°, 64.49°, 77.36° and 81.47° corresponded to phase planes 111, 200, 220, 311 and 222, respectively, revealed the FCC structure of Aq-AgNPs.

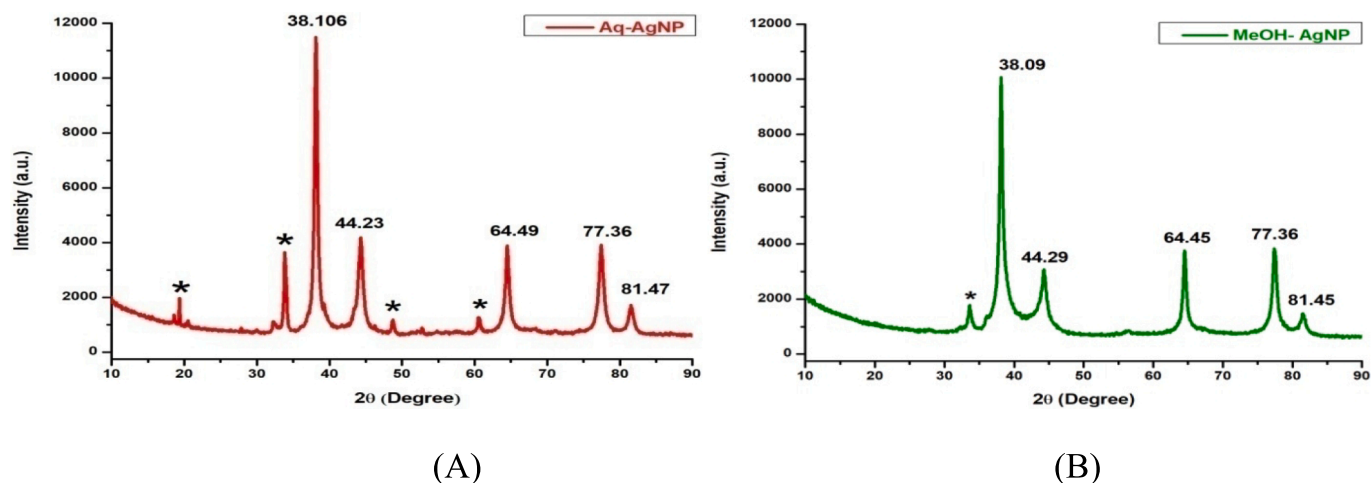


Fig. 7. Pictorial representations of X-Ray diffraction spectra (A) Aq-AgNPs (B) MeOH-AgNPs.

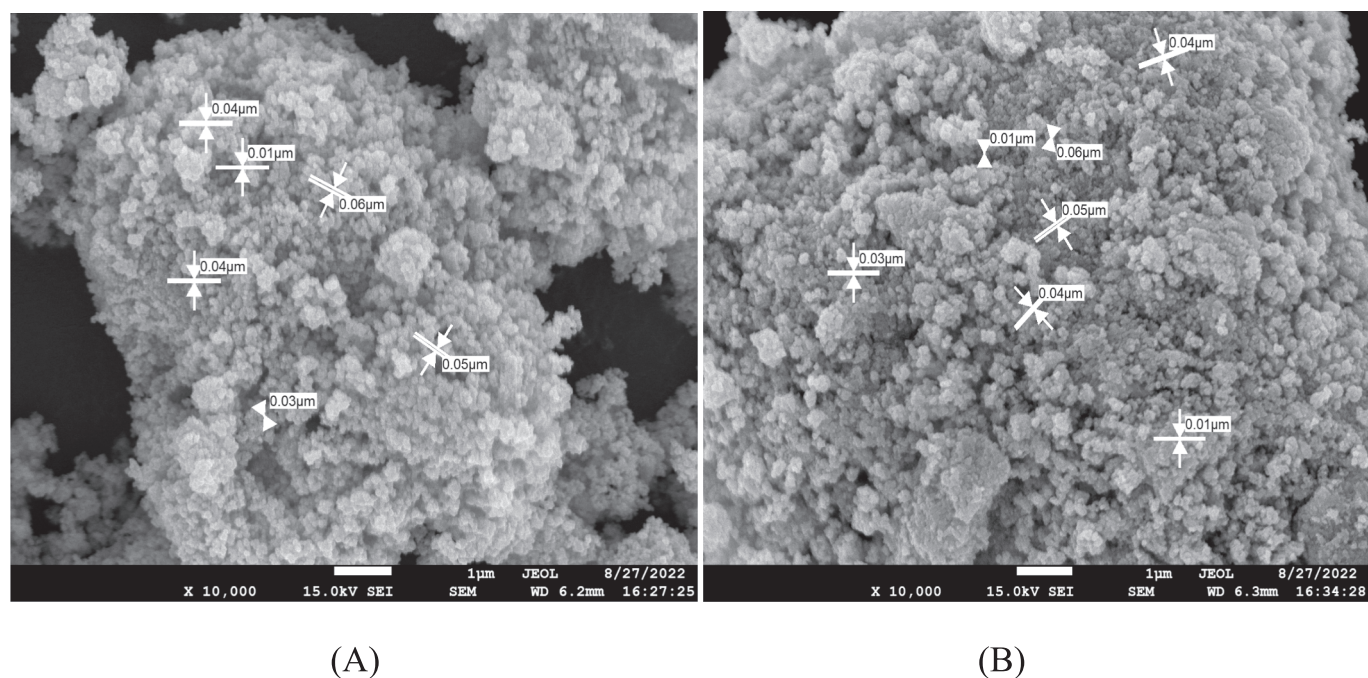


Fig. 8. Showing the size and shape of (A) Aq-AgNPs and (B) MeOH-AgNPs examined by FESEM.

Likewise, MeOH-AgNPs show diffraction peaks at 38.09° , 44.29° , 64.45° , 77.36° and 81.45° their respective planes 111, 200, 220, 311 and 222. Other peaks marked with * were due to the presence of bio-inorganic material. The resultant values assured the FCC structure and confirmed the crystalline nature of Aq-AgNPs and MeOH-AgNPs. The obtained result favors the existing report [41] that synthesized silver nanoparticles using *Lycium barbarum* dried fruits. Dong et al. [49] synthesized silver nanoparticles from fruit extract of *Lycium barbarum* and confirmed the crystalline structure of AgNPs.

FESEM analyzes the surface morphology, shape and size of silver nanoparticles. The size of Aq-AgNPs ranges from 10 nm to 60 nm and MeOH-AgNPs from 10 nm to 60 nm (Fig. 8). FESEM images show aggregated and individual nanoparticles. Aq-AgNPs and MeOH-AgNPs exhibit spherical shapes.

3.4. Antimicrobial efficacy of crude extract and AgNPs

Agar well diffusion method was used to check the efficacy of crude

extracts (aqueous and methanolic) and AgNPs (Aq-AgNPs and MeOH-AgNPs) act as an antimicrobial agent against pathogenic microbes. After incubation, aqueous extract (50 mg/ml) exhibited a zone of inhibition (ZOI) of 4.6 ± 0.4 mm for *E.coli*, 6 ± 0.81 mm for *P. fluorescens*, 13 ± 0.18 mm for *S. enterica*, 4.5 ± 0.7 for *B. subtilis* and 6 ± 0.70 for *S. aureus*, 7.3 ± 0.62 for *R. oryzae*, 7 ± 0.81 mm for *A. niger* and no zone of inhibition was observed against *C. albicans*. AgNPs are employed for biocidal activity because of their stability and potential for use against gram + ve and gram -ve bacterial cells [9]. Aq-AgNPs (50 mg/ml) has revealed an increase in zone of inhibition 11 ± 0.8 mm for *E.coli*, 12 ± 0.47 mm for *P. fluorescens*, 13 ± 0.23 mm for *S. enterica*, 7.8 ± 0.62 for *B. subtilis* and 8.3 ± 0.47 for *S. aureus*, 13.83 ± 0.23 for *R. oryzae*, 10.5 ± 0.7 mm for *A. niger* and 5.6 ± 0.94 mm for *C. albicans*.

Similarly, methanolic extract (50 mg/ml) showed ZOI 6.1 ± 0.23 mm for *E.coli*, 6 ± 0.7 mm for *P. fluorescens*, 17.6 ± 0.94 mm for *S. enterica*, 6.5 ± 0.7 mm for *B. subtilis* and 6.3 ± 0.47 mm for *S. aureus*, 6.83 ± 0.62 mm for *R. oryzae*, 9.6 ± 0.23 mm for *A. niger* and 2.83 ± 0.4 mm for *C. albicans*. To get excellent biocidal efficacy MeOH-AgNPs (50

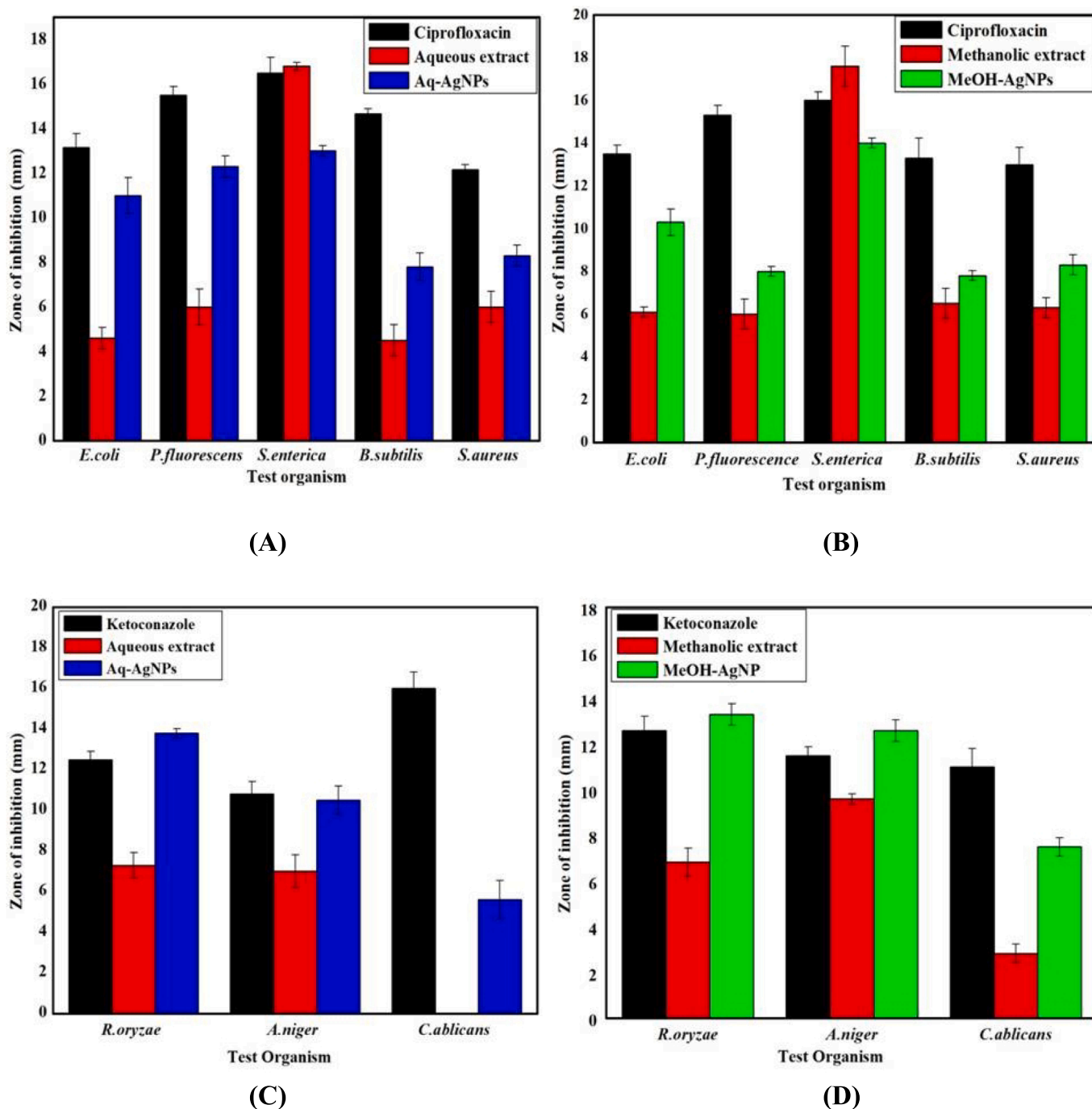


Fig. 9. Representing the standard error bar graph for Antimicrobial activity. Graphs (A) and (B) express antibacterial activity. Diagrams (C) and (D) showing antifungal activity.

mg/ml) were evaluated and the observed zone of inhibition was 10.3 ± 0.62 mm for *E.coli*, 8.1 ± 0.23 mm for *P. fluorescens*, 14 ± 0.23 mm for *S. enterica*, 7.8 ± 0.23 mm for *B. subtilis*, 8.3 ± 0.47 mm for *S. aureus*, 13.3 ± 0.47 mm for *R. oryzae*, 12.6 ± 0.4 mm for *A. niger* and 7.5 ± 0.4 mm for *C. albicans*. The graphical representation of the observed antimicrobial results are shown in Fig. 9. Maximum ZOI was observed against *S. enterica* for crude extract (aqueous and methanolic) and AgNPs (Aq-AgNPs and MeOH-AgNPs) among the used microbes. In case of *S. enterica*, aqueous and methanolic extract exhibit more significant activity than the positive control (Ciprofloxacin). Methanolic extract and MeOH-AgNPs exhibit more significant activity than crude aqueous extract and Aq-AgNPs. Small AgNPs can have a greater bactericidal

impact than large due to the greater surface area available for interaction and simpler absorption [50]. The results of present research favor the existing report on the biofabrication of AgNPs using *Lycium barbarum* shows effective results against *Salmonella enterica* [18]. The mechanism followed by AgNPs is described as penetrating the cell membrane, causing intracellular ATP leakage and cell, which alters the membrane's permeability [51]. The pictorial representation of the zone of inhibition shown in Fig. 10.

MIC of the crude plant extracts and nanoparticles were also reported. It was found that nanoparticles from both the extracts exhibited quite low MIC as compared to the crude extracts. The observed results clearly indicated that the synthesized nanoparticles have strong antimicrobial



Fig. 10. Pictorial representation of Antibacterial activity.

Table 2

Observed MIC of Crude Aqueous Extract, Crude methanolic Extract, Aq-AgNPs and MeOH-AgNPs against different human pathogens.

	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. enterica</i>	<i>P. florescence</i>	<i>S. aureus</i>	<i>R. oryzae</i>	<i>A. niger</i>	<i>C. albicans</i>
Crude Aqueous Extract	12 mg/ml	13 mg/ml	2 mg/ml	10 mg/ml	2 mg/ml	15 mg/ml	16 mg/ml	12 mg/ml
Aq-AgNPs	1 mg/ml	0.8 mg/ml	3 mg/ml	1 mg/ml	0.8 mg/ml	2.0 mg/ml	1.5 mg/ml	1.2 mg/ml
Crude methanolic Extract	9 mg/ml	10 mg/ml	10 mg/ml	12 mg/ml	2 mg/ml	13 mg/ml	15 mg/ml	10 mg/ml
MeOH-AgNPs	0.8 mg/ml	0.8 mg/ml	4 mg/ml	2 mg/ml	1 mg/ml	1.5 mg/ml	1.2 mg/ml	1.5 mg/ml

potential. Hence can be used as a good source for the development of new drugs against resistant microbes. The observed MIC against different pathogens is mentioned in Table 2.

3.5. Antioxidant activity of crude extract and AgNPs

The results revealed by DPPH and hydrogen peroxide methods confirmed the scavenging and antioxidant potential of crude extracts (Aqueous and methanolic extract) and AgNPs (Aq-AgNPs and MeOH-AgNPs) in contrast with ascorbic acid. By the DPPH method, scavenging activity was calculated as 45.94 ± 1.33 % for aqueous extract, 51.44 ± 0.62 % for Aq-AgNPs, 95.42 ± 0.57 % for methanolic extract, 82.95 ± 1.92 % for MeOH-AgNPs and 98.1 ± 0.12 % for ascorbic acid at 1 mg/ml concentration (Fig. 11). The results of present work are concurrent with the prior studies, as Kharat et al. [52] analyzed scavenging potential 72 % and 85 % for aqueous extract of *Elephantopus scaber* and AgNPs, respectively, at 250 μ g/ml concentration. With an increase in nanoparticle and crude extract concentration, the percentage of DPPH scavenging activity rose linearly [53]. Instead of this, the hydrogen peroxide method confirmed scavenging potential at 1 mg/ml as 73.42 ± 0.88 % for aqueous extract, 75.95 ± 0.69 % for Aq-AgNPs, 81.33 ± 0.40 % for methanolic extract, 82.32 ± 0.58 % for MeOH-AgNPs and 78.74 ± 0.41 % for ascorbic acid (Fig. 12). While methanolic extract, MeOH-AgNPs exhibit more significant scavenging potential than aqueous extract and Aq-AgNPs. The results revealed by Keshari et al. [35] exhibited 45.41 % scavenging activity of *Cestrum*-mediated AgNPs at 250 μ g/ml. Bedlovicova et al. [54] suggested that the adsorption of bioactive chemicals from leaf extract onto spherically shaped nanoparticles is the cause of the enhanced antioxidant capabilities of

nanoparticles compared to extract. The different phytochemicals, phenolics, flavonoids, alkaloids, saponins, and tannins were present in crude extract, which act as capping and stabilizing agents for synthesizing Aq-AgNPs and MeOH-AgNPs. Meanwhile, due to the presence of phytoconstituents, the antioxidant activity of NPs gets enhanced [55].

4. Conclusion

In this present study, leaves of *Lycium shawii* were used to analyze the qualitative and quantitative content of bioactive compounds. *L. shawii* aqueous and methanolic extract successfully synthesized Aq-AgNPs and MeOH-AgNPs as the green synthesis is cost-effective, environment-friendly and simple. Further, nanoparticles were characterized using bio-analytical tools ultraviolet spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, particle size analyzer, zeta potential and field emission scanning electron microscope. Different biological activities of extracts and nanoparticles were evaluated, like antibacterial, antifungal, DPPH scavenging activity, and hydrogen peroxide scavenging activity of extracts and nanoparticles. Significantly, methanolic extract and MeOH-AgNPs exhibit more significant activity among aqueous extract and Aq-AgNPs. The crude extract and nanoparticles showed remarkable biological activities. Overall the green synthesized nanoparticles from *Lycium shawii* can be used alone or in combination with antibiotics to combat the effect of multidrug-resistant pathogens.

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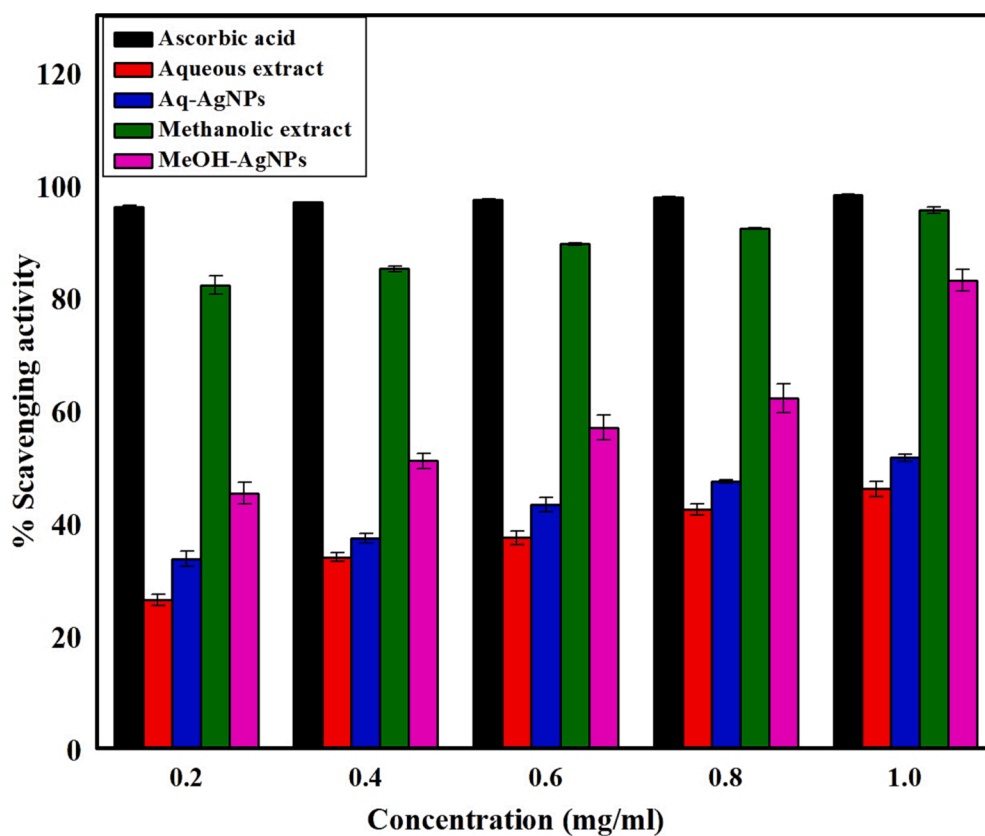


Fig. 11. Standard error bar of antioxidant activity depicted by the DPPH method.

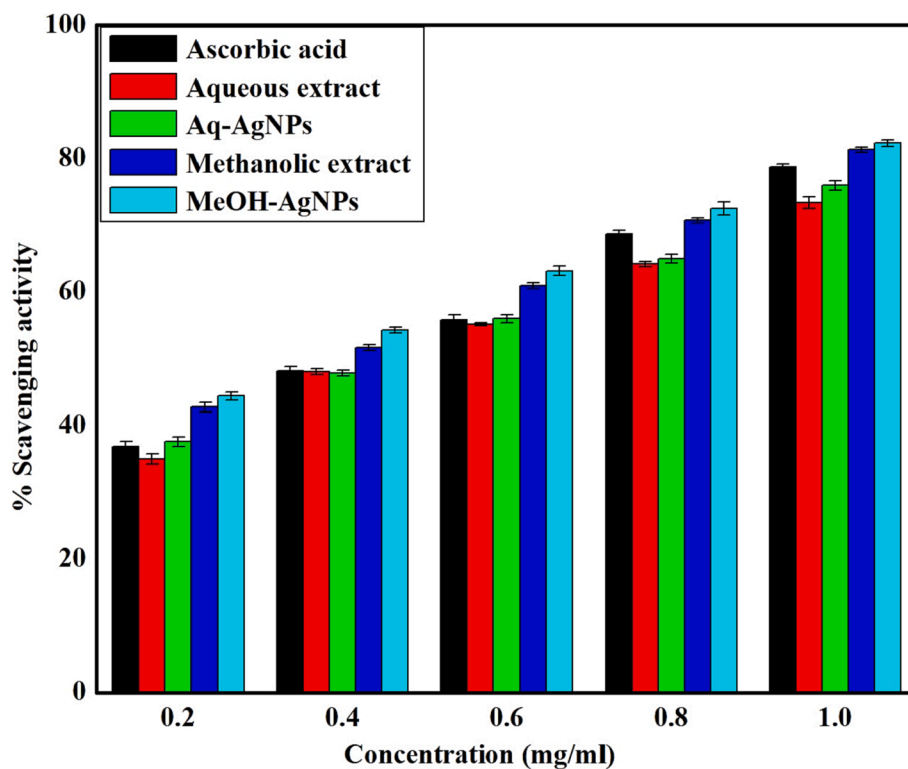


Fig. 12. The standard error bar of antioxidant activity analyzed by the hydrogen peroxide method.

CRediT authorship contribution statement

Navdeep Kaur: Conceptualization, Investigation, Methodology, Writing – original draft. **Ravinder Kumar:** Conceptualization, Formal analysis, Investigation, Writing – original draft. **Sarita Alhan:** Formal analysis, Validation. **Himanshu Sharma:** Formal analysis. **Nitesh Singh:** Formal analysis, Writing – review & editing. **Rajesh Yogi:** Investigation, Writing – review & editing. **Vinod Chhokar:** Conceptualization, Writing – review & editing. **Vikas Beniwal:** Writing – review & editing, Validation. **Mithun Kumar Ghosh:** Methodology, Validation. **Sandip Kumar Chandraker:** Methodology, Validation. **Sarvesh Rustagi:** Methodology, Validation. **Anil Kumar:** Conceptualization, Formal analysis, Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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