Phyto-extract-mediated Synthesis of Silver Nanoparticles using *Blumea lacera* Leaf Extract and Antimicrobial Activity Screening

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Green synthesis of silver nanoparticles (AgNPs) has been proven as an alternative method owing to its environmentally benign nature. This study used an eco-friendly method utilising *Blumea lacera* leaf extracts to prepare AgNPs which were then characterized by UV-Vis, FTIR, XRD, SEM, EDX, SAED and TEM, and evaluated for their antimicrobial activity. The formation of *Bl*-AgNPs was primarily confirmed by the UV-Vis absorption maximum at 454 nm, while FTIR spectra confirmed the presence of biomolecules, e.g., ketones, ethers, esters, flavonoids, polyphenols or alkaloids that facilitated the green synthesis of AgNPs. SEM and TEM analyses determined that AgNPs had a spherical shape, with an average size of 19 ± 7 nm. Antibacterial tests were performed using disc diffusion and macro-broth dilution assays against both gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli, Salmonella typhi, Pseudomonas sp.*) bacterial strains, in which *Bl*-AgNPs showed substantial antibacterial activity. Antifungal tests conducted on *Aspergillus nigar* and *Candida albicans* fungal strains showed that their growths were inhibited by up to 72.97 % and 78.19 %, respectively, at a concentration of 650 µg/mL. *Bl*-AgNPs exhibited good antimicrobial action in a dose-dependent manner. The present study demonstrates that *Bl* leaf extracts may be employed to fabricate stable AgNPs for use in biomedical materials.

Keywords: Green synthesis; ·*Blumea lacera;* Leaf extract; Silver nanoparticles; Antibacterial; Antifungal

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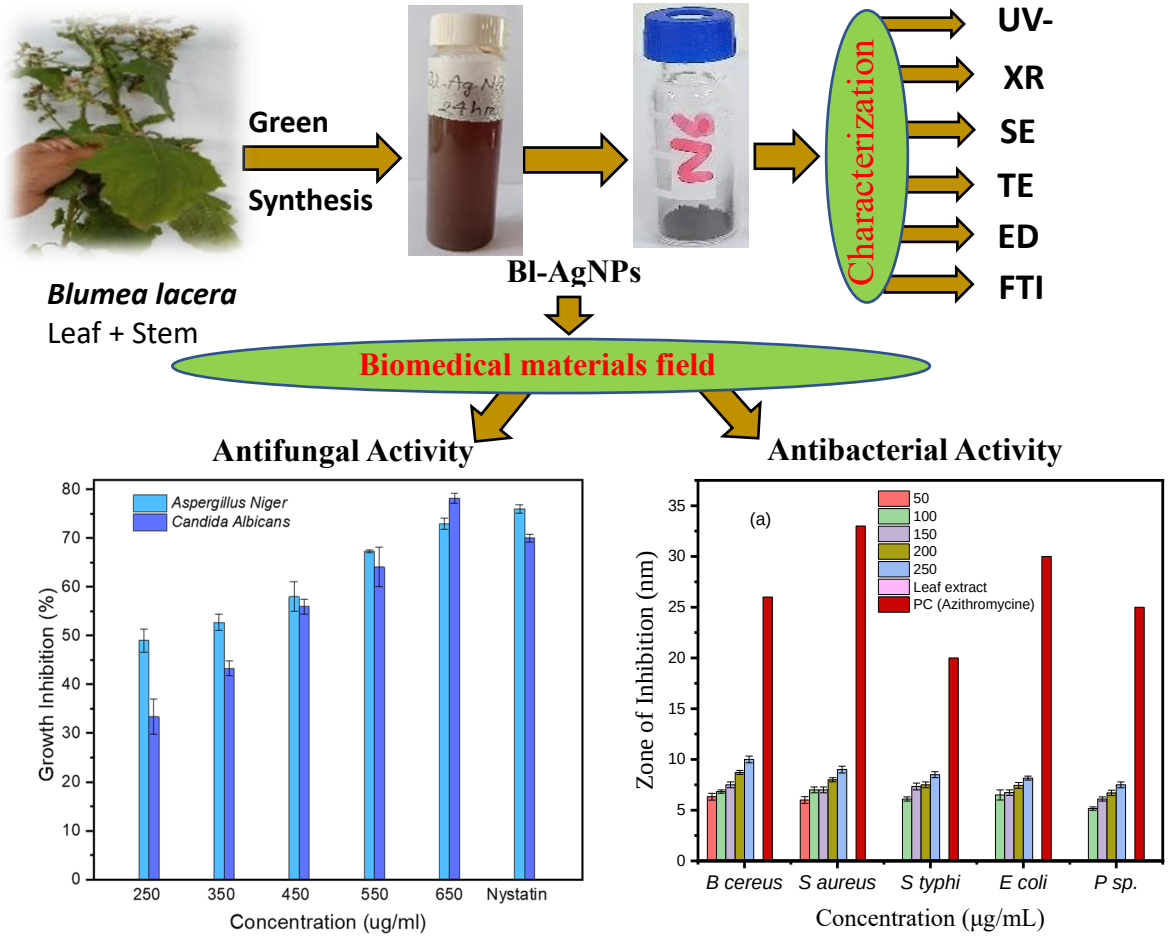
Highlights

- Green synthesis of stable silver nanoparticles (AgNPs) from *Blumea lacera* aqueous leaf extract by simple treatment of silver nitrate under mild reaction conditions, without using alkali or acid.
- SEM and TEM analysis confirmed spherically shaped AgNPs of 19 ± 7 nm.
- EDX, XRD and SAED analysis revealed the formation of pure and crystalline AgNPs.

- Synthesized AgNPs showed good antibacterial and antifungal properties.

Graphical Abstract



Nanotechnology is an interdisciplinary research area that involves the creation of nanostructures in the range of 1-100 nm. This technology involves research on the fabrication of noble metal nanoparticles (NPs) [1]. The synthesis and applications of metal NPs are now a growing field in nanoscience. Among the noble metal NPs, silver nanoparticles (AgNPs) have been synthesized and investigated widely because of their versatility [2]. Physical methods e.g., laser ablation, vapour-phase deposition, plasma-assisted vapour and chemical methods, are mainly used to synthesize NPs [3, 4]. However, physical routes need large spaces, a long time, and involve sophisticated machinery which is costly [5]. On the other hand, chemical synthesis involves toxic and hazardous materials, causing huge amounts of waste materials, in addition to preparative methods that are time-consuming and costly. Furthermore, chemically synthesized NPs may have a chemical layer on their surfaces which makes them unsuitable for medical purposes [6]. To overcome these drawbacks, researchers have widely used green methods to synthesize metal NPs due to their simplicity and environmentally benign nature [7].

Bacteria, fungi, plants, cellulosic materials, yeast, algae, and actinomycetes have been used as green materials to synthesize metal NPs [8, 9]. Among these green materials, the synthesis of metal NPs using plant extracts requires less time and can be easily handled [10]. Notably, different parts of plants such as leaves, seeds, bark, stems, fruits, flowers, rhizomes, peels and latex have been used for the biosynthesis of metal NPs [11-15]. Secondary metabolites such as terpenoids, flavonoids, phenolics, terpenoids, polysaccharides, flavones, alkaloids, amino acids and alcoholic compounds are primarily responsible for reducing metals into metal NPs while also acting as capping and stabilizing agents [16, 17]. There have been numerous studies focused on the biosynthesis of AgNPs using aqueous leaf extracts such as *Acalypha indica*, [16], *Azadirachta indica* [18], *Argemone mexicana and Turnera ulmifolia* [19], *Camellia sinensis* [20], *Desmodium triflorum* [21], *Dioscorea batatas* [22], *Morinda lucida* [23], *Ocimum sanctum* [24],

Pedalium murex [25], *Punica granatum* [26], *Pterocarpus santalinus* [27], and *Tribulus terrestris* L. [28] This highlights the broader relevance and importance of green nanotechnology. In fact, potential antimicrobial, antioxidant, and even biocompatibility properties have been observed very recently in rat models using bio-inspired AgNPs which are relevant to the present study [29-33].

Blumea lacera (*Bl*) is a yearly flowering plant known for its strong turpentine scent. It belongs to the genus *Blumea* and the family *Asteraceae*. In Bangladesh, it is recognized by various names such as bonomula, siyalmutra, shealmoti, tamrachura, and kukursunga. *Bl* is a common herb that is found in Africa, tropical Asia, New Guinea and northern Australia. It is also found in Sundarban and the hilly area of Chattrogram in Bangladesh [34] and is abundant in the Chittagong University campus. Most importantly, past studies have found the presence of various phytochemicals viz., flavonoids, alkaloids, terpenoids, glycosides, phenolic compounds, carbohydrates, saponins, amino acids, reducing sugars and steroids in *Bl* leaf extracts [35]. Therefore, it is anticipated that such phytochemicals have reduction potential, stabilizing properties and capping capability towards AgNPs. Recently, Pandey *et al*. reported the synthesis of *Bl*-AgNPs with an average size of 12.52 nm using a leaf extract of *Bl* with a pH value of 9, which exhibited good cytotoxic activity against the lung cancer cell A549 [36]. The synthesized *Bl*-AgNPs also exhibited better antioxidant activity than the pure *Bl* extract. Dubey et al. reported the synthesis of *Bl*-mediated AgNPs using an ethanolic extract of *Bl* with an adjusted pH value of 6.5 at 60 °C [37]. The synthesized AgNPs showed good antioxidant, antibacterial, and in-vitro anti-inflammatory activity. However, the synthesis of *Bl*-AgNPs using a water extract of *Bl* without the use of alkali/acid, as well as its effectiveness as an antibacterial and antifungal agent have not been explored to date. Recently, we demonstrated a simple, one-step biosynthesis of stable AgNPs from three plant species, using *Ophiorrhiza mungos, Ophiorrhiza harrisiana* and *Ophiorrhiza rugosa* leaf extracts as the reducing and capping agents to control particle size without using alkali/acid [38]. The biosynthesized AgNPs showed effective antimicrobial activity against a wide range of bacteria and fungi.

Owing to the potential effectiveness of the phytochemicals present in *Bl* leaf extracts [35, 37], the goal of the present study was to examine the utilization of *Bl* leaf extract, using water as a reducing and capping agent, in the synthesis of AgNPs without alkali/acid. To assess the potential application of the synthesized AgNPs in the field of biomedical materials, particularly in the treatment of microbial diseases, the antimicrobial activity of the *Bl*-AgNPs was evaluated for both gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli, Salmonella typhi, Pseudomonas sp*.) bacterial strains using disc diffusion and macro-broth dilution assays. The antifungal activity was also evaluated for the *Aspergillus nigar* and *Candida albicans* fungal strains using the poisoned food technique.

MATERIALS AND METHODS

1. Materials

Silver nitrate was purchased from Merck, Germany. Trypan blue stain solution (4%) was purchased from Bio-Rad, Hercules, CA, USA. Bactotrypton, yeast extract and bacto agar were purchased from Difco Laboratories, Detroit, MI, USA. Sodium chloride was purchased from Wako Pure Chemical Company, Osaka, Japan. Fresh leaves of *Bl* were collected from the hilly area of the University of Chittagong (CU), Bangladesh. Distilled water (DI) was used for all experiments. The *Bl* leaves were identified and authenticated by the Department of Botany, University of Chittagong, Bangladesh. All glassware was washed with DI and dried in the oven before use.

2. Preparation of Leaf Extract

Preparation of the leaf extract was carried out as described in our earlier work [38]. **Figure 1** shows the schematic illustration of the synthesis of *Bl*-AgNPs. The fresh leaves were washed with water (tap and bi-distilled) thoroughly several times to prevent contamination, and then dried under sunlight. The dried leaves were ground to a fine powder which was kept in a sampling bottle at room temperature. Subsequently, 1 g of leaf powder was mixed with 100 mL DI under constant stirring (80 °C for 30 min). The supernatant was separated using Whatman No. 1 filter paper after cooling.

3. Synthesis of *Bl*-AgNPs

The fresh leaf extract was added slowly (20 min) into the $AgNO_3$ (1 mM) solution at a 20:1 ratio for the reduction of Ag ions. The mixture was stirred for 60 minutes with a hotplate magnetic stirrer at 80 °C. The formation of Ag ions (Ag^+) to AgNPs (Ag^0) was primarily confirmed by the change in colour of the mixture, from yellowish-brown to reddish-brown or colloidal brown. The dilute colloidal suspension was cooled to room temperature and left for 24 hours for complete bio-reduction, followed by centrifugation at 5000 rpm for 20 min. After washing, the separated *Bl*-AgNPs were dried overnight at 60 °C and stored at room temperature for future use.

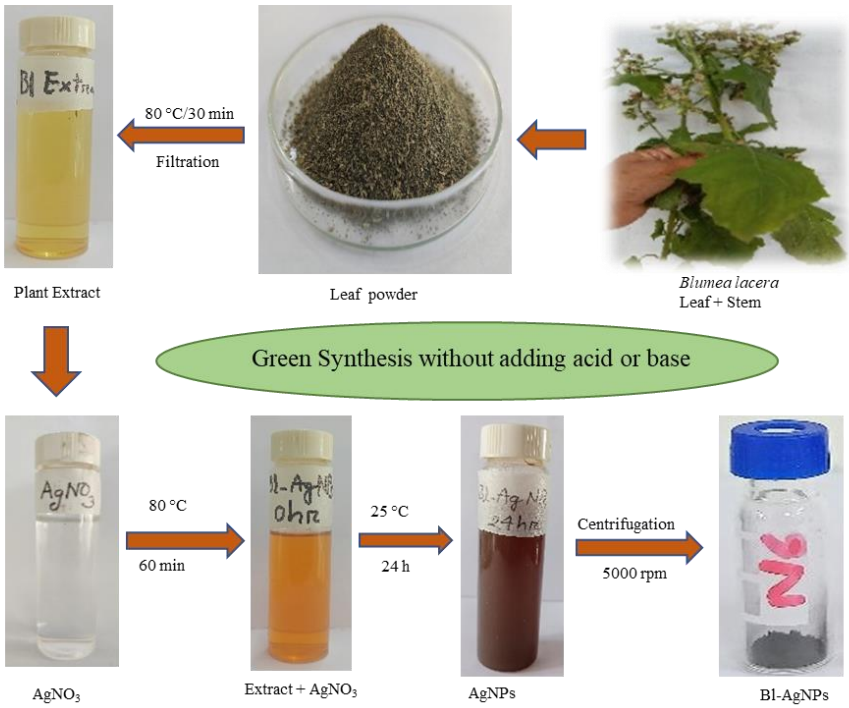


Figure 1. Schematic illustration of the synthesis of *Bl*-AgNPs.

4. Characterization Methods

Electronic spectra were obtained using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). X-ray diffraction (XRD) was performed using a Rigaku Ultima IV diffractometer with Cu-Kα (λ = 1.5406 Å) radiation at 0.2 min^{-1}. Fourier Transform Infrared Spectrophotometer (FTIR) spectra were recorded using KBr discs on an IR Prestige (Shimadzu) spectrometer. Scanning Electron Microscope (SEM) micrographs were obtained with JSM-6490LA. Transmission Electron Microscope (TEM) micrographs were obtained with FEI (Bellaterra, Spain). Energy-dispersive X-ray (EDX) data were recorded using a BRUKER system attached to the SEM.

5. Antimicrobial Potential

Disc diffusion assays were used to assess the anti-bacterial activity of *Bl*-AgNPs [39]. The antibacterial potential of the *Bl*-AgNPs was tested against five strains of bacteria, *Bacillus cereus* (ATCC 14574), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 9027). The macro broth dilution method was employed to confirm antibacterial activity [40].

The poisoned food technique was performed to ascertain the antifungal activity using potato dextrose agar (PDA) [41]. To evaluate the antifungal activity of *Bl*-AgNPs, *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231) were used as model test strains. Deionized water and nystatin were used as positive and negative controls respectively. The inhibition capacity of mycelium growth was calculated using the equation:

% of Inhibition = {1- Theoretical colony diameter/ Control colony diameter}/100

Experiments were performed in triplicate and the results were reported as mean ± SD.

RESULTS AND DISCUSSION

1. Visual Examination and UV-Vis Spectral Analysis

The biosynthesis of AgNPs was carried out using aqueous *Bl* leaf extract at pH 5.6 under mild reaction conditions. The pH of the mixture was measured as 4.6 at the beginning of the reaction (after 5 min); it reached 3.9 after 1 h, and then remained constant afterward. At the onset of the study, a visual examination and UV-vis spectroscopic measurements were performed to check for the formation of *Bl*-AgNPs. **Figure 2 (a)** shows the colour changes in the colloidal suspension of *Bl*-AgNPs at different time intervals. The colour of the suspension was yellowish just after mixing with the plant extract, but turned a deep brown after 24 hours. This was due to the formation of surface plasmon resonance (SPR) during the bio-reduction of Ag^{+} to Ag^{o} [27].

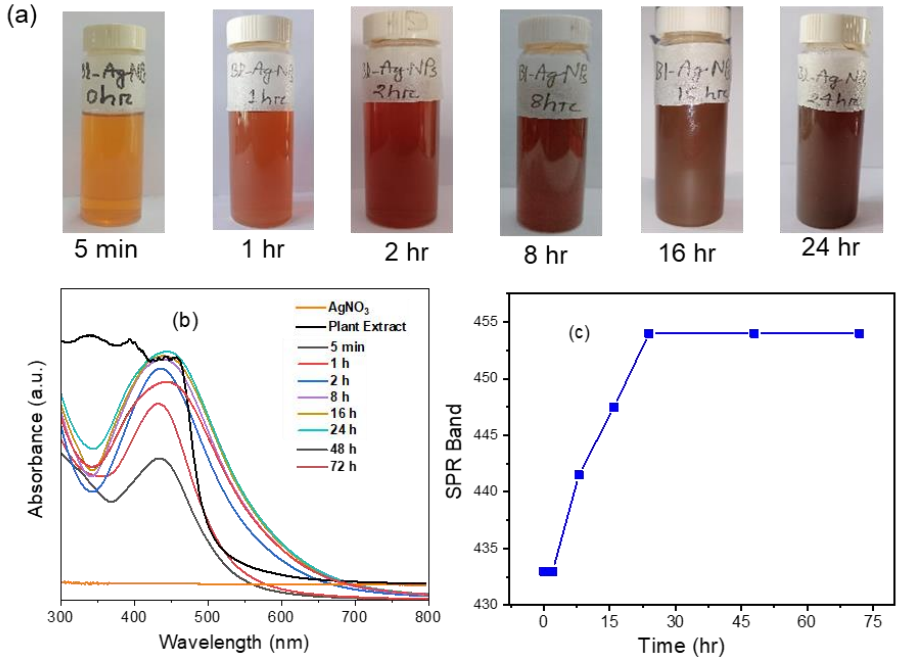


Figure 2. (a) Colour change at 5 min, 1 h, 2 h, 8 h, 16 h and 24 h, (b) UV-Visible spectra at different time intervals, and (c) SPR adsorption peak value against reaction time.

UV-Vis spectra of the colloidal suspension of *Bl*-AgNPs at different time intervals are shown in **Figure 2 (b)**. Interestingly, peaks were observed at 434 nm, 436 nm, 443 nm and 447 nm after 1, 2, 8 and 16 hours of reaction, respectively. This phenomenon was also observed in other studies [42] indicating the size of the NPs increased with the red shift of the SPR band. However, in the present case, the peak maxima then shifted to 454 nm after 24 hours and remained the same afterward, suggesting AgNP nucleation started with time while size was not affected throughout the reaction [38]. These findings were also supported by our recent reports [38]**.** Ganguli *et al*. reported synthesis of AgNPs at different sizes using the *Ophiorrhiza* genus, which simultaneously showed a red shift of the peak maxima with higher values and an increase in size of the AgNPs [38]. The reaction profile for the adsorption peak maximum over time is shown in **Figure 2 (c)**. The initial shift in peak position (within 24 hours) was attributed to Ostwald ripening and coalescence [43]. This effect can be explained by the fact that initially, small-sized AgNPs were formed and these aggregated with incubation time to form larger NPs [43]. As reported by Pandey *et al.*, the peak of AgNPs was detected at 430 nm only after a synthesis period of 3 h at pH 9. In the present case, a peak was observed at 436 nm after 2 h, which is similar, even though the reaction pH was different (pH ~5.6). After 24 h, a peak at 454 nm was detected and remained almost the same after 72 h. This phenomenon can be explained by the pH of the medium as well as Ostwald ripening and coalescence effects. Melkamu and Bitew also reported the shifting of centred peak maxima to a higher wavelength [42], as was observed in our study. The UV-Vis spectra of the leaf extract and $AgNO_3$ did not show any absorbance peak at around 434 to 454 nm (**Figure 2 (b)**). The above findings confirmed the successful formation of AgNPs when compared with previous reports [38, 44–47], while the single SPR band indicated the formation of spherical AgNPs.

2. XRD Analysis

XRD analysis was performed to examine the crystal lattice indices, crystalline phases and crystallinity of the *Bl*-AgNPs (**Figure 3 (a)**). The pattern obtained showed peaks at 27.64 º, 32.11 º, 37.95 º, 43.98 º, 46.02 º, 54.63 º, 57.4 º, 64.33º and 77.26º, which corresponded to the (210), (122), (111), (200), (231), (142), (241), (220) and (311) planes of a face-centred cubic lattice, according to JCPDS card no 04-0783 (**Figure 3 (b)**).

It is well established that crystalline size can be estimated by the Debye-Scherrer equation (D-S) [48].

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

where k, the Scherrer constant = 0.94, ƛ, the X-ray wavelength = 1.5418 Aº, β is the line broadening at half the maximum intensity (FWHM), and θ is the Bragg angle. **Table 1** displays the results. The estimated average crystalline size of the *Bl*-AgNPs was 18.52 nm. The XRD analysis data revealed that *Bl*-AgNPs were crystalline in nature, which is consistent with earlier reports as well as the TEM data in the present study [49, 50].

Table 1. XRD peak parameter details of *Bl*-AgNPs.

Peak Position	Plane (hkl)	2-theta (θ°)	FWHM (θ°)	Rel. int. I (a.u.)	Crystalline Size (nm)	Average size (nm)	W-H Plot (nm)
1	(210)	27.64	0.299	17.66	25.80		
2	(122)	32.11	0.260	40.56	29.36		
3	(111)	37.95	0.49	100.00	15.33		
4	(200)	43.98	1.00	27.92	7.37		
5	(231)	46.02	0.352	21.35	20.77	18.52	18.31
6	(142)	54.63	0.34	6.56	20.76		
7	(241)	57.37	0.44	5.91	15.84		
8	(220)	64.33	0.38	26.65	17.70		
9	(311)	77.26	0.45	39.95	13.79		

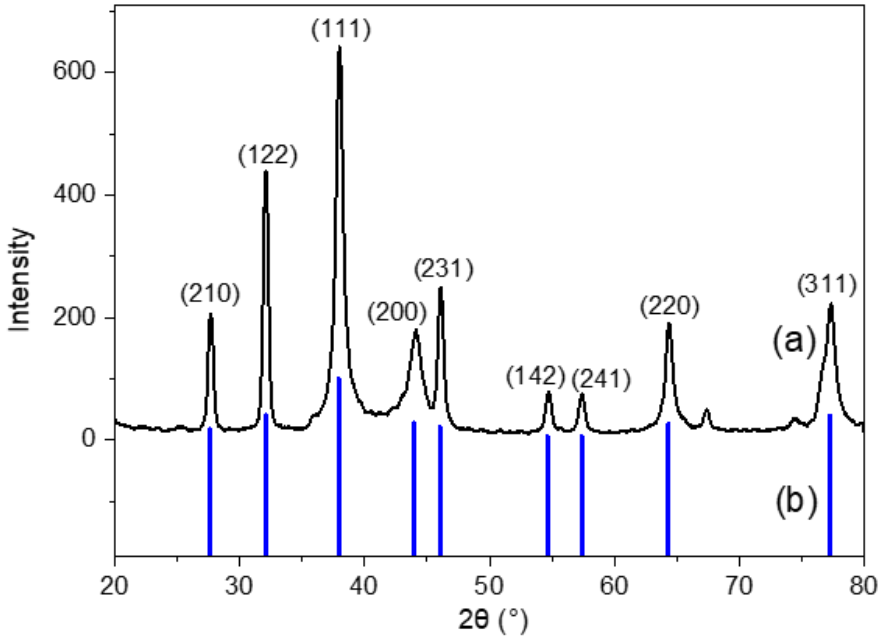


Figure 3. XRD patterns of (a) *Bl*-AgNPs and (b) Simulated AgNPs.

3. SEM and EDX Analysis

Figure 4 (a-c) illustrates the morphology of the *Bl*-AgNPs at various magnifications using SEM. Rough surfaces and agglomerated particles can be observed in the SEM images. In contrast, higher magnification SEM images (**Figure 4 (c)**) displayed individual *Bl*-AgNPs joining each other to form cluster-like morphologies. The formation of nano-clusters could also be a result of the closeness of the nanoparticles due to their high surface energies. Furthermore, secondary metabolites such as polyphenols, alkaloids, carbohydrates, and proteins on the surface of the AgNPs may form a network with neighbouring NPs to form clusters.

Nanocrystals of metallic silver commonly exhibit characteristic optical absorption peaks, typically at 3 keV due to the SPR band [51, 52]. The *Bl*-AgNPs had an elemental composition of 92.56 % Ag, 4.31 % C and 3.13 % O (**Figure 4(d)**). The weaker signals for C and O in the EDX spectrum indicate the possible existence of biomolecules on the surface of the AgNPs [53].

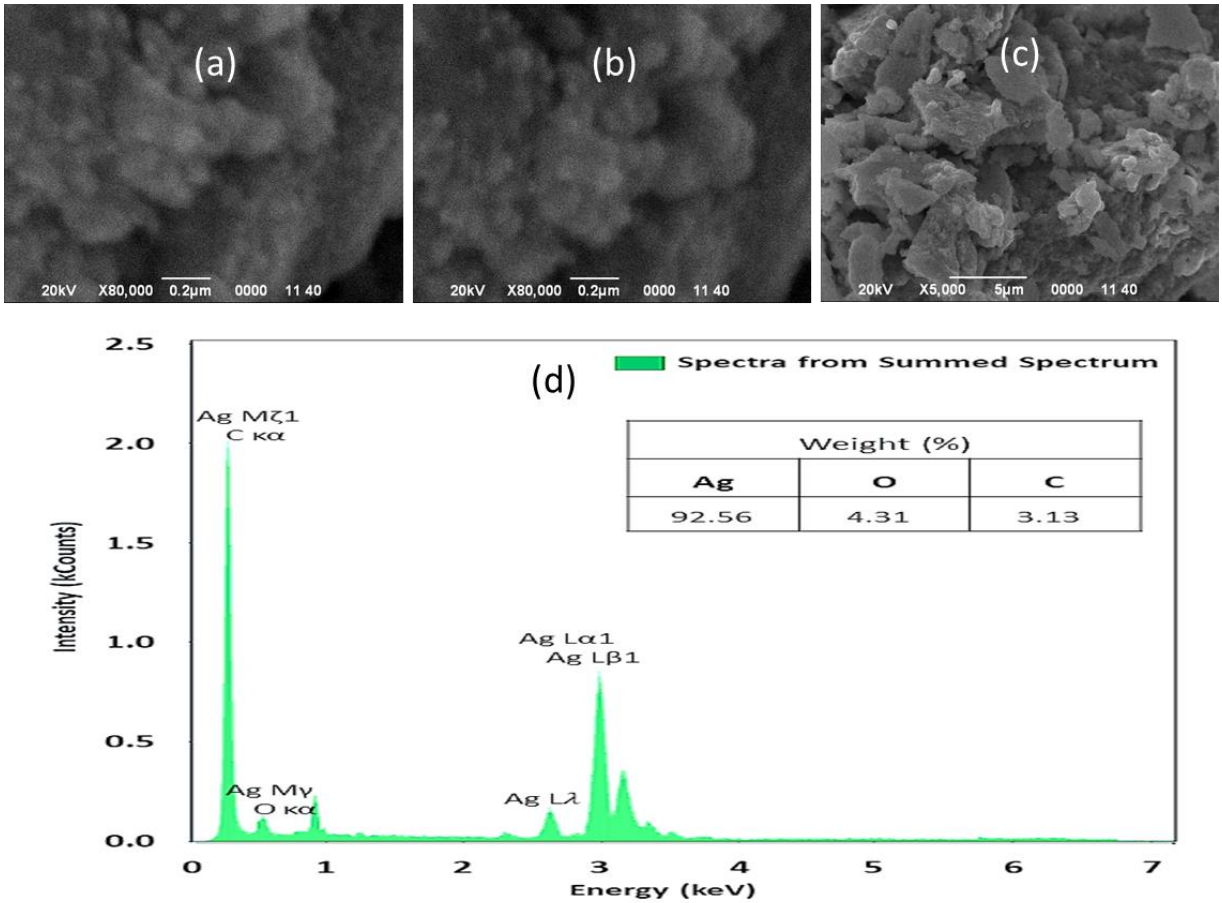


Figure 4. (a-c) Scanning electron micrographs at different magnifications and (d) EDX spectra of *Bl*-AgNPs with its elemental analysis.

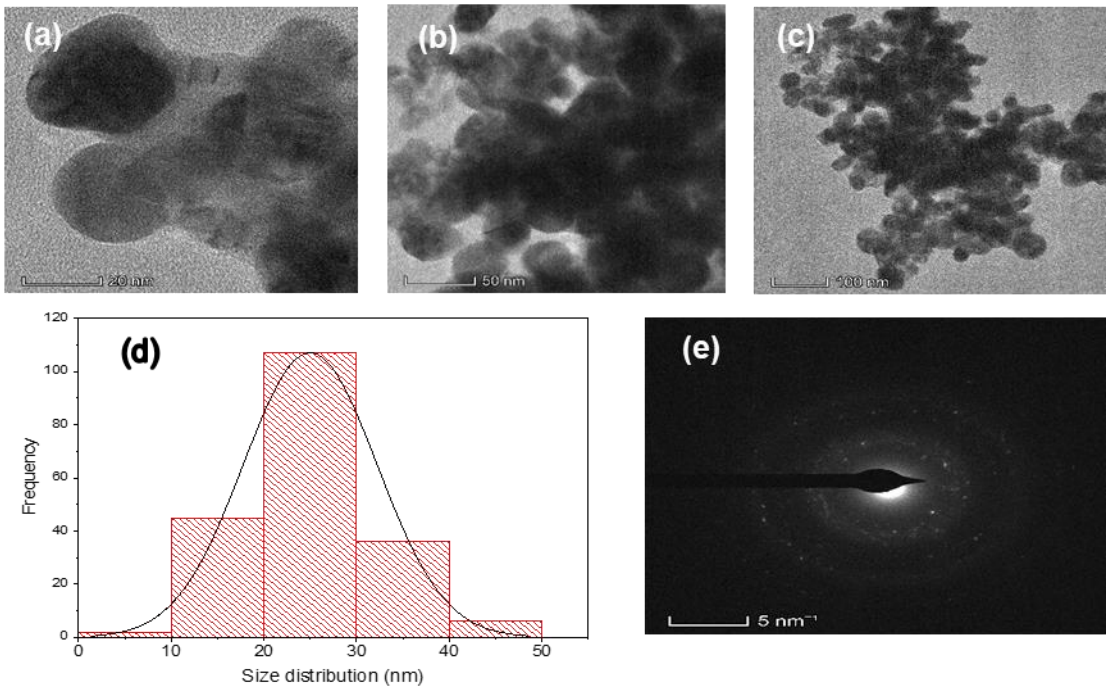


Figure 5. (a-c) TEM images at different magnifications, (d) particle size distribution, and (e) SAED pattern of *Bl*-AgNPs.

4. TEM and Selected Area Electron Diffraction (SAED) Analysis

TEM images of the synthesized *Bl*-AgNPs at varying magnifications in Figure 5 (a-c) display spherical particles of varying sizes. The particle size distribution diagram in **Figure 5 (d)** indicates the average size of the *Bl*-AgNPs was approximately 19 ± 7 nm, similar to the size estimated by XRD (18.52 nm). This is larger than the size reported by Pandey and his colleagues, which was 12.52 nm [36]. This is possible because of the influence of pH in the reaction medium (this study: pH 5.6; Pandey *et al.*: pH 9). At a higher pH or basic conditions, biomolecules are more available than at a lower pH or acidic conditions. So, biomolecules under basic conditions effectively reduce the Ag^+ ion and entrap the AgNPs [54]. Thus, smaller and more stable AgNPs are generally obtained at higher pH.

Additionally, the absorption maxima obtained in the current study at 454 nm and 430 nm, which were both noted by Pandey et al. [36], roughly correspond to the sizes of the AgNPs, suggesting that higher wavelengths support the formation of larger-sized NPs while lower wavelengths suggest the formation of smaller-sized NPs [55]. The sizes calculated from the D-S equation and W-H plot in both studies (this study: 18.31 nm; Pandey et al.: 9.12 nm; both studies: 18.52 nm and 10.96 nm) were also well matched in terms of this phenomenon. The SAED (**Figure 5 (e)**) pattern showed that the diffraction rings could be divided into reflections with the numbers (101), (111), (200), (220) and (311) that indicates a face-centred cubic poly-crystalline Ag from inner to outer with a small variance in d-spacing, which is consistent with the XRD spectra of the *Bl*-AgNPs.

5. FTIR Analysis

FTIR analysis was performed to determine if bio-molecules were attached to the bio-reduced AgNPs. **Figure 6 (a)** and **Figure 6 (b)** represent the FTIR spectra of *Bl* powder and *Bl*-AgNPs, respectively. *Bl*-AgNPs exhibited bands at 1620, 1267 and 1078 cm^{-1}, corresponding to the stretching modes of C=O ketone groups, C-N of amine, and C-O of ether, respectively [56], indicating the presence of phytochemicals like flavonoids, polyphenols or alkaloids on the *Bl*-AgNP surface. The EDX profile of *Bl*-AgNPs showed carbon and oxygen, further confirming the presence of these phytochemicals in synthesized AgNPs which can act as reducing and capping agents, and also promote stability [57].

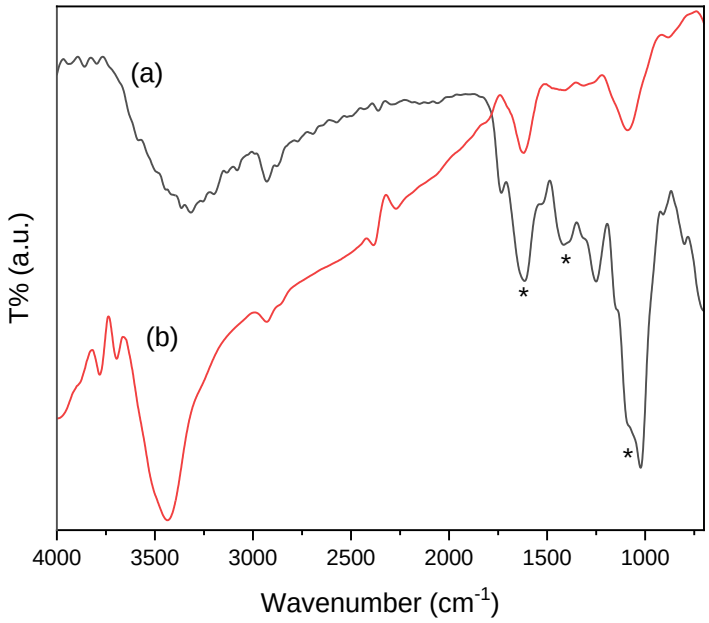


Figure 6. FTIR spectra of (a) leaf extracts and (b) synthesized Bl-AgNPs.

6. Antibacterial Activity

The antibacterial activity of the *Bl*-AgNPs was evaluated against gram-positive (*Bacillus cereus, Staphylococcus aureus*) and gram-negative (*Escherichia coli, Salmonella typhi, Pseudomonas sp.*) bacteria by disc diffusion assay **(Figure 7 (a))** and macro broth dilution method (**Figure 7 (b)**).

The results indicated that *Bl*-AgNPs possessed efficient antibacterial activity against both gram-negative and gram-positive bacterial strains. The leaf extract did not display any antibacterial activity against the bacteria, which implied that the AgNPs were solely responsible for the antibacterial activity. At times the disc diffusion test may give false positive or negative results, so to get more reliable information and confirm the antibacterial potential of *Bl*-AgNPs, we next used the macro broth dilution method [58]. The antimicrobial activity results in terms of percentage (%) of inhibition are displayed in **Figure 7 (b)** [40]. In both cases, we found activity against gram-positive and gram-negative bacteria, while the inhibition rate increased with concentration. The highest activity was observed at 250 µg/mL concentration. Half-maximal inhibitory concentration (IC_{50}) is the most widely used method to measure drug efficacy and it indicates the optimum dosage needed to inhibit a biological process. The IC_{50} values for the *Bl*-AgNPs revealed that AgNPs could inhibit by 50 % the growth of *B. cereus, S. typhi, S. aureus, E. coli* and *P. aeruginosa sp.* bacterial strains at concentrations of 103.51 ± 2.01 µg/ml, 86.42 ± 1.93 µg/ml, 75.96 ± 1.88 µg/ml, 68.41 ± 1.83 µg/ml and 67.82 ± 1.83 µg/ml, respectively.

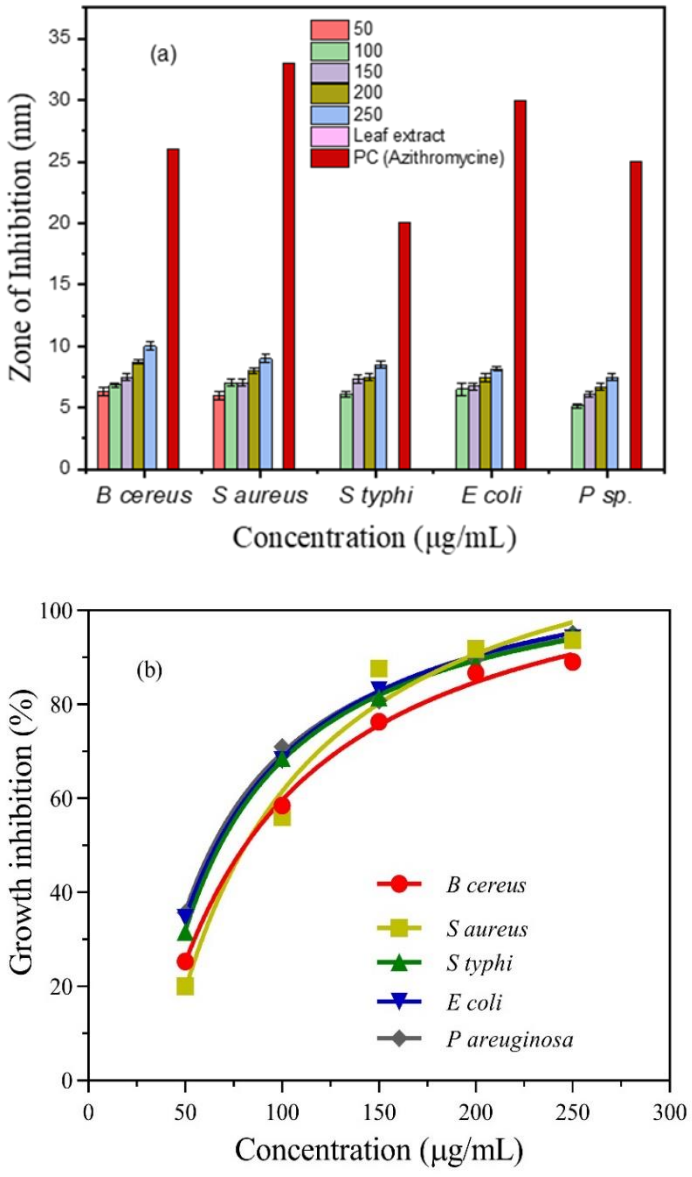


Figure 7. Bactericidal effects of *Bl*-AgNPs as measured by (a) Disc diffusion assay (b) Macro broth dilution method.

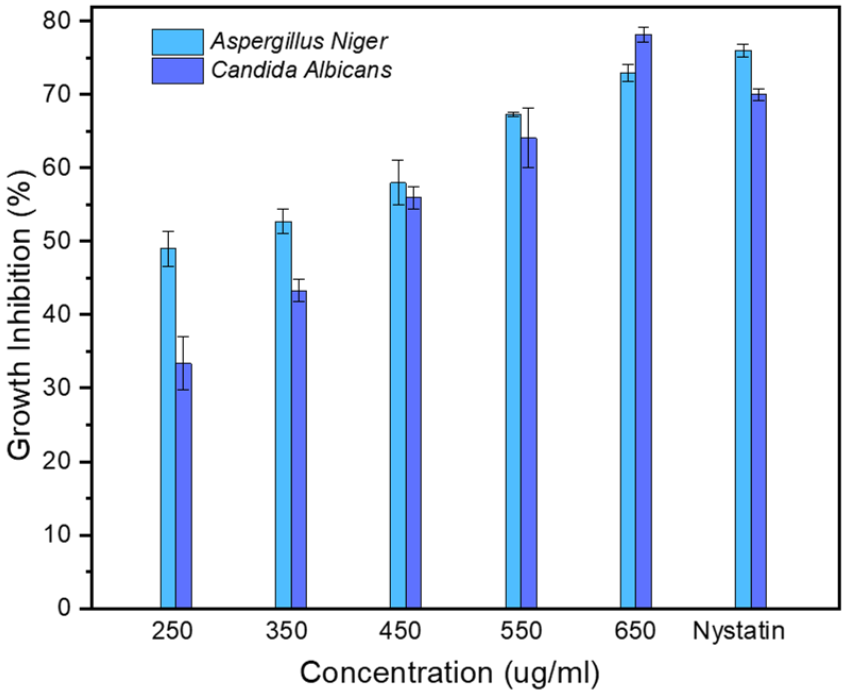


Figure 8. Effects of *Bl*-AgNPs on the mycelium growth of *Aspergillus nigar* and *Candida albicans*. The results were reported as mean values ± SDs of three independent experiments.

7. Antifungal Activity

The antifungal activity of the *Bl*-AgNPs is illustrated in **Figure 8.** In our study, significant antifungal activity was exhibited against the *Aspergillus nigar* and *Candida albicans* fungal strains by the *Bl*-AgNPs compared to the positive control, nystatin. The highest activity was observed at 650 µg/mL against *Candida albicans.* IC_{50} values of *Bl*-AgNPs against *Aspergillus nigar* and *Candida albicans* were 471.60 ± 1.71 µg/mL and 444.5 ± 0.99 µg/mL, respectively. From the IC_{50} values, it can be concluded that growth inhibition increased with the concentration of *Bl*-AgNPs. The *Bl* leaf extract also exhibited 37.33 ± 0.57 % and 34.45 ± 0.50 % growth inhibition against *Aspergillus nigar* and *Candida albicans*, respectively, indicating that the synthesized AgNPs showed higher antifungal activity compared to the pure leaf extract.

CONCLUSION

The present work describes the synthesis of AgNPs using *Bl* leaf extract without alkali or acid. This study confirms that under biologically friendly conditions, the aqueous *Bl* leaf extract could be used to synthesize AgNPs with potential antibacterial and antifungal activities. Mostly pure crystalline spherical particles with a mean size of 19 ± 7 nm were obtained. The FTIR spectra and EDX profile showed the presence of phytochemicals such as flavonoids, polyphenols or alkaloids on the surface of the *Bl*-AgNPs which may act as reducing and capping agents and promote stability. *Bl*-AgNPs exhibited inhibitory activity against a wide range of gram-positive and gram-negative bacteria and fungi in a dose-dependent manner. The present study indicates that AgNPs synthesized under physiological conditions using plant extracts could be good prospects for new antimicrobial agents. This observation suggests that the research on plant-based AgNPs should be up-scaled to search for potential applications in various areas ranging from photo-catalysis, chemical separation, biosensors and bio-medical materials.

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Data availability: Data will be made available on request.

Declarations

Competing interests: 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.

Ethical approval: Not applicable.

REFERENCES

1. Vijayaram, S., Razafindralambo, H., Sun, Y. Z. (2023) Applications of Green Synthesized Metal Nanoparticles - a Review. *Biol Trace Elem Res*, 2023.

2. Abdelghany, T. M., Al-Rajhi, A. M. H., Al Abboud, M. A., Alawlaqi, M. M., Ganash, G. A., Helmy, E. A. M., Mabrouk, A. S. (2018) Recent advances in green synthesis of silver nanoparticles and their applications: About future directions. A review. *In Bio Nano Science*, **8**, 5–16.

3. Pokropivny, V. (2007) Introduction to nanomaterials and nanotechnology. *Tartu University Press*.

4. Baig, N., Falath, W., Kammakakam, I. (2021) Nanomaterials: A review of synthesis methods, properties, recent progress, and challenges. *Materials Advances*, **2**,1821–1871.

5. Abdellatif, A. A. H., Alhathloul, S. S., Aljohani, A. S. M., Maswadeh, H., Abdallah, E. M., Hamid, M., el Hamd, M. A. (2022) Green synthesis of silver nanoparticles incorporated aromatherapies utilized for their antioxidant and antimicrobial activities against some Clinical bacterial isolates. *Bioinorganic Chemistry and Applications*, 2432758.

6. Pirtarighat, S., Ghannadnia, M. & Baghshahi, S. (2019) Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. *Journal of Nanostructure in Chemistry*, **9(1)**, 1–9.

7. Giri, A. K., Jena, B., Biswal, B., Pradhan, A. K., Arakha, M., Acharya, S. & Acharya, L. (2022) Green synthesis and characterization of silver nanoparticles using Eugenia roxburghii DC. extract and activity against biofilm-producing bacteria. *Scientific Reports*, **12(1**), 8383.

8. Rafique, M., Sadaf, I., Rafique, M. S. & Tahir, M. B. (2017) A review on green synthesis of silver nanoparticles and their applications. *Artificial Cells, Nanomedicine and Biotechnology*, **45(7)**, 1272–1291.

9. Rauwel, P., Küünal, S., Ferdov, S. & Rauwel, E. (2015) A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. *Advances in Materials Science and Engineering*, 682749.

10. Ajitha, B., Reddy, Y. A. K., Jeon, H. J., Ahn, C. W. (2018) Synthesis of silver nanoparticles in an eco-friendly way using Phyllanthus amarus leaf extract: Antimicrobial and catalytic activity. *Advanced Powder Technology*, **29**, 86–93.

11. Karthiga, P. (2018) Preparation of silver nanoparticles by Garcinia mangostana stem extract and investigation of the antimicrobial properties. *Biotechnology Research and Innovation*, **2(1)**, 30–36.

12. Varadharaj, V., Christina, C., Kavasseri Ganesan, K. & Krishna Murthy, G. (2014) Silver nanoparticle synthesis from aqueous leaf extract of Annona squamosa (L) and assessment of its antimicrobial activity. *International Journal of Frontiers in Science and Technology*, **2**, 2321-0494.

13. Jagtap, U. B. & Bapat, V. A. (2013) Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. seed extract and its antibacterial activity. *Industrial Crops and Products*, **46**, 132–137.

14. Gul, S. F., Muzaffar, R., Nawaz, M., Gul, S. & Asim, M. R. (2018) Synthesis, Characterization and Anti-microbial Activity of Citrus limon Mediated Nanoparticles, **3**, 139.

15. Gour, A. & Jain, N. K. (2019) Advances in green synthesis of nanoparticles. In *Artificial Cells, Nanomedicine and Biotechnology*, **47**, 844–851.

16. Krishnaraj, C., Jagan, E. G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P. T. & Mohan, N. (2010) Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces B: Biointerfaces*, **76(1)**, 50–56.

17. Ahmed, R. H. & Mustafa, D. E. (2020) Green synthesis of silver nanoparticles mediated by traditionally used medicinal plants in Sudan. *International Nano Letters*, **10(1)**, 1–14.

18. Ahmed, S., Saifullah, Ahmad, M., Swami, B. L. & Ikram, S. (2016) Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *Journal of Radiation Research and Applied Sciences*, **9(1)**, 1–7.

19. Chandrasekhar, N. & Vinay, S. P. (2017) Yellow colored blooms of argemone mexicana and turnera ulmifolia mediated synthesis of silver nanoparticles and study of their antibacterial and antioxidant

activity. *Applied Nanoscience (Switzerland)*, **7(8)**, 851–861.

20. Loo, Y. Y., Chieng, B. W., Nishibuchi, M. & Radu, S. (2012) Synthesis of silver nanoparticles by using tea leaf extract from Camellia Sinensis. *International Journal of Nanomedicine*, **7**, 4263–4267.

21. Ahmad, N., Sharma, S., Singh, V. N., Shamsi, S. F., Fatma, A. & Mehta, B. R. (2011) Biosynthesis of Silver Nanoparticles from Desmodium triflorum: A Novel Approach Towards Weed Utilization. *Biotechnology Research International*, 1–8.

22. Lee, K. D. & Nagajyothi, P. C. (2011) Synthesis of plant-mediated silver nanoparticles using dioscorea batatas rhizome extract and evaluation of their antimicrobial activities. *Journal of Nanomaterials*, **7**.

23. Labulo, A. H., David, O. A. & Terna, A. D. (2022) Green synthesis and characterization of silver nanoparticles using Morinda lucida leaf extract and evaluation of its antioxidant and antimicrobial activity. *Chemical Papers*, **76(12)**, 7313–7325.

24. Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A. R. & Singh, R. P. (2011) Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. *Journal of Nanoparticle Research*, **13(7)**, 2981–2988.

25. Anandalakshmi, K., Venugobal, J. & Ramasamy, V. (2016) Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. *Applied Nanoscience (Switzerland)*, **6(3)**, 399–408.

26. Saratale, R. G., Shin, H. S., Kumar, G., Benelli, G., Kim, D. S. & Saratale, G. D. (2018) Exploiting antidiabetic activity of silver nanoparticles synthesized using punica granatum leaves and anticancer potential against human liver cancer cells (HepG2). *Artificial Cells, Nanomedicine and Biotechnology*, **46(1)**, 211–222.

27. Gopinath, K., Gowri, S. & Arumugam, A. (2013) *Phytosynthesis of silver nanoparticles using Pterocarpus santalinus leaf extract and their antibacterial properties*, **3**, 68.

28. Gopinath, V., MubarakAli, D., Priyadarshini, S., Priyadharsshini, N. M., Thajuddin, N. & Velusamy, P. (2012) Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: A novel biological approach. *Colloids and Surfaces B: Biointerfaces*, **96**, 69–74.

29. Dilbar, S., Sher, H., Ali, H., Ullah, R., Ali, A., Ullah, Z. (2023) Antibacterial Efficacy of Green Synthesized Silver Nanoparticles Using Salvia nubicola Extract against Ralstonia solanacearum, the Causal Agent of Vascular Wilt of Tomato. *ACS Omega*, **8**, 31155–31167.

30. Li, Z., Ma, W., Ali, I., Zhao, H., Wang, D., Qiu, J. (2020) Green and Facile Synthesis and Antioxidant and Antibacterial Evaluation of Dietary Myricetin-Mediated Silver Nanoparticles. *ACS Omega*, **5**, 32632–32640.

31. Mahmud, K. M., Hossain, M. M., Polash, S. A., Takikawa, M., Shakil, M. S., Uddin, M. F., Alam, M., Shawan, M. M. A. K., Saha, T., Takeoka, S., Hasan, M. A., Sarker, S. R. (2022) Investigation of Antimicrobial Activity and Biocompatibility of Biogenic Silver Nanoparticles Synthesized using Syzigyum cymosum Extract. *ACS Omega*, **7**, 27216–27229.

32. Rodríguez-Felix, F., Lopez-Cota, A. G., Moreno-Vasquez, M. J., Graciano-Verdugo, A. Z., Quintero-Reyes, I. E., Del-Toro-Sanchez, C. L., Tapia-Hernandez, J. A. (2021) Sustainable-green synthesis of silver nanoparticles using safflower (Carthamus tinctorius L.) waste extract and its antibacterial activity. *Heliyon*, **7**, e06923.

33. Hanafiah, R. M., Ghafar, S. A. S., Lim, V., Musa, S. N. A., Yakop, F., Anuar, A. H. H. (2023) Green synthesis, characterisation and antibacterial activities of Strobilanthes crispus-mediated silver nanoparticles (SC-AGNPS) against selected bacteria. *Artificial Cells, Nanomedicine, and Biotechnology*, **51**, 549–559.

34. Ibrahim, M., Qamrul, A. M., Homa, Z., Ruhul, K. M., Khair, A., Ahsan, Q., Bin R. R., Abdur, R. M. (2014) Pharmacological Activities of Blumea lacera (Burm f) DC: A medicinal plant of Bangladesh. *Original Research Article British Journal of Pharmaceutical Research*, **4**,1677–1687.

35. Agarwal, R., Singh, R., Siddiqui, I. R., Singh, J. (1995) Triterpenoid and prenylated phenol glycosides from Blumea lacera. *Phytochemistry*, **38**, 935–938.

36. Pandey, P. K., Gangwar, C., Yaseen, B., Kumar, I., Nayak, R., Kumar, S., Naik, R. M., Banerjee,

M., Sarkar, J. (2023) Anticancerous and antioxidant properties of fabricated silver nanoparticles involving bio-organic framework using medicinal plant Blumea lacera. *Chemical Papers*, **77**,3603–3617.

37. Tarkeshwar, D., Kancharla, B., Kuna, D., Siva, H. (2023) Development and Evaluation of Bio fabricated Silver Nanoparticles from Blumea lacera for In-vitro Antibacterial, Antioxidant and Anti-inflammatory Activity. *Pharmacognosy Journal*, **15**, 266–278.

38. Ganguli, S., Howlader, S., Ullah, A. K. M. A., Bhuiyan, F. R., Akhi, A. A., Hasan, A., Dey, K., Islam, S., Ali, F., Chakraborty, A. K., Bhattacharjee, S., Dey, B. K. (2023) Size controlled biosynthesis of silver nanoparticles using Ophiorrhiza mungos, Ophiorrhiza harrisiana and Ophiorrhiza rugosa aqueous leaf extract and their antimicrobial activity. *Heliyon*, **9**, e16072.

39. Kazlagić, A, Abud, O. A., Ćibo, M., Hamidović, S., Borovac, B. & Omanović-Mikličanin, E. (2019) Green synthesis of silver nanoparticles using apple extract and its antimicrobial properties. *Health and Technology* **2016**, 4102196.

40. Mohanta, Y. K., Panda, S. K., Bastia, A. K., Mohanta, T. K. (2017) Biosynthesis of silver nanoparticles from Protium serratum and investigation of their potential impacts on food safety and control. *Frontiers in Microbiology*, **8**.

41. Mittal, A. K., Chisti, Y., Banerjee, U. C. (2013) Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, **31**, 346–356.

42. Melkamu, W. W., Bitew, L. T. (2021) Green synthesis of silver nanoparticles using Hagenia abyssinica (Bruce) JF Gmel plant leaf extract and their antibacterial and anti-oxidant activities. *Heliyon*, **7**, e08459.

43. Anowar, H. M., Sultana, M. N., Al-Ghorbani, M., Baashen, M., Almalki, F. A., Ben, H. T., Ali, F., Mahmud, S., Abu, S. M., Laaroussi, H., Kawsar, S. M. A. (2022) Synthesis, antimicrobial, molecular docking and molecular dynamics studies of lauroyl thymidine analogs against SARS-CoV-2: POM study and identification of the pharmacophore sites. *Bioorganic Chemistry*, **125**.

44. Banerjee, P., Satapathy, M., Mukhopahayay, A., Das, P. (2014) Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, **1**.

45. Muthu, K., Priya, S. (2017) Green synthesis, characterization and catalytic activity of silver nanoparticles using Cassia auriculata flower extract separated fraction. Spectrochimica Acta - Part A: *Molecular and Biomolecular Spectroscopy*, **179**, 66–72.

46. Maghimaa, M., Alharbi, S. A. (2020) Green synthesis of silver nanoparticles from Curcuma longa L and coating on the cotton fabrics for antimicrobial applications and wound healing activity. *Journal of Photochemistry and Photobiology B: Biology*, **204**.

47. Ssekatawa, K., Byarugaba, D. K., Kato, C. D., Wampande, E. M., Ejobi, F., Nakavuma, J. L., Maaza, M., Sackey, J., Nxumalo, E., Kirabira, J. B. (2021) Green strategy–based Synthesis of silver nanoparticles for antibacterial applications. *Frontiers in Nanotechnology*, **3**.

48. Banne, S. V., Patil, M. S., Kulkarni, R. M., Patil, S. J. (2017) Synthesis and characterization of silver nano particles for EDM applications. *Materials Today: Proceedings*, **4**, 12054–12060.

49. Sivaramakrishnan, M., Jagadeesan, S. V., Karaiyagowder, G. D., Meganathan, Y., Devaraj, B. S., Natesan, S., Kothandan, R., Kandaswamy, K. (2019) Green synthesized silver nanoparticles using aqueous leaf extracts of Leucas aspera exhibits antimicrobial and catalytic dye degradation properties. *SN Applied Sciences*, 2019.

50. Uddin, A. K. M. R., Siddique, M. A. B., Rahman F., Ullah, A. K. M. A., Khan, R. (2020) Cocos nucifera leaf extract mediated green synthesis of silver nanoparticles for enhanced antibacterial activity. *Journal of Inorganic and Organometallic Polymers and Materials*, **30**, 3305–3316.

51. Muthukrishnan, S., Bhakya, S., Senthil, K. T., Rao, M. V. (2015) Biosynthesis, characterization and antibacterial effect of plant-mediated silver nanoparticles using Ceropegia thwaitesii - An endemic species. *Industrial Crops and Products*, **63**, 119–124.

52. Adebayo-Tayo, B., Salaam, A., Ajibade, A. (2019) Green synthesis of silver nanoparticle using Oscillatoria sp extract, its antibacterial, antibiofilm potential and cytotoxicity activity. *Heliyon*, **5**, e02502.

53. Jyoti, K., Baunthiyal, M., Singh, A. (2016) Characterization of silver nanoparticles synthesized using Urtica dioica Linn leaves and their synergistic effects with antibiotics. *Journal of Radiation Research and Applied Sciences*, **9**, 217–227.

54. Niloy, M. S., Hossain, M. M., Takikawa, M., Shakil, M. S., Polash, S. A., Mahmud, K. M., Uddin, M. F., Alam, M., Shubhra, R. D., Shawan, M. M. A. K., Saha, T., Takeoka, S., Hasan, M. A., Ranjan Sarker, S. (2020) Synthesis of Biogenic Silver Nanoparticles Using Caesalpinia digyna and Investigation of their antimicrobial activity and in vivo biocompatibility. *ACS Applied Bio Materials*, **3**, 7722-7733.

55. Kredy, H. M, (2018) The effect of pH, temperature on the green synthesis and biochemical activities of silver nanoparticles from Lawsonia inermis extract. *Journal of Pharmaceutical Sciences and Research*, **10**, 2022–2026.

56. Bhakya, S., Muthukrishnan, S., Sukumaran, M., Muthukumar, M. (2016) Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. *Applied Nanoscience (Switzerland)*, **6**, 755–766

57. Ullah, A. K. M. A., Kabir, M. F., Akter, M., Tamanna, A. N., Hossain, A., Tareq, A. R. M., Khan, M. N. I., Kibria, A. K. M. F, Kurasaki, M., Rahman, M. M. (2018) Green synthesis of biomolecule encapsulated magnetic silver nanoparticles and their antibacterial activity. *RSC Advances*, **8**, 37176–37183

58. Jorgensen, J. H., Ferraro, M. J. (2009) Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases*, **49**,1749–1755.