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Flacourtia indica based biogenic nanoparticles: development, characterization, and bioactivity against wound associated pathogens

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Abstract

Surface engineered nanoparticles (NPs) are of great attention due to their targeted medical applications. The nature of the functionalized surface plays a vital role in achieving the required functionalities of engineered NPs. Owing to the biofilm formation capabilities of wound associated pathogens, impaired wound healing is a major complication in the medical field. In this context, herein, we report the biogenic synthesis of Flacourtia indica (FI) based NPs, i.e., FI-AgNPs using the aqueous leaf extract of this anti-bacterial herb. The newly developed FI-AgNPs were characterized using various analytical and imaging techniques such as UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The FI-AgNPs showed potent anti-microbial activity and anti-biofilm potential which were examined through a test tube adherence method and congo red agar method. It has been observed that synthesized FI-AgNPs inhibit the formation of a biofilm of observed bacteria, even at a minimum concentration of 80 μgml−1. These findings suggest that synthesized FI-AgNPs could be used against wound associated microbes, especially bacterial coating on medical devices, to prevent antibiotic-resistant biofilm infections. Further development and research are obligatory to decode this skill into preventive and therapeutic strategies.

1. Introduction

The methods impending due nanoparticles (NPs) like a chemical, photo-reduction, and thermal decomposition have been limited to non-eco-friendly and expensive pathways [1–3]. Due to increasing hazards using toxic chemicals, capping agents, oxidizing and reducing agents in the synthesis of NPs, researchers have proposed new engineering strategies using the combination of natural resources and nano-biotechnology [4, 5]. The nano-biotechnology is promisingly based on the eco-friendly and green routes [6, 7]. The biogenic routes have considered as leading paths to get the desired product having a specific shape, size, and surface chemistry, which are still challengeable in the chemical routes [8, 9]. The synthesis of noble metal NPs using plant extract as a reducing agent is advancing the topic due to their remarkable applications in medicine sector [10, 11]. Among all the noble metal NPs, the silver nanoparticles (AgNPs) are well known for their potential against microbial infections and bacterial colonization on different surface devices (biofilms) [12–14]. Therefore, the synthesis of AgNPs coupled with unique biological activities is much focused in recent years [15]. AgNPs have remarkable
antimicrobial properties when synthesized using medicinal plants compared to other biological synthesis methods. Microbial infections are being treated with several non-conventional remedies including antimicrobial peptides, naturopathic therapy, metallic particles, phytotherapy and small molecule inhibitors [16, 17]. Formerly it is considered that AgNPs can remove the microbial infections [18]. Many antimicrobial activities of Ag against pathogens, including Gram-positive and Gran-negative bacteria are present in the literature. Complexation of Ag with other material has good record of anti-bacterial activities [19, 20]. For example, in case of wound dressing, Ag-saturated nylon fibers have good antimicrobial potential particularly against Candida albicans and Staphylococcus aureus [21, 22]. The ability of AgNPs to damage bacterial cell wall is highly appreciated against the Escherichia coli [23]. Presently, silver-coated medical devices including vascular grafts, catheters, endotracheal tubes, AgNPs in wound dressing and burn ointments are used to stop and treat bacterial infections [24, 25].

Many studies have been made to fabricate the AgNPs using plant leaves, stem, roots, and fruit such as Dalbergia spinosa, Sapindus emarginatus, Ficus hispida, Diospyros paniculata, and Berberis vulgaris [26–30]. These plant extracts are used as reducing as well as a capping agent for the AgNPs fabrication. Recently, plants of the Flacourtiaceae family have gained much attention in the fabrication of AgNPs, owing to its wide medicinal applications for the cure of conditions such as diarrhea, fever and intestinal worms [31, 32]. Anti-microbial activity of biogenic AgNPs makes them potential candidates for water filtration plants, food industries and textiles [33]. Using the green synthesis route, Rasheed et al [34] used a methanolic leaf extract of Taraxacum officinale plant for the fabrication of AgNPs. As developed AgNPs of Taraxacum officinale plant showed broad antibacterial activities against Gram-positive and Gram-negative bacterial strains and antiproliferative activity against MCF-7 breast cancer cell line. Recently, Nandhini et al used the methanolic extract of Flacourtia indica to synthesize the AgNPs as anti-proliferative agents [35].

Following careful consideration of the literature discussed above, we theorized that the practice of this plant material for the preparation of FI-AgNPs could increase the anti-bacterial efficacy as it capped and stabilize the NPs. Thus, the present work was designed to use the aqueous leaf extract for the synthesis of FI-AgNPs as a potential candidate against the biofilm formation by bacteria colonization.

2. Materials and method

2.1. Chemicals/reagents
All the reagents and silver nitrate (99.8%) were procured from Sigma-Aldrich and employed as received without further refinement. Deionized water was used for all the experiments. All other chemicals or reagents used in the current experimental work were of analytical laboratory grade and used as received unless otherwise specified.

2.2. Plant collection and extract preparation
The leaves of Flacourtia indica were obtained from the Department of Agriculture, The Islamia University of Bahawalpur, Pakistan. The leaves were washed three times using distilled water and dried in a shadow for a week. The dried leaves were ground into a fine powder using a domestic pulverizer and stored for further use. Around 10 g of freshly prepared powder was added in 100 ml of deionized water and boiled for 90 min at 70 °C. The filtrate (extract) was collected through filter paper and stored at room temperature for subsequent experimental work.

2.3. Biogenic synthesis of FI-AgNPs
Initially, 50 ml of 1 mM silver nitrate solution was added in 50 ml of plant extract. The mixture was stirred (160 rpm) at 70 °C. The color changed from yellow to dark brown that ratifies the AgNPs synthesis. The resulting bio-reduced FI-AgNPs were centrifuged at 6000 rpm for 10–15 min. The precipitates thus obtained were filtered, washed with deionized water, and were dried at room temperature for further use. The FI-AgNPs were re-dispersed in de-ionized water at the 1 mg ml⁻¹ ratio for UV-visible spectrophotometer. The absorption spectrum was recorded after 10, 30, 60 min, 24 h. As developed FI-AgNPs were then subjected to characterization using analytical and imaging techniques, as discussed in the following section. Figure 1 shows a schematic illustration of the synthesis of FI-AgNPs and its characterization.

2.4. Characterization of FI-AgNPs
2.4.1. UV-Visible spectroscopy
The formation of FI-AgNPs by reduction of silver nitrate into silver ion was confirmed by UV-Visible spectroscopy (Cecil 7500 UV-vis spectrophotometer). The data was recorded from 200 to 800 nm.
2.4.2. Fourier transform infrared spectroscopy

Fourier Transform Infra-Red Spectrometer (Bruker Tensor 27 FTIR) was used for the determination of reduction of silver nitrate into AgNPs by reducing agents present in the aqueous leaves extract of Flacourtia indica. The data was collected in the range of 400 to 4000 cm\(^{-1}\).

2.4.3. Powder x-ray diffraction analysis

The crystallite size of as prepared FI-AgNps was determined by Powder x-ray Diffractometer (Bruker D8 Advance PXRD) with high-resolution LynxEye detector and Cu radiation source.

2.4.4. SEM and TEM—morphological analysis

Scanning Electron Microscope (SEM) MIRA-III TESCON was employed to observe the morphology of FI-AgNps. The samples were carbon coated, and their morphology was probed using FESEM working at an operating voltage of 20 kV. The suspensions were dropped onto copper grids; the ethanol was eliminated via the freeze-drying technique; the specimens were subjected to morphological observations on the Transmission Electron Microscope (JEOL JEM 2100 F TEM) with an accelerating voltage of 120 kV.

2.5. Anti-pathogenic activities

Antimicrobial susceptibility testing of biogenic FI-AgNPs was done using Kirby Bauer well diffusion method [36], where Mueller Hinton Agar was taken as a medium. Four different wound associated pathogens, i.e., Proteus mirabilis (P. mirabilis), Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus saprophyticus (S. saprophyticus), and Streptococcus pyogenes (S. pyogenes) were tested for antimicrobial potential of FI-AgNPs. Solvent blank was utilized as a negative control. Silver nitrate salt solution and leaf extract of Flacourtia indica plant and drug povidone-iodine were utilized as a positive control.

2.5.1. Tube adherence method

The potential of biogenic FI-AgNPs has examined against five strains of bacteria, i.e., Acinetobacter baumannii SABS (A. baumannii), Pseudomonas aeruginosa ETPS11 (P. aeruginosa), Klebsiella pneumoniae SKP7 (K. pneumoniae), Proteus mirabilis PPM8 (P. mirabilis) and Escherichia coli ETEC12 (E. coli). The method by
Hasan et al. was employed after little modifications [37]. In labeled pre-sterilized test tubes sterilized trypticase soy broth (TSB) media, 2 ml, and each freshly cultured stain, 50 μl were added followed by FI-AgNPs (15, 30, 60, 80 and 100 μg ml⁻¹). All the test tubes were incubated for 24 h to allow the formation of biofilm. After that, phosphate buffer saline (pH = 7.3) was used to wash the cultural broth. The dried test tubes were stained with 0.1% of crystal violet, washed with de-ionized water, and dried. Biofilm was characterized positive when the film appeared at the bottom and the wall of the test tube. Ring formation at the surface of liquid was not considered as biofilm formation.

2.5.2. Congo red agar method
Congo red agar was prepared by mixing 50 g sucrose, 10 g agar, 37 g brain heart infusion broth, and 0.8 g Congo red dye in one-liter distilled water. The inoculums from the above-stated tube method were inoculated into Congo red agar and incubated aerobically at 37 °C for 24 h. Biofilm producing strains results in the formation of black colonies, while others appear in the form of red colonies [38, 39].

2.6. Statistical analysis
Statistical analysis was performed using the origin and Microsoft Excel software. The data was collected and presented as an average ± standard deviation. The whole presented experimental work was performed in triplicate samples.

3. Results
For the biomedical application, small-sized silver nanoparticles are reported to have more penetrating potential, but this small size fetches toxicity. Hence, researchers are ambitious to achieve the appropriate size of nanoparticles for specialized biological applications. This control magnitude of nanoparticles was achieved herein by using the appropriate quantity of leaf extract of Flacourtia indica. The FI-AgNPs may be noteworthy regarding the biomedical application, antimicrobial, antifungal, anti-bacterial as well as for anti-malarial due to medicinal bio-reducing plant, Flacourtia indica. Confirmation of the reduction of Ag⁺ to Ag° was rectified by a change in color from brown to dark red due to surface Plasmon resonance of newly synthesized AgNPs (figure 2).

3.1. UV-visible spectroscopy
Moreover, UV-Vis analysis endorses the visual results and stabilization of biogenic FI-AgNPs at 420 nm when scanned between 300 – 800 nm. The Silver nitrate solution did not show any peak from 350–700 nm (figure 3 A), revealing no formation of AgNPs without reducing agent while mediated FI-AgNPs using plant extract exhibited a sharp peak around 420 nm (figure 3(B)). These results convinced that biomolecules in the plant leaf extract to possess significant potential for reduction of Ag⁺ to Ag° metal. Different parameters studied exposed that the factors such as the concentration of salt precursor and aqueous extract of leaves of Flacourtia indica greatly influence the yield and growth of AgNPs. The 1–5 mM concentration of AgNO₃ was employed at ambient conditions, resulted that by adding a higher concentration of AgNO₃, exhibited a bit strong and sharp absorbance at 410 nm (owing to the enriched concentration of Ag nanoparticles) that refer to the surface Plasmon vibration in AgNPs [40]. The reference samples AgNO₃, deionized water, and plant extract did not
evidence for any peak in the range of 400 to 700 nm region. Similarly, at low concentration of plant extract results does not exhibit any peak in the UV-Vis region, convincing that at low concentration of extract the bio-reducing molecules do not potentially reduce silver ions to metallic AgNPs.

3.2. FT-IR spectroscopy
In FT-IR spectrum, aqueous leaves extract of Flacourtia indica shows peaks at 3805 cm\(^{-1}\), 3731 cm\(^{-1}\), 2926 cm\(^{-1}\), 2350 cm\(^{-1}\), 1743 cm\(^{-1}\), 1457 cm\(^{-1}\), 1054 cm\(^{-1}\) and 702 cm\(^{-1}\). Whereas new biogenic FI-AgNPs show peaks at 3877 cm\(^{-1}\), 3503 cm\(^{-1}\), 2969 cm\(^{-1}\), 2353 cm\(^{-1}\), 1598 cm\(^{-1}\), 1046 cm\(^{-1}\), and 663 cm\(^{-1}\) (figure 4).

Hence the chief functional groups that entail in bio-reducing of AgNPs are phenolic hydroxyl groups and amino groups that exist herein due to the presence of xanthones and proteins. Moreover, some peaks in the FT-IR spectrum intimate the presence of flavonoids and aromatic groups (2353 cm\(^{-1}\) and 663 cm\(^{-1}\)). It is documented that the superficial peak arose due to shifting in the absorbance from 3800–3600 cm\(^{-1}\) is mainly concerned with hydroxyl groups on the surface of the newly synthesized nanoparticles. Its broad intensity might be due to moisture in the environment. The carboxylic groups present in the proteins and carboxylic acids adhere to the as-synthesized AgNPs hence granting them stability during the synthesis. Moreover, the biomolecules in the plant leaf extract, including amino acids, enzymes polysaccharides, and proteins, are thought to be another reason for better stabilization of FI-AgNPs. The peak at 3503 cm\(^{-1}\) is attributed to phenolic hydroxyl groups indicating the presence of xanthones. Stretching vibrations at 2969 cm\(^{-1}\) are assigned to C–H bonds from plant metabolites. A sharp and characteristic peak at 2353 cm\(^{-1}\) arises due to carboxylic acids and/or amide linkages of protein that can cause these peaks. All these findings are much consistent with the literature \[41\] in which hydroxyl and ketone groups and flavonoids are reported to the bio-reducing agent and carboxylic acid as the capping agent.

3.3. Powder x-ray diffraction analysis
The PXRD spectrum (figure 5) portrays the face-centered cubic (FCC) structure of the biogenic FI-AgNPs. The characteristic peaks in the XRD pattern of biologically fabricated AgNPs exhibit a peak at 2\(\theta\) of 38°, 45°, 65° and 77° corresponding to the lattice planes of (111), (200), (220) and (311), respectively. The plant-mediated AgNPs...
depict sharp and more peaks in the XRD spectrum compared to chemically synthesize AgNPs, substantiating the better crystallinity of plant-mediated silver nanoparticles. The sharp peaks give an indication about the Face Centered Cubic (FCC) structure of biogenic FI-AgNPs [42]. There is no impurity peak observed in the XRD pattern of FI-AgNPs. The biogenic AgNPs are a pure crystal with an average crystalline magnitude of 54.82 nm, calculated from the Scherer’s equation.

$$D = \frac{k\lambda}{B \cos \theta}$$  \hspace{1cm} (1)

3.4. SEM and TEM—morphological analysis
Scanning Electron Microscopy (SEM) was employed to clarify the shape, size, and morphology of the as-prepared AgNPs. As shown in figure 6(A), SEM images confirms the size of the nanoparticles as 45.96 to 64.88 nm with an average size of 55.42 nm, which may attribute to the aggregation of smaller particles or overlapping of capping agents on one another. The existence of aggregated AgNPs endorses the accomplishment of the synthesis of elemental silver nanoparticles and their capping through phytochemicals present in the aqueous extract of leaves of *Flacourtia indica* (figure 6(A)). Transmission Electron Microscopy (TEM) was

![Figure 4](image4.png)  
**Figure 4.** The typical FT-IR spectral scans of freshly prepared plant leave extract (A), and newly engineered FI-AgNPs (B).

![Figure 5](image5.png)  
**Figure 5.** X – ray diffraction patterns of newly engineered FI-AgNPs (JCPDS: 04-0783).
employed to explore the surface shape and size of the individual FI-AgNPs. TEM results (figure 6(B)) support the aggregation of nanoparticles in SEM images, as in TEM images, the size of individual FI-AgNPs appears in the range of 2.08 to 7.89 nm with spherical and uniform shape. The small size nanoparticles will certainly make them very active in the biomedical field.

3.5. Anti-microbial activity by FI-AgNPs
The inhibitory effect of silver has already been recognized against the microbial pathogens that are associated with industrial and medical processes [43, 44]. Antimicrobial activity of FI-AgNPs exposed that FI-AgNPs has noteworthy antibacterial activity against wound associated bacteria as compared to the plant extract and presubstituting drug (povidone-iodine) (table 1). Biogenic FI-AgNPs showed good antibacterial activity against S. saprophyticus, P. aeruginosa and S. pyogenes, i.e. 14, 15, and 16 mm, respectively. In integration to that povidone-iodine presented no activity against P. aeruginosa and displayed less activity against S. pyogenes, S. saprophyticus, and P. mirabilis, i.e., 8, 11, and 8 mm respectively. Moreover, Silver nitrate solution displayed no activity against P. aeruginosa and S. pyogenes and gives little inhibition to S. saprophyticus and P. mirabilis, i.e. 9 and 10 mm.

3.6. Anti-biofilm activity by FI-AgNPs
The potential of FI-AgNPs has been analyzed on the formation of biofilm by five strains of bacteria, i.e., Acinetobacter baumannii (SAB5), Pseudomonas aeruginosa (ETPS11), Klebsiella pneumoniae (SKP7), Proteus mirabilis (PPM8) and Escherichia coli (ETEC12) has been observed. Two methods were employed, i.e. ‘tube adherence and Congo red agar method’.

Figure 6. Morphological evaluation of newly engineered FI-AgNPs using SEM (A), and TEM (B).
3.6.1. Tube adherence method

In table 1, strong biofilm formation is represented by (A), moderate biofilm formation by (B), and very weak biofilm formation by (C). The results were calculated after 24 h exposing of a bacterial strain by different concentrations of FI-AgNPs. There was no effect of 15 and 30 $\mu$g ml$^{-1}$ concentrations of FI-AgNPs on bacterial strains, but overall 100 $\mu$g ml$^{-1}$ concentration of FI-AgNPs strongly inhibits the formation of biofilm in all five types of bacterial strains (figure 7(A)). Surprisingly, Escherichia coli (ETEC12) showed complete inhibition against biofilm formation at 80 $\mu$g ml$^{-1}$ FI-AgNPs and represented as N. The moderated, and weak biofilms were produced at 60 $\mu$g ml$^{-1}$ concentration. Pseudomonas aeruginosa (ETPS11) appeared as a weak biofilm producer while Proteus mirabilis (PPM8) and others appeared as a moderate biofilm producer at 60 $\mu$g ml$^{-1}$ concentration. In the positive control with 0 $\mu$g ml$^{-1}$ FI-AgNPs, very strong biofilm appeared, and no biofilm was produced in negative control for all bacterial strain (table 2). The increasing concentration of FI-AgNPs progressively inhibits biofilm formation. The FI-AgNPs impede the biofilm formation [45].

3.6.2. Congo red agar method

A biofilm is a layer of exopolysaccharides produced by bacteria for their protection. In this method five strains of bacteria (A. baumannii SAB5, P. aeruginosa ETPS11, K. pneumoniae SKP7, P. mirabilis PPM8, and E. coli ETEC12) were observed in Congo red agar media in the presence and absence of FI-AgNPs and observed after 24 h incubation period. At 15 $\mu$g ml$^{-1}$ concentration of FI-AgNPs, there was the appearance of black colonies indicating the growth of bacteria and the production of exopolysaccharide (EPSs), which is the initiation of biofilm formation. The same condition was observed in strains without FI-AgNPs. At 30 to 60 $\mu$g ml$^{-1}$ concentration of FI-AgNPs, the bacteria’s colonies were grown up without the intense display of dry crystalline

| Bacterial strain | Sets | 0.1 mg ml$^{-1}$ | 0.3 mg ml$^{-1}$ | 0.5 mg ml$^{-1}$ | 0.7 mg ml$^{-1}$ | 0.9 mg ml$^{-1}$ |
|------------------|------|----------------|----------------|----------------|----------------|----------------|
| P. mirabilis     | Set 1| P              | P              | N              | N              | N              |
|                  | Set 2| P              | P              | N              | N              | N              |
|                  | Set 3| P              | P              | N              | N              | N              |
| S. pyogenes      | Set 1| P              | P              | N              | N              | N              |
|                  | Set 2| P              | P              | N              | N              | N              |
|                  | Set 3| P              | P              | P              | N              | N              |
| P. aeruginosa    | Set 1| P              | P              | N              | N              | N              |
|                  | Set 2| P              | P              | P              | N              | N              |
|                  | Set 3| P              | P              | P              | N              | N              |
| S. saprophyticus | Set 1| P              | P              | P              | N              | N              |
|                  | Set 2| P              | P              | N              | N              | N              |
|                  | Set 3| P              | P              | N              | N              | N              |

N = no turbidity, P = microbial growth turbidity.
toxicity and mainly due to environmentally friendly properties. Biosynthesis of NPs is preferred over other methods owing to its biocompatibility, low cost, non-nanoparticles using algae, plants, bacteria, fungi, or other biological material is not only economic but also eco-harms with toxic chemicals that remain in biological systems. So, the researchers concluded that biosynthesis of Synthesis of nanoparticles using physical methods requires a high amount of energy likewise chemical methods antibiotic resistance. This antibiotic resistance takes the high cost of public health care due to prolonged treatment consisting of new and more effective drug designing and applying more efficient and widespread methods to control the spreading of pathogens antibiotic resistance. The resistance is mainly due to improper or over the use of antibiotics. This antibiotic resistance takes the high cost of public health care due to prolonged treatment consisting of new and more effective drug designing and applying more efficient and widespread methods to control the spreading of pathogens antibiotic resistance.

Another problem associated with the antibiotics is the appearance of dangerous and even life-threatening side effects that is majorly classified as hematologic side effects or hypersensitivity side effects including hypersensitivity reaction (Anaphylactic shock), growth inhibition or liver and kidney failure in some patients. No doubt these complications arise less common in patients but cannot be ignored due to their life-threatening nature. With the progress of nanotechnology science, the biosynthesis of AgNPs and their demonstration against these biomedical issues has increased dramatically due to their notable antimicrobial property. The presence of reducing agents or biologically active compounds may play vital role in the preparation, capping, and stabilization of AgNPs. Unique biomedical applications especially antibiotic

| Table 2. Antibiofilm activity of FI-AgNPs by tube adherence method. |
|-----------------------------------------------|
| **Sr. No.** | **SBP-MDR Strains** | **-ve control** | **+ve control (μg ml⁻¹)** |
| Drug strains generating a strong biofilm |
| 1. | *E. coli* | N | A |
| 2. | *P. aeruginosa* | N | A |
| 3. | *P. mirabilis* | N | A |
| 4. | *K. pneumoniae* | N | A |
| 5. | *A. baumannii* | N | A |

SBP = ‘strong biofilm producing’, A = ‘strong biofilm producer’, B = ‘moderate biofilm producer’, C = ‘weak biofilm producer’, N = ‘Non-biofilm producing’, MDR = ‘multidrug-resistant strains’.  

| Table 3. Antibiofilm activity of FI-AgNPs by Congo red Agar method. |
|-----------------------------------------------|
| **Sr. No** | **SBP-MDR Strains** | **-ve control** | **+ve control (μg/ml)** |
| Drug strains generating a strong biofilm |
| 1. | *Acinetobacter baumannii* | R | VB |
| 2. | *Pseudomonas aeruginosa* | R | VB |
| 3. | *Klebsiella pneumoniae* | R | VB |
| 4. | *Proteus mirabilis* | R | VB |
| 5. | *Escherichia coli* | R | VB |

SBP = strong biofilm producing, MDR = multidrug-resistant strains, VB = ‘strong biofilm producer’, AB = ‘moderate biofilm producer’, B = ‘weak biofilm producer’, R = ‘Non-biofilm producer’.  

black colonies because FI-AgNPs inhibited the synthesis of glycocalyx matrix. Whereas FI-AgNPs at 80 and 100 μg ml⁻¹ concentrations depicted completely inhibited the growth of bacteria and block the formation of biofilm (table 3, figure 7(B)).

4. Discussion

Synthesis of nanoparticles using physical methods requires a high amount of energy likewise chemical methods harms with toxic chemicals that remain in biological systems. So, the researchers concluded that biosynthesis of nanoparticles using algae, plants, bacteria, fungi, or other biological material is not only economic but also eco-friendly. Biosynthesis of NPs is preferred over other methods owing to its biocompatibility, low cost, non-toxicity and mainly due to environmentally friendly properties [46–48]. Plants leaves and fruits have anciently been used as medicine because they are rich in antibiotics, flavonoids, amino acids, terpenoids, steroids, alkaloids, and polysaccharides that are used for the production of useful herbal medicines for various diseases including cancer [49, 50]. Strong antimicrobial property of the herbal medicines is always required because of resistance of microbes to the antibiotic which is still a major issue in medical science. The resistance is mainly due to improper or over the use of antibiotics. This antibiotic resistance takes the high cost of public health care due to prolonged treatment consisting of new and more effective drug designing and applying more efficient and widespread methods to control the spreading of pathogens antibiotic resistance.

Another problem associated with the antibiotics is the appearance of dangerous and even life-threatening side effects that is majorly classified as hematologic side effects or hypersensitivity side effects including hypersensitivity reaction (Anaphylactic shock), growth inhibition or liver and kidney failure in some patients. No doubt these complications arise less common in patients but cannot be ignored due to their life-threatening nature. With the progress of nanotechnology science, the biosynthesis of AgNPs and their demonstration against these biomedical issues has increased dramatically due to their notable antimicrobial property. The presence of reducing agents or biologically active compounds may play vital role in the preparation, capping, and stabilization of AgNPs [15]. Unique biomedical applications especially antibiotic
therapy can excite researchers for new findings, purification, and standardization of nano-products synthesized from a herbal plant-like Flacourtia indica.

In this study, the antibacterial activity of the biosynthesized AgNPs prepared using aqueous leaves extract of the Flacourtia indica was investigated against various microbes for biofilm inhibition. The mechanism of interaction of the Fl-AgNPs is mainly concern with the compounds of thiol groups in the bacterial cell enzymes associated with respiration. Fl-AgNPs attach with the thiol groups present in the bacterial cell wall and inhibit respiration result in the decline phase of bacteria. Sulfur and phosphorus are the main components present throughout the bacterial cell walls. Fl-AgNPs possess high antibacterial activity due to the interaction with phosphorus and sulphur. Microbial cell walls consisting of sulfur are decomposed and their growth is inhibited as the concentration of Fl-AgNPsis increases.

Small AgNPs are more active because of their high penetration power, large surface area, and easy absorption. The potential release of silver ions greatly influences the reduction of nanoparticles size. So, the toxicity mainly associated with the size of AgNPs and the rate of emission of ions. Moreover, the antibacterial effects of the Fl-AgNPs are owing to the interaction of AgNPs with the three-dimensional microbial structure of the cell walls of bacteria. The destruction of the bacterial cell wall may also be attributed to the release of ROS (reactive oxygen species) that can damage membrane protein and nucleic acid. Bagherzadeh et al. compared the antibacterial effects of biosynthesized AgNPs prepared using saffron extract and market purchased AgNPs and showed that the extract of the saffron plant and purchased AgNPs did not show enough antibacterial effects but biosynthesized AgNPs exhibited promising antibacterial activity. Anandalakshmi et al. synthesize AgNPs using aqueous leaves extract of plant Pedalium murex, characterized them and investigate their antibacterial activity and concluded that green synthesis is eco-friendly and produce AgNPs that have a potential antibacterial effect against S. aureus, P. aeruginosa, E. coli, Mariniluteicoccus flavus, Bacillus subtilis, and Bacillus pumilus. Likewise, Espenti et al. explored antimicrobial effects of biogenic nanoparticles by (chebula Retz, Myrobalan), and investigated its antimicrobial activity against Bacillus subtilis and E. coli and resulted that AgNPs have greater antimicrobial activity than aqueous leaves extract of the plant. Like our experimental results, it is mentioned in the literature that increased concentration of AgNPs formed by the application of medicinal plants, increase the percentage inhibition of biofilm.

5. Conclusions

In this work, the air-stable Fl-AgNPs were successfully synthesized from the aqueous leaf extract of Flacourtia indica. The nanoparticles size appeared in TEM analysis is 2.08 nm to 7.89 nm, with an average size of 5.0 nm having a spherical shape. The sharp peak at 419 nm in the UV-Vis spectrum endorses the formation of biogenic Fl-AgNPs. The following study justified the synthesis of stable nanoparticles, which could be due to the presence of stabilizing and capping materials such as flavonoids and terpenoids within the plant extract. Additionally, the Fl-AgNPs have shown strong anti-microbial properties against four wound associated pathogen growth. Furthermore, Fl-AgNPs enhances the inhibition of biofilm formation in various bacterial strains of infectious disease. E. coli ET-EC12 appeared to be more sensitive and affected in the presence of Fl-AgNPs. Conclusively, the biogenic Fl-AgNPs exhibited noteworthy anti-microbial potential, especially anti-biofilm activity, and can open a new arena to clear production, eco-friendly, biocompatible, homogenous and pure Fl-AgNPs with low cost and propitious throughput rendering an authentic way to acquire green chemistry.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Geoprincy G, Srit B V, Poonguzhali U, Gandhi N N and Renganathan S 2013 A review on green synthesis of silver nanoparticles Asian Journal of Pharmaceutical and Chemical Research 6 8–12
[2] Lu F, Sun D, Huang J, Du M, Yang F, Chen H, Hong Y and Li Q 2014 Plant-mediated synthesis of Ag–Pd alloy nanoparticles and their application as catalyst toward selective hydrogenation ACS Sustainable Chemistry & Engineering 2 1212–8
[3] Kumar V, Pammi S, Kollu F, Satyanarayana K and Shameem U 2014 Green synthesis and characterization of silver nanoparticles using Borahavai diffusia plant extract and their anti bacterial activity Ind. Crops Prod. 52 562–6
[4] Salem W, Leitner D R, Zingl F G, Schratzer G, Prassl R, Goessler W, Reidl I and Schilld S 2015 Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli Int. J. Med. Microbiol. 305 85–95
[5] Bilal M, Rasheed T, Iqbal H M, Li C, Hu H and Zhang X 2017 Development of silver nanoparticles loaded chitosan–aginate constructs with biomedial potentials Int. J. Biol. Macromol. 105 393–400
[6] Arevalo-Gallegos A, Garcia-Perez J S, Carrillo-Nieves D, Ramirez-Mendoza R A, Iqbal H M and Parra-Saldivar R 2018 Botryococcus braunii as a bioreactor for the production of nanoparticles with antimicrobial potentialities Int. J. Nanomed. 13 5591
[7] Rasheed T, Nabeel F, Bilal M and Iqbal H M 2019 Biogenic synthesis and characterization of cobalt oxide nanoparticles for catalytic reduction of direct yellow-142 and methyl orange dyes. Biocatalysis and Agricultural Biotechnology 19 101154
[8] Mosayebi J, Kiyasatfar M and Laurent S 2017 Synthesis, functionalization, and design of magnetic nanoparticles for theranostic applications Adv. Healthcare Materials 6 1700306
[9] Sau T K and Rogach A L 2010 Nonspherical noble metal nanoparticles: colloid-chemical synthesis and morphology control Adv. Mater. 22 1781–804
[10] Ahmed S, Ahmad M, Swami B L and Ikram S 2016 A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise J. Adv. Res. 7 17–28
[11] Stark W 2011 Nanoparticles in biological systems Angew. Chem. Int. Ed. 50 1242–58
[12] Karhan O, Cemal O, Sera O N and Şimşek B 2017 Response surface methodology based desirability function approach to investigate optimal mixture ratio of silver nanoparticles synthesis process Ind. Eng. Chem. Res. 56 1810–9
[13] Furno F, Morley K S, Wong B, Sharp B L, Arnold P L, Howdle S M, Bayston R, Brown P D, Winship P D and Reid H J 2004 Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection J. Antimicrob. Chemother. 51 1019–24
[14] Vasilev K, Cook J and Grieser H J 2009 Antibacterial surfaces for biomedical devices Expert Review of Medical Devices 6 533–67
[15] Bharathi D, Josephin M D, Vasanharaj S and Bhuvaneshwari V 2018 Biosynthesis of silver nanoparticles using stem bark extracts of Diospyros montana and their antioxidant and antibacterial activities Journal of Nanostructure in Chemistry 8 83–92
[16] Makeboongo M O, Gilbreath J and Merrell D S 2014 Nontraditional therapies to treat Helicobacter pylori infection Journal of Microbiology 52 2359–72
[17] Ramesh S, Sivasamy A, Rhee K, Park S and Hui D 2015 Preparation and characterization of maleimide–polystyrene/SiO2–Al2O3 hybrid nanocomposites by an in situ sol–gel process and its antimicrobial activity Composites Part B: Engineering 75 167–75
[18] Prucke K, Tuček J, Kilianová M, Panaček A, Kvitík L, Filip J, Kolář M, Tománková K and Zbořil R 2011 The targeted antibacterial and antifungal properties of magnetic nanocomposite of iron oxide and silver nanoparticles Biomaterials 32 4704–13
[19] Liu Y, Yan J, Miao Y–E, Huang Y and Liu T 2015 Catalytic and antibacterial activities of green-synthesized silver nanoparticles on electropun polystyrene nanofiber membranes using tea polyphenols Composites Part B: Engineering 79 217–23
[20] Khan B A, Chevali V S, Na H, Zhu J, Warner P and Wang H 2016 Processing and properties of antibacterial silver nanoparticle-loaded hemp hurd, (poly lactic acid) biocomposites Composites Part B: Engineering 88 18–8
[21] Wang C, Flynn N T and Langer R 2004 Controlled structure and properties of thermoresponsive nanoparticle–hydrogel composites Adv. Mater. 16 1074–9
[22] Kang Y O, Im J N and Park W H 2015 Morphological and permeable properties of antibacterial double-layered composite nonwovens consisting of microfibers and nanoparticles Composites Part B: Engineering 75 236–63
[23] Liu F, Yuan Y, Li, Shang S, Yu X, Zhang Q, Jiang S and Wu Y 2015 Synthesis of polypyrrole nanocomposites decorated with silver nanoparticles with electrocatalysis and antibacterial property Composites Part B: Engineering 69 232–6
[24] Silver S, Phung L T and Iqbal G W 2006 Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds J. Ind. Microbiol. Biotechnol. 33 627–34
[25] Johnson J R, Kusowski M A and Witt J T 2006 Systematic review: antimicrobial urinary catheters to prevent catheter-associated urinary tract infection in hospitalized patients Annals of Internal Medicine 144 116–26
[26] Mishra V K, Husen A, Rahman Q I, Iqbal M, Sohrab S S and Yassin M O 2019 Nanomaterials and Plant Potential (Springer) pp. 135–75
[27] Jaffri S B and Ahmad K S 2018 Phytofunctionalized silver nanoparticles: green biomaterial for biomedical and environmental applications Reviews in Inorganic Chemistry 38 127–49
[28] Khan S U, Saleh T A, Wahab A, Khan M H U, Khan D, Khan W U, Rahim A, Kamal S, Khan F U and Fahad S 2018 Nanosilver: new ageless and versatile biomedical therapeutic scaffold Int. J. Nanomed. 13 733–62
[29] Bilal M, Mehmoond S, Rasheed T and Iqbal H 2019 Bio-catalysis and biomedical perspectives of magnetic nanoparticles as versatile carriers Magneticotechnology 5 42
[30] Shamaila S, Sajjad A K L, Farooqi S A, Jabeen N, Majed S and Farooq I 2016 Advancements in nanoparticle fabrication by hazard free eco-friendly green routes Applied Materials Today 5 150–99
[31] Bilal M, Zhao Y, Rasheed T, Ahimed I, Hassan S T, Nawaz M Z and Iqbal H 2019 Biogenic nanoparticle–chitosan conjugates with antimicrobial, antibiofilm, and anticancer potentialities: development and characterization International Journal of environmental research and public health 16 598
[32] Johnson-Culton S B 2014 Systematics, Bioecology, and Ethnobotany of the Puntropical Family Cochlospermaceae (Malvales) (Oxford, OH 45056, United States: Miami University
[33] Durán N, Nakazato G and Seabra A B 2016 Antimicrobial activity of biogenic silver nanoparticles, and silver chloride nanoparticles: an overview and comments Appl. Microbiol. Biotechnol. 100 6555–70
[34] Rasheed T, Bilal M, Li C and Iqbal H 2017 Biomedical potentialities of taxacum officinale-based nanoparticles biosynthesized using methanic leaf extract Current pharmaceutical biotechnology 18 1116–23
[35] Nandini T, Monajkumar S, Vadivel V, Devipriya N and Meena Devi J 2019 Synthesis of spheroid shaped silver nanoparticles using Indian traditional medicinal plant Flacourtia indica and their in vitro anti-proliferative activity Mater. Res. Express 6 045032
[36] Lekshmi N P, Sumi S B, Viveka S, Jeera S and Brindha J R 2017 Antibacterial activity of nanoparticles from Allium sp Journal of Microbiology and Biotechnology Research 2 115–9
[37] Kulshreshtha S, Khan S, Hasan S, Khan M E, Misra L and Khan A U 2016 Calcium fluoride nanoparticles induced suppression of Streptococcus mutans biofilm: an in vitro and in vivo approach Appl. Microbiol. Biotechnol. 100 1901–14
[38] Çiftçi A, Findik A, Onuk E E and Savasan S 2009 Detection of methicillin resistance and slime factor production of Staphylococcus aureus in bovine mastitis Brazilian Journal of Microbiology 40 254–61
[39] Kaiser T D L, Pereira E M, dos Santos K R N, Maciel E L N, Schuenck R P and Nunes A P F 2013 Modification of the Congo red agar method to detect biofilm production by Staphylococcus epidermidis Diagnostic Microbiology and Infectious Disease 75 235–9
[40] Bharath D, Kalaichelvan P, Atmaram V and Anbu S 2016 Biogenic synthesis of silver nanoparticles from aequous flower extract of Bougainvilleas spectabilis and their antibacterial activity J. Med. Plants 4 248–52
[41] Rasheed T, Bilal M, Iqbal H M and Li C 2017 Green biosynthesis of silver nanoparticles using leaves extract of Artemisia vulgaris and their potential biomedical applications Colloids Surf., B 158 408–15
[42] Bhakya S, Muthukrishnan S, Sukumar M and Muthukumar M 2016 Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity Applied Nanoscience 6 755–66
[43] Emam H E and Zahran M K 2015 Ag0 nanoparticles containing cotton fabric: synthesis, characterization, color data and antibacterial action Int. J. Biol. Macromol. 75 106–14
[44] Lok C-N, Ho C-M, Chen R, He Q-Y, Yu W-Y, Sun H, Tam P K-H, Chiu J-F and Che C-M 2007 Silver nanoparticles: partial oxidation and antibacterial activities JBIC Journal of Biological Inorganic Chemistry 12 527–34
[45] Kalishwaralal K, BarathManiKanth S, Pandian S R K, Deepak V and Gurunathan S 2010 Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis Colloids Surf., B 79 340–4
[46] Shahverdi A R, Minaeian S, Shahverdi H R, Jamalifar H and Nohi A-A 2007 Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach Process Biochem. 42 919–23
[47] Tsuji T, Kakita T and Tsuji M 2003 Preparation of nano-size particles of silver with femtosecond laser ablation in water Appl. Surf. Sci. 206 314–20
[48] Gajbiye M, Kesharwani J, Ingle A, Gade A and Rai M 2009 Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole Nanomed. Nanotechnol. Biol. Med. 5 382–6
[49] Goswami H K and Ram H K 2017 Ancient food habits dictate that food can be medicine but medicine cannot be ‘food’!! Medicines (Basel) 4, 82
[50] Behravan M, Panahi A H, Naghizadeh A, Ziae M, Mahdavi R and Mirzapour A 2019 Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity Int. J. Biol. Macromol. 124 148–54
[51] Cunha B A 2001 Antibiotic side effects Medical Clinics of North America 85 149–85
[52] Carlson C, Hussain S M, Schrand A M, Braydich-Stolle L K, Hess K L, Jones R L and Schlager J J 2008 Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species The Journal of Physical Chemistry B 112 13608–19
[53] Azizi M, Sedaghat S, Tahvidari K, Derakshi P and Ghacesi A 2017 Synthesis of silver nanoparticles using peganum harmala extract as a green route Green Chem. Lett. Rev. 10 620–7
[54] Bagherzade G, Tavakoli M M and Namai M H 2016 Green synthesis of silver nanoparticles using aqueous extract of saffron (Crocus sativus L.) wastages and its antibacterial activity against six bacteria Asian Pacific Journal of Tropical Biomedicine 7 227–33
[55] Anandalakshmi K, Venugobal J and Ramasamy V 2016 Characterization of silver nanoparticles by green synthesis method using Pedaliun murex leaf extract and their antibacterial activity Applied Nanoscience 6 399–408
[56] Espenti CS, Rao K K and Rao K M 2016 Bio-synthesis and characterization of silver nanoparticles using terminalia chebula leaf extract and evaluation of its antimicrobial potential Mater. Lett. 174 129–33
[57] Bharath D, Vasantharaj S and Bhuvaneswari V 2018 Green synthesis of silver nanoparticles using Cordia dichotoma fruit extract and its enhanced antibacterial, anti-biofilm and photo catalytic activity Mater. Res. Express 5 055404
[58] Doan V D, Nguyen T D, Nguyen T L H and Nguyen H T 2019 Green synthesis of silver nanoparticles using aganonerion polymorphum leaves extract and evaluation of their antibacterial and catalytic activity Mater. Res. Express 6 1150g1