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A Comparative Analysis of Antimicrobial, Antibiofilm and Antioxidant Activity of Silver Nanoparticles Synthesized from *Erythrina Suberosa* Roxb. and *Ceiba Pentandra*

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Abstract: Biofilm forming bacteria can cause serious health problems that are difficult to combat. Silver nanoparticles (Ag-NPs) synthesized from plant extracts have potential to fight and eradicate biofilm-forming bacteria. In the present research, AgNPs were synthesized using leaf and bark extract of *Erythrina suberosa* Roxb. and *Ceiba pentandra* L. and their antibiofilm, antioxidant and antibacterial activity was checked. Phytochemical analysis of the plant extracts showed important bioactive compounds such as tannins, saponins, steroids, phenolics, alkaloids, flavonoids and glycosides. The AgNPs were synthesized and confirmed by visual color observation and UV-Vis spectrophotometer. Visual color observation showed that the color of the leaf and bark extracts of *E. suberosa* and *C. pentandra* turned into brown. UV-Vis spectra analysis showed absorbance peak range between 430-450 nm. The antioxidant activity of the AgNPs was determined by FRAC (Ferrous reducing antioxidant capacity) assay. Synthesized AgNPs from all sources showed significant antioxidant activity. However, antioxidant activity of *E. suberosa* AgNPs was significant compared to other sources. Antibacterial activity and biofilm forming assay was analyzed against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The synthesized AgNPs silver nanoparticles showed significant ($p \leq 0.05$) antibacterial activity against all the bacteria. The maximum zone of inhibition was found in case of *E. suberosa* AgNPs bark extract against *P. aeruginosa* was 20±1.154 mm. The results of biofilm forming assay showed that the AgNPs from all sources significantly ($p \leq 0.05$) inhibited the activity of biofilms by all the tested bacteria. From results, it can be concluded that AgNPs synthesized from both plants can be used in developing antimicrobial compounds.

Key words: silver nanoparticles, *Erythrina suberosa*, *Ceiba pentandra*, phytochemicals, biofilm

1 Introduction

Nanotechnology is a science associated with broad-ranging physiochemical properties of nanoparticles (NPs). Nanoparticles are extremely small particles of size 1–100 nm[1, 2]. Nanoscale materials in this size range exhibit distinctive properties[3–5]. Metal nanoparticles are the most studied nanoparticle materials because they are easy to make. Furthermore, metal NPs show many applications such as detectors, antimicrobials/antibacterial, agents for surface coating, catalysts. Following are the most studied metallic NPs: silver nanoparticles (AgNPs)[6, 7], platinum (Pt)[8, 9] and palladium nanoparticles (PdNPs)[10]. The most widely used metallic NPs, AgNPs, are widely used in the medical industry for the production of creams and ointments which are used in healing of wounds and burns to prevent infections[11]. Silver is documented for its anti-bac-

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terial potential against a wide-ranging spectrum of disease causing bacteria[12].

Use of plants in the synthesis of NPs has attracted a lot of interest as it requires less work than growing and keeping microbes. Plant metabolites are organic and usually have little toxicity to live cells, making their usage in the production of AgNPs advantageous[13]. Plant's biomolecules are involved in the biosynthesis of AgNPs from plants. Secondary metabolites such as flavonoids, phenolic compounds, glycosides, and sterols are mainly involved in the biosynthesis process of NPs because these molecules reduce and stabilize the Ag^{+} [14-16].

Erythrina suberosa Roxb. (*E. suberosa*) is a member of Fabaceae family (sub-family Leguminasae) and commonly it is called as coral tree in English and Gul-e-nishtar in Urdu. *E. suberosa* is used as useful herbal drug and widely used as medicinal plant[17]. Various studies have confirmed the various medicinal uses such as its leaves can be used to suppress the sperm production, in the treatment of high blood pressure, inhibits the production of androgens and gonadotropins[18, 19]. Stem bark has anti–tumor, hypoglycemic, cytotoxic, anti-spasmodic, antibacterial, anticancer activities[20, 21]. *Ceiba pentandra* L. (*C. pentandra*) is a member of family Malvaceae and is commonly called as "silk cotton tree" or "kapok tree". Bark and leave extracts *C. pentandra* are conventionally used in the treatment of diabetes mellitus, gonorrhea, malaria fever, syphilis, wound healing, gastrointestinal infections and asthma in various ways[22, 23].

Biosynthesis of AgNPs using plant concentrates and their reduction is ecofriendly, quick, single step without the need of any specific physical and chemical methods[24]. Present study deals with the green synthesis of AgNPs utilizing leaf and bark extracts of *E. suberosa* and *C. pentandra* along with determination of antimicrobial, antibiofilm and antioxidant activity.

2 Materials and Methods

2.1 Collection and extraction of plant material

Fresh leaves and stem bark of *C. pentandra* and *E. suberosa* Roxb. were collected from Bagh-e-Jinnah Lahore, Pakistan. The leaves and bark materials were dried under shade at room temperature to prevent degradation of phytochemicals. The dried materials were grinded in a mill to form powder. After obtaining crude methanolic extract, it was filtered. This process was repeated for 3-5 times. The extract was concentrated in rotary evaporator at 40℃ to get crude extract[25].

2.2 Phytochemical screening of plant extracts

Phytochemical screening was done both qualitatively and quantitatively. Qualitative analysis was performed to detect the presence of different phytochemicals such as tannins, saponins, steroids, phenolics, alkaloids, flavonoids and glycosides.

2.2.1 Test for tannins

For 10 minutes, 0.30 g powdered plant material was boiled in a water bath and filtered. Few drops (2-4) of 0.1% $FeCl_3$ solution were added in the filtrate. The appearance of a greenish brown or a blue black coloration was considered as positive test for tannins[26].

The tannins were quantified by using Folin-Ciocalteu (FC) reagent[27]. 0.1 mL of the plant extract, 7.5 mL of distilled water, 0.5 mL of FC reagent and 1 mL of (35%) sodium carbonate solution were mixed and final volume was made up to 10 mL by adding distilled water. The mixture was shaken and was left at room temperature for 30 minute. At 725 nm absorbance was measured against blank using water. Gallic acid was used as standard to construct calibration curve under the same conditions as described above and TTC was calculated from this curve using the equation: $y = 0.561x + 0.239$ $R^2 = 0.947$. Where y is absorbance and x is the concentration of Gallic acid.

2.2.2 Test for saponins

Froth Test was performed to detect saponins in which 3 mL of concentrate was poured in 20 mL of distilled water. Formation of 1 cm foam layer persistent for ten minutes confirmed the saponins[19].

2.2.3 Test for steroids

For the detection of steroids test, concentrated sulfuric acid (4-5 drops) was added in 2 mL of extract. Red color appearance showed the positive test for steroids[28].

2.2.4 Test for phenolics

For the detection of phenolics, 5% ferric chloride solution was added in 2 mL of extract. Formation of deep blue black coloration indicated the existence of phenolics[19].

Quantitatively, total phenolic content (TPC) was determined by using Folin-Ciocalteu (FC) reagent[29]. When phenols react with phosphotungstate-phosphomolbdate complex, the reduction of this complex takes place and blue color appears. In this method, the 20 μL extract was poured in distilled water and volume increased up to 158 μL of distilled water and 100 μL of FC reagent was mixed and incubated for 10 minutes at room temperature. 300 μL of 25% w/v solution of sodium bicarbonate was dissolved into the mixture, incubated at 40℃ for 30 minutes and allowed to cool. Absorbance was noted at 765 nm using methanol as blank. To construct calibration curve, tannic acid was used as standard and TTC was calculated from this calibration curve using the equation: $y = 0.980x + 0.822$ $R^2 = 0.938$. Where y represents the absorbance and x is the concentration of tannic acid.

2.2.5 Tests for alkaloids

For detection of alkaloids, Dragendroff's test was used in which 1 mL plant extract was added with 3-4 drops of Dragendroff's reagent[19]. Formation of orange red color gave

positive result for alkaloids.

2.2.6 Test for flavonoids

To detect flavonoids, few drops of 1% aluminium chloride solution were added to a plant extract. A yellow coloration was observed indicating the presence of flavonoids[28].

Total flavonoid content (TFC) in each extract was determined by using $AlCl_3$[30]. 5% w/v 90 μL sodium nitrate solution was mixed and allowed to settle for six minutes. 185 μL of 15% w/v $AlCl_3$ was mixed and incubated for 5 minutes at room temperature and 600 μL of 1M sodium hydroxide solution was added. By adding de-ionized water, the total volume of reaction mixture was made up to 3 mL. At 510 nm, absorbance was noted against methanol as blank. To construct calibration curve, quercitin was used as standard and TFC was calculated from this calibration curve using the equation: $y = 0.582x + 1.705$ $R^2 = 0.803$. Where y represents absorbance and x denotes the quercetin concentration.

2.2.7 Test for glycoside

To detect the presence of glycosides, 2 g of sample was added to 25 mL of distilled water, boiled for 5 minutes on a water bath, and filtered. 5 mL of this filtrate was mixed with 0.2 mL Fehling's solution. On heating, appearance of a brick-red coloration indicated the presence of glycosides[30].

2.3 Biosynthesis of silver nanoparticles

For the synthesis of AgNPs, 20 g sterilized powder was mixed into 200 mL deionized water. Following heating at 60℃–70℃ for 15 minutes, mixture was filtered, and 100 mL was poured into 400 mL (pre-warmed) of 1 mM $AgNO_3$ solution with constant stirring. The mixture color change indicated the formation of AgNPs[25].

2.4 UV-Vis spectra analysis

The reduction of silver ions was confirmed by examining the reaction medium's UV–Vis spectra after an overnight incubation. Coloration was detected during the synthesis step since the color variations may be seen visually. As previously reported, the concentration of generated AgNPs was determined using a UV spectrophotometer with a 1 nm resolution between 200 and 500 nm[31].

2.5 Antioxidant activity of synthesized silver nanoparticles

Antioxidant potential of plant extracts was determined by the ferrous reducing antioxidant capacity (FRAC) assay[32]. In this method, 0.25 mL of synthesized AgNPs at different concentration (31, 63, 125, 250, 500 μg/mL), was mixed with 0.625 ml of potassium phosphate buffer (0.2 M) and 0.625 mL of 1% potassium ferricyanide, $[K_3Fe(CN)_6]$ solution. Reaction mixture was incubated at 50℃ for 20 minute. 0.625 mL of 10% trichloro acetic acid (TCA) solution was mixed. Following addition of TCA at 3000 rpm for 10 minute mixture was centrifuged. Then 1.8 mL supernatant was mixed with 1.8 mL of distilled water and 0.36 mL of 0.1% ferric chloride ($FeCl_3$) solution was added. A spectrophotometer was used to test the absorbance of the mixture at 700 nm against a blank. The same solution mixture without the AgNP was used as blank solution, and incubated under the same conditions. At 700 nm, the absorbance of the blank solution was measured. Increased reaction mixture absorbance suggests increased reducing capability. At each concentration, the experiment was performed three times.

2.6 Determination of antibacterial potential of synthesized AgNPs by agar well diffusion method

The antimicrobial potential of the synthesized AgNPs was checked using the well diffusion assay[24] against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In brief, L agar slants were used to grow the tested bacteria. The L Agar medium was autoclaved and was allowed to cool down at 40-45℃. Test organisms (old cultures) were added. After mixing well, L Agar medium containing test bacteria was transferred into petri dishes. Media plates were allowed to solidify. With the help cork borer, wells were cut properly in petri dishes. The 0.1 mL of synthesized AgNPs were added to the wells. Ciprofloxcin (50 μg/mL) was used as control. Plates were kept in the refrigerator for 30 minutes before being incubated at 37℃ for 24 hours for diffusion. The diameter of the inhibition zone established around the well was measured to determine antibacterial activity.

2.7 Disc diffusion method and minimum inhibitory concentration (MIC) determination

In this method, several aqueous solutions of AgNPs comprising of the following concentrations: 15, 31, 62, 125, 250 and 500 μg/mL were made. Sterile filter paper discs were impregnated with test concentration and air dried. Ciprofloxacin (50 μg/mL) was used as control. For antimicrobial screening, these discs were placed in petri dishes (120 mm in diameter) containing an appropriate agar medium inoculated with test organisms via a sterile transfer loop. After that, the plates were held at 370℃ to allow for maximum diffusion. Silver nanoparticles showing antimicrobial potential suppressed the development of microorganisms, forming a visible, discrete zone known as the zone of inhibition. The diameter of the zone of inhibition in terms of mm was used to measure the tested pathogens' antibacterial activity. The experiments are repeated three times, with the average readings recorded. The minimum inhibitory concentration (MIC) was determined by measuring the diameter of zone of inhibition. MIC value represents the lowest concentration of the synthesized NPs that inhibited visible growth of the test organisms.

2.8 Biofilm forming assay and antibiofilm potential of plant based AgNPs

The isolates from clinical specimens consisting of *E. coli*, *P. aeruginosa* and *S. aureus* were used for their biofilm forming ability. The biofilm was determined by spectrophotometric analyses using 96 well microtiter plates[33, 34]. The bacterial culture was inoculated in LB medium in microtiter plate wells and was permitted for formation of biofilms 24 hours by incubating at 37℃. After successful incubation, the wells were rinsed with water and air dried for 20-25 minutes. Moreover, the crystal violet was added into wells and kept for 15 minutes at 37℃. The wells were again rinsed with water to wash out the extra stain and air dried. Finally, the wells were de-stained with absolute ethanol and the absorbance of stained cells was recorded using spectrophotometer at 570 nm. Biofilm was expressed as normalized values (OD_{570} / OD_{600}) to overcome the differences in bacterial growth. To determine the biofilm inhibitory concentration (BIC) and efficacy of AgNPs, AgNPs were added during biofilm formation.

2.9 Statistical analysis

All experiments were done in triplicates and values were expressed as average values. Standard errors of values were calculated and expressed in spread of bars in each graph. Analysis of Variance (ANOVA) followed by post hoc Tureky test was performed to statistically analyze the data with using level of significance ($p \leq 0.05$). The difference between specific groups was determined using LSD (least significant differences).

3 Results

3.1 Extraction of plant material

The dry yield of the methanolic leaf and bark extracts of the plants showed that *E. suberosa* yielded more extracts than *C. pentandra*. The results of dry yield for both plant extract are given in Table 1.

3.2 Phytochemical screening of plant extracts

3.2.1 Test for tannins

Tannins were present in both leaf and bark extracts of *E. suberosa* and *C. pentandra*. Leaf and bark extracts of both plants showed blue black coloration indicating positive result for tannins. Total tannin content was found to be highest in *E. suberosa* leaf extract and lowest in *C. pentandra* bark extract. Results of total tannin content in plant extracts are shown in Fig. 1.

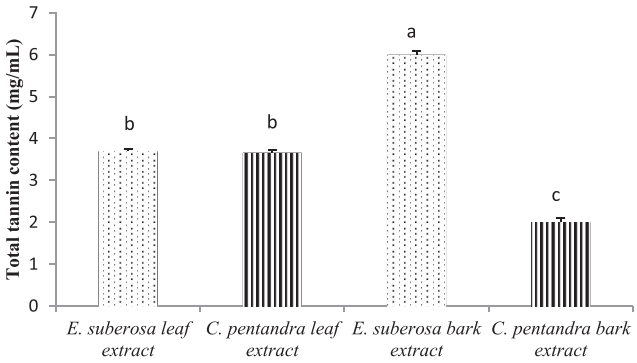

Fig. 1 Total tannin content in leaf and bark extracts of *E. suberosa* and *C. pentandra*. *E. suberosa* bark extract showed significantly high tannin content compared to other plant sources. One Way Analysis of Variance (ANOVA) with post hock Tukey was used to determine the total tannin content. $P<0.05$ was considered significant in all tests.

3.2.2 Test for saponins

Saponins were present in all plant extracts as foam layer appeared in all plant extracts and persisted for 10 minutes which confirmed the presence of saponins.

3.2.3 Test for steroids

Steroids were present in bark extracts of *E. suberosa* and *C. pentandra* as red coloration which confirmed the positive result for steroids in bark extracts of *E. suberosa* and *C. pentandra*. Steroids were absent in leaf extracts of *E. suberosa* and *C. pentandra*.

3.2.4 Tests for alkaloids

Phenolics were present in bark extracts of *E. suberosa* and *C. pentandra* as Orange red color appeared in bark extracts of *E. suberosa* and *C. pentandra*.

3.2.5 Test for phenolics

Total phenolic content was found significantly high ($p \leq 0.05$) in bark extract of *E. suberosa* and lowest in *C. pentandra* as indicated by appearance of deep blue color (Fig. 2)

3.2.6 Test for flavonoids

A yellow coloration confirmed the presence of flavonoids in all plant extracts s. Maximum total flavonoid content

Table 1 Dry yield of plant extract.

	Fresh weight (g)	Fresh weight (g)	Crude methanol	% Yield
E. suberosa leaf extract	140.0 ± 0.6	101.0 ± 0.5	2.65 ± 0.1	2.6[a]
C. pentandra leaf extract	250.0 ± 0.4	114.0 ± 0.6	1.94 ± 0.0	1.7[b]
E. suberosa bark extract	140.0 ± 0.9	120.0 ± 0.2	2.17 ± 0.0	1.8[b]
C. pentandra bark extract	250.0 ± 1.2	105.0 ± 0.4	1.86 ± 0.1	1.8[b]

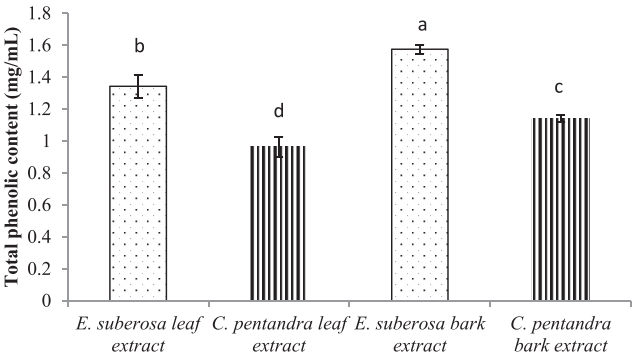

Fig. 2 Total phenolic content in leaf and bark extracts of *E. suberosa* and *C. pentandra*. *E. suberos*a leaf and bark extracts showed significantly high total phenolic contents compared to *C. pentandra* leaf and bark extracts. One Way Analysis of Variance (ANOVA) with post hock Tukey was used to determine the total phenolic content. $P<0.05$ was considered significant in all tests.

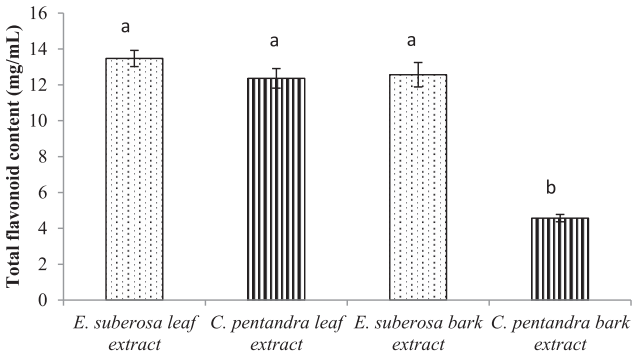

Fig. 3 Total flavonoid content in leaf and bark extracts of *E. suberosa* and *C. pentandra*. Significant high total flavonoid contents were observed in *E. suberosa* leaf-bark extracts and *C. pentandra* bark extract. One Way Analysis of Variance (ANOVA) with post hock Tukey was used to determine the total flavonoid content. $P<0.05$ was considered significant in all tests.

was found in *E. suberosa* leaf extract and lowest in bark extract of *C. pentandra* (Fig. 3).

3.2.7 Test for glycoside

Glycosides were present in leaf extracts of *E. suberosa* and *C. pentandra* because brick red coloration which confirmed the presence of glycosides was appeared in leaf extracts of *E. suberosa* and *C. pentandra*.

3.3 Formation of nanoparticles

After the mixing of leaf and bark extracts with the 1 mM $AgNO_3$ solution, the formation of NPs started due to reduction of silver ions. The visible color changes from light yellow to reddish brown of *E. suberosa* leaf and *C. pentandara* bark extract while for *E. suberosa* bark and *C. pentandara* leaf extract colorless to reddish brown color change was noted.

3.4 UV-Vis spectra analysis

The confirmation of synthesized AgNPs was analyzed using UV-Vis spectrophotometer. To determine the characteristics of the peak spectrum wavelength of the AgNPs, UV-Vis spectra analysis was done. In this study, a peak specific for the synthesis of AgNPs was appeared at 430-450 nm for each extract by UV-Visible spectroscope. The UV-Visible absorption spectrum of the AgNPs of leaf and bark extracts of *C. pentandara* and *E. suberosa* are shown in Figs. 4a-4d.

3.5 Antioxidant activity of synthesized silver nanoparticles

With increasing concentration of synthesized AgNPs using leaf and bark extracts of *E. suberosa* and *C. pentandra*, the antioxidant potential of NPs significantly increased. In general, among all NPs sources, 125 μg/mL

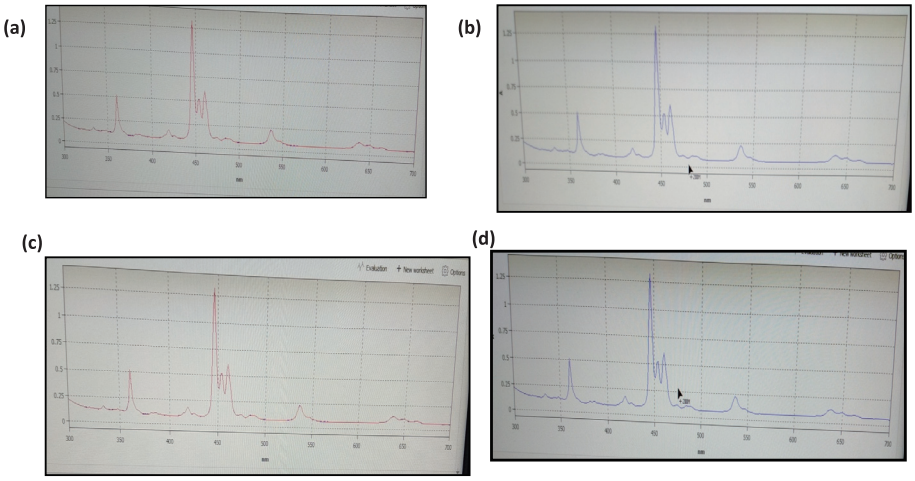

Fig. 4 UV–Vis absorption spectra of silver nanoparticles biosynthesized by (a) *E. suberosa leaf* extract (b) *E. suberosa bark* extract, (c) *C. pentandara* leaf extract, (d) *C. pentandara* leaf extract.

concentration was optimum for antioxidant activity by both plant extracts biosynthesized NPs. Though, *E. suberosa* bark extract AgNPs showed antioxidant activity at all concentrations. The results of antioxidant activity are given in Table 2.

3.6 Antibacterial potential of AgNPs by agar well diffusion method

The biosynthesized AgNPs showed significant inhibitory activity against all the bacteria. Plant extracts showed no zone of inhibition against all bacteria. The maximum zone of inhibition was found in case of AgNPs of *E. suberosa* bark extract against *P. aeruginosa* (18.7 ± 1.2) and lowest was found in AgNPs of *C. pentandra* leaf extract against *P. aeruginosa* (8.6 ± 1.2). AgNPs of *E. suberosa* leaf and bark extract showed comparatively high antibacterial activity than AgNPs of *C. pentandra* leaf and bark extract (Table 3).

3.7 Minimum Inhibitory Concentration (MIC) determination by disc diffusion method

E. suberosa bark AgNPs showed effective MIC at 63, 250 and 125 μg/mL concentrations against *E. coli*, *P. aeruginosa* and *S. aureus*, respectively. *E. suberosa* leaf AgNPs showed effective MIC value at 250 μg/mL against *E. coli* and 125 μg/mL against *S. aureus* and *P. aeruginosa* (Table 4). The MIC values of 63 μg/mL against *S. aureus*, 125 μg/mL against *P. aeruginosa* and *E. coli* were found effective by *C. petandara* bark AgNPs. *C. petandara* leaf AgNPs showed promising MIC at 125 μg/mL against *E. coli*, *S. aureus* and 250 μg/mL against *S. aureus* and *P. aeruginosa* (Table 5).

3.8 Biofilm forming assay

Silver nanoparticles synthesized from leaf and bark extracts *E. suberosa* and *C. pentandra* significantly inhibited the biofilm formed by *E. coli*, *S. aureus*, *P. aeruginosa* compared to control. The results of this assay showed that the AgNPs from all plant sources significantly inhibited ($p < 0.05$) the activity of biofilms for all the tested bacterial isolates. In general, among all the bacteria, greater inhibition was observed for AgNPs of *E. suberosa* bark and leaf against *P. aeruginosa* (Figs. 5a, 5b). When *C. pentandara* bark was used to prepare AgNPs, it showed greater inhibition against *E. coli* (Fig. 5c), however, *C. pentandara* leaf AgNPs showed variable results for all bacterial isolates (Fig. 5d).

Table 2 Absorbance of FRAC of *E. suberosa* and *C. pentandra* leaf and bark extract biosynthesized AgNPs.

Concentration (μg/mL)	AgNPs of *E. suberosa* leaf extract	AgNPs of *E. suberosa* bark extract	AgNPs of *C. pentandra* leaf extract	AgNPs of *C. pentandra* bark extract
31	2.19 ± 0.1^{b}	3.76 ± 0.2^{a}	1.84 ± 0.2^{c}	2.05 ± 0.1^{b}
63	2.26 ± 0.2^{b}	3.14 ± 0.1^{a}	2.01 ± 0.0^{b}	2.21 ± 0.1^{b}
125	3.89 ± 0.2^{a}	3.13 ± 0.1^{a}	3.44 ± 0.1^{a}	3.28 ± 0.0^{a}
250	4.12 ± 0.1^{a}	3.77 ± 0.4^{b}	2.51 ± 0.0^{c}	3.59 ± 0.0^{b}
500	4.78 ± 0.1^{a}	3.15 ± 0.2^{b}	3.18 ± 0.1^{b}	3.47 ± 0.1^{b}

Table 3 Antibacterial activity of biosynthesized AgNPs by agar well diffusion method.

Components	Zone of inhibition (mm)		
	P. aeruginosa	*E. coli*	*S. aureus*
E. suberosa leaf extract	No zone	No zone	No zone
E. suberosa leaf AgNPs	14.7 ± 1.2^{b}	16.7 ± 2.31^{a}	17.6 ± 1.2^{a}
E. suberosa bark extract	No zone	No zone	No zone
E. suberosa bark AgNPs	18.7 ± 1.2^{a}	16 ± 1.2^{b}	14.7 ± 11.6^{b}
C. pentandra leaf extract	No zone	No zone	No zone
C. pentandra leaf AgNPs	8.6 ± 1.2^{b}	15.0 ± 1.0^{a}	9.3 ± 2.3^{b}
C. pentandra bark extract	No zone	No zone	No zone
C. pentandra bark AgNPs	14.7 ± 1.2^{a}	15.3 ± 0.6^{a}	12.7 ± 2.3^{b}
Control (Ciprofloxicin)	19.3 ± 1.2^{a}	20.3 ± 0.6^{a}	19.3 ± 1.6^{a}
LSD for components = 1.722; LSD for isolates = 2.81			

Table 4 Minimum inhibitory concentration of synthesized from *E. suberosa* bark and leaf AgNPs by disc diffusion method.

	AgNPs of *E. suberosa* bark extract (μg/mL)						
	15	31	63	125	250	500	Control 50
E. coli	4.0 ± 0.0^{d}	11.3 ± 1.2^{b}	17.3 ± 1.2^{b}	4.7 ± 0.5^{d}	9.3 ± 1.2^{c}	7.3 ± 1.2^{c}	19.0 ± 1.1^{a}
S. aureus	3.3 ± 1.2^{c}	5.7 ± 1.2^{b}	6.0 ± 1.2^{b}	9.3 ± 0.5^{b}	15.3 ± 1.2^{a}	6.0 ± 0.0^{b}	14.4 ± 1.5^{a}
P. aeruginosa	5.7 ± 1.5^{b}	5.3 ± 1.2^{b}	7.33 ± 1.2^{b}	16.3 ± 0.5^{a}	9.3 ± 1.2^{b}	6.3 ± 0.5^{b}	15.6 ± 1.5^{a}
	AgNPs of *E. suberosa* leaf extract (μg/mL)						
E. coli	3.3 ± 1.2^{d}	13.0 ± 1.7^{b}	8.0 ± 0.0^{c}	6.2 ± 1.2^{c}	16.7 ± 1.2^{a}	6.7 ± 1.2^{c}	20.0 ± 1.1^{a}
S. aureus	2.7 ± 0.1^{c}	13.3 ± 1.2^{a}	4.7 ± 1.2^{c}	14.0 ± 0.0^{b}	7.33 ± 1.2^{c}	4.7 ± 1.2^{c}	17.0 ± 2.1^{a}
P. aeruginosa	1.7 ± 0.5^{c}	4.0 ± 0.0^{c}	4.0 ± 0.0^{c}	11.3 ± 1.2^{a}	10.0 ± 0.0^{b}	5.3 ± 1.2^{c}	14.0 ± 2.2^{a}
LSD for source: 3.081							
LSD for microorganisms: 4.2							

Table 5 Minimum inhibitory concentration of AgNPs of *C. pentandara* bark and leaf AgNPs by disc diffusion method.

	AgNPs of *C. pentandara* bark (μg/mL)						
	15	31	63	125	250	500	Control 50
E. coli	5.3 ± 1.2^{c}	3.3 ± 1.1^{c}	6.33 ± 1.2^{c}	14.7 ± 1.2^{b}	10.0 ± 0.0^{b}	9.3 ± 0.5^{c}	20.0 ± 1.1^{a}
S. aureus	8.7 ± 1.2^{b}	9.0 ± 1.1^{b}	16.0 ± 1.2^{a}	10.7 ± 1.2^{b}	9.3 ± 1.2^{b}	4.7 ± 1.2^{c}	17.8 ± 1.5^{a}
P. aeruginosa	3.4 ± 1.2^{c}	5.3 ± 1.2^{c}	8.7 ± 1.2^{b}	14.7 ± 1.2^{a}	6.8 ± 1.2^{c}	4.6 ± 1.2^{c}	17.7 ± 0.8^{a}
	AgNPs of *C. pentandara* leaf (μg/mL)						
E. coli	7.3 ± 1.2^{c}	3.7 ± 1.2^{d}	8.7 ± 1.2^{c}	12.3 ± 0.5^{b}	7.3 ± 1.2^{c}	4.8 ± 1.2^{d}	19.0 ± 0.9^{a}
S. aureus	3.3 ± 1.2^{c}	6.0 ± 0.0^{b}	7.3 ± 1.2^{b}	5.7 ± 1.2^{b}	11.3 ± 1.2^{a}	7.7 ± 1.2^{b}	13.0 ± 2.8^{a}
P. aeruginosa	4.7 ± 1.2^{d}	2.7 ± 1.2^{d}	5.3 ± 0.5^{d}	9.3 ± 1.2^{b}	9.4 ± 0.5^{b}	7.3 ± 1.2^{c}	13.3 ± 2.4^{a}
LSD for source: 3.89							
LSD for microorganisms: 5.95							

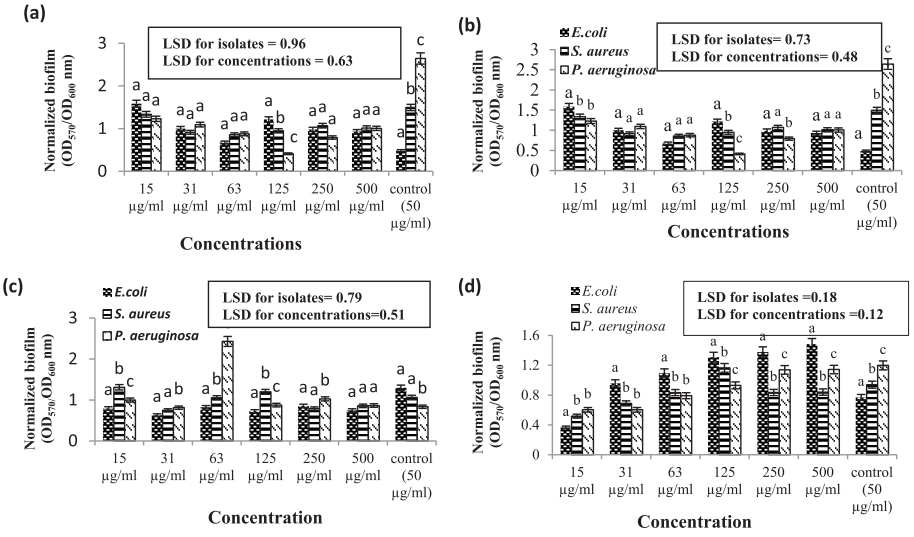

Fig. 5 Biofilm formation by (a) *E. suberosa* bark AgNPs (b) *E. suberosa* leaf AgNPs (c) *C. pentandara* bark AgNPs (d) *C. pentandara* leaf AgNPs. One Way Analysis of Variance (ANOVA) with post hock Tukey was used to determine the antibiofilm effect. $P < 0.05$ was considered significant in all tests.

4 Discussion

In the current study, the antimicrobial, antibiofilm and antioxidant potential of Ag-NPs synthesized from leaf and bark extract from *E. suberosa* Roxb and *C. pentandra* was determined. Plants are considered useful and economically beneficial in the field of medicine because they contain many active compounds that have been proved very effective to treat many human diseases. Plant's primary and secondary metabolites are involved in many biological activities and are considered as valuable natural resources for the formation of NPs. The employ of plant concentrates in the production of AgNPs has gotten a lot of interest because it's a quick, environmentally benign, non-pathogenic, and cost-effective method for biosynthetic processes. Furthermore, unlike microbiological cultures, plant extracts are simple and safe to handle[35]. Antibiotic resistance will inevitably rise as a result of continued antibiotic use around the world. There is a need to increase the antibacterial armament with the emergence of new virulent strains[36]. Nanoparticles having antibacterial potential have recently gained a lot of interest as a way to combat drug resistance in a variety of pathogenic microbes. Antimicrobial and anti-biofilm properties of silver ions and salts are well recognized[37].

Phytochemical analysis of leaf and bark extract of both plants revealed the presence of tannins, steroids, alkaloids, saponins, phenolics, flavonoids and glycosides. Tannins, saponins, phenolics, flavonoids were present in both leaf and bark extracts of *E. suberosa* and *C. pentandra*. Steroids and alkaloids were present in bark extracts of *E. suberosa* and *C. pentandra*. Glycosides were present in leaf extracts of both plants. Total tannin content was found to be highest in *E. suberosa* leaf extract and lowest d in *C. pentandra* bark extract. Likewise, highest total phenolic content was found in bark extract of *E. suberosa* than other plant extracts and the highest total flavonoid content was found in *E. suberosa* leaf extract and lowest was found in bark extract of *C. pentandra*. According to a study in which phytochemical evaluation of *Ceiba pentandra* extract indicated the presence of phenolic compounds, alkaloids, saponins, tannins, anthocyanin, proanthocyanin, carbohydrates, flavonoids, glycosides and proteins[38]. Results of another study confirmed the presence of tannins, steroids, alkaloids, saponins, phenolics, flavonoids and glycosides in *E. suberosa*[19].

A visible color change after mixing extract with silver nitrate solution was observed which confirmed the formation of AgNPs. After the mixing of leaf and bark extracts with the 1 mM $AgNO_3$ solution the formation of nanoparticles started due to Ag^+ ions reduction. The visible color changes from light yellow to reddish brown of *E. suberosa* leaf and *C. pentandara* bark extract while for *E. suberosa* bark and *C. pentandara* leaf extract from colorless to reddish brown were recorded. Many previous studies observed the color change from light yellow to dark brown during synthesis of AgNPs[39–41]. The color change was due to surface plasmon resonance (SPR) and bioreduction of Ag^+ ions by phytochemicals[42].

The confirmation of synthesized AgNPs was analyzed using UV-Vis spectrophotometer in which a peak specific for the synthesis of AgNPs was appeared at 430-450 nm for each extract. Maximum absorbance between 430-475 nm for AgNPs prepared from plants, Simul (*Bombax ceiba*), *Picea abies*, *E. suberosa* (Roxb.), *Achillea millefolium* was observed[40, 43–45].

In current study antioxidant activity of AgNPs at different concentrations was also evaluated. Silver nanoparticles of *E. suberosa* showed the good antioxidant activity than *C. pentandra* AgNPs. This might be due to the highest phenolic content present in *E. suberosa*. The antioxidant potential and role of phenolics as reducing agents which are responsible in the production of AgNPs have been reported in a previous research[46].

The synthesized AgNPs showed significant antimicrobial activity against all the bacteria. Plant extracts showed no zone of inhibition against all bacteria. In the previous studies, antibacterial activity of AgNPs synthesized from both plants against *P. aeruginosa*, *E. coli* and *S. aureus* was also documented[39–41, 45]. The maximum zone of inhibition was found in case of *E. suberosa* AgNPs bark extract against *P. aeruginosa* that was 20 ± 1.154 mm. According to a previous research, the zone of inhibition 24 ± 0.8 mm of AgNPs synthesized from *E. suberosa* was measured against *P. aeruginosa*[19]. Antibacterial potential was also checked by the minimum inhibitory concentration (MIC) value determination against *P. aeruginosa*, *E. coli* and *S. aureous* by disc diffusion assay. Silver nanoparticles synthesized from both plants showed significant inhibitory activities against *S. aureus*, *E. coli* and *P. aeruginosa*. This might be due to the presence of active biomolecules, present in plant extracts. The presences of such phytochemicals like alkaloids, flavonoids, tannins, glycosides, saponins, steroids have been documented to have antimicrobial activities against pathogenic bacteria[19, 47, 48].

Biofilm formation is strong adaptation of bacteria to survive under harsh conditions[49]. The biofilm forming assay showed the significant results of AgNPs from all sources against *P. aeruginosa*, *E. coli*, and *S. aureus*. However greater inhibition was observed for AgNPs of *E. suberosa* bark and leaf against *P. aeruginosa*. In a previous study, the AgNPs synthesized from extracts of *Semecarpus anacardium*, *Glochidion lanceolarium*, and *Bridelia retusa* were screened for anti-biofilm activity against human pathogens *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Greater inhibition of biofilm was observed against *P. aeruginosa*[19]. Based upon the results of current research, AgNPs synthesized from *E. suberosa* and *C. pentandara* may serve as a

future direction in biomedical nanotechnology in developing antimicrobial compounds.

5 Conclusion

Present work describes the synthesis of AgNPs from the leaf and bark extract of *E. suberosa* and *C. pentandara*. The leaf and bark extract of both plants possess bioactive molecules which are responsible for reduction of silver ions. The biosynthesized AgNPs from both plant sources showed significant antioxidant activity. It was observed that AgNPs synthesized from both plants may be useful preventing the harmful effects of oxidation processes. Silver nanoparticles contained in the plant extracts showed growth inhibition against *S. aureus* and *E. coli*, *P. aeruginosa*, though the best antibacterial activity was exhibited by the *E. suberosa* AgNPs. Regarding biofilm inhibition, AgNPs synthesized from leaf and bark extracts of both plant sources significantly inhibited the biofilm formed by *E. coli*, *S. aureus*, *P. aeruginosa*. However, *E. suberosa* bark and leaf AgNPs showed greater biofilm inhibition formed by *P. aeruginosa*. Overall, the current study suggests that AgNPs prepared from both plant extracts possess significant antioxidant, antibacterial and antibiofilm potential hence recommending their toxicity evaluation using *in vivo* study.

Conflict of Interests

Authors declare no conflicts of interests.

Author Contributions

IL and AWQ designed research; SQ, BS performed research; IS, MM and SA contributed analytic tools; IL, AWQ and BS analyzed data; IL and AWQ wrote the manuscript.

References

1) Tufail, M.S.; Liaqat, I. Silver nanoparticles and their applications-A comprehensive review. *Pure Appl. Biol.* **11**, 315-330 (2021).
2) Tufail, S.; Liaqat, I.; Ibrahim, S.; Younas, S.; Fatima N. *et al.* Biogenic synthesis, characterization and antibacterial properties of silver nanoparticles against human pathogens. *J. Oleo Sci.* **71**, 257-265 (2022).
3) Jeevanandam, J.; Barhoum, A.; Chan, Y.S.; Dufresne A.; Danquah, M.K. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein J. Nanotechnol.* **9**, 1050-1074 (2018).
4) Zia-ur-Rehman, M.; Naeem, A.; Khalid, H.; Rizwan, M.; Azhar, M. Responses of plants to iron oxide nanoparticles. *Nanomat. Plants Algae Microorg.* **12**, 221-238 (2018).
5) Teulon, J.M.; Godon, C.; Chantalat, L.; Moriscot, C.; Cambedouzou, J. *et al.* On the operational aspects of measuring nanoparticle sizes. *Nanomaterials* **9**, 18 (2019).
6) Feng, S.; Wu, S.; Chen, H.; Zhang W.; Shao, Z. Synthesis of silver nanoparticles with tunable morphologies via a reverse nano-emulsion route. *Mater. Trans.* **54**, 1145-1148 (2013).
7) Ajitha, B.; Reddy, Y.A.K.; Reddy P.S. Green synthesis and characterization of silver nanoparticles using *Lantana camara* leaf extract. *Mater. Sci. Eng. C* **49**, 373-381 (2015).
8) Aritonang, H.F.; Onggo, D.; Ciptati C.; Radima, C.L. Synthesis of platinum nanoparticles from K_2PtCl_4 solution using bacterial cellulose matrix. *J. Nanopart.* **28**, 1- 6 (2014).
9) Aritonang, H.F.; Kamu, V.S.; Ciptati, C.; Onggo, D.; Radiman, C.L. Performance of platinum nanoparticles/multiwalled carbon nanotubes/bacterial cellulose composite as anode catalyst for proton exchange membrane fuel cells. *Bull. Chem. React. Eng. Catal.* **12**, 287-292 (2014).
10) Raut, R.W.; Haroon, A.S.M.; Malaghe, Y.S.; Nikan B.T.; Kashid S.B. Rapid biosynthesis of platinum and palladium metal nanoparticles using root extract of *Asparagus racemosus* Linn. *Adv. Mater. Let.* **4**, 650-654 (2013).
11) Khatoon, N.; Mazumder J.A.; Sardar, M. Biotechnological applications of green synthesized silver nanoparticles. *J. Nanosci. Curr. Res.* **2**, 275-286 (2017).
12) Mohanta, Y.; Singdevsachan, S.; Parida, U.; Panda, S.; Mohanta T.; Bae, H. Green synthesis and antimicrobial activity of silver nanoparticles using wild medicinal mushroom *Ganoderma applanatum* (Pers.) Pat. from Similipal Biosphere Reserve, Odisha, India. *IET Nanobiotechnol.* **10**, 184-189 (2016).
13) Mittal, A.; Chisti K, Y.; Banerjee, U.C. Synthesis of metallic nanoparticles using plant extracts. *Biotechnol. Adv.* **31**, 346-356 (2013).
14) Liaqat, I.; Arshad, N.; Arshad, M.; Mirza, S.A.; Ali, N.M.; Shoukat, A. Antimicrobial activity of some medicinal plants extracts against food industry isolates. *Pak. J. Zool.* **49**, 565-572 (2017).
15) Chandran, S.P.; Chaudhary, P.M.; Ahmad, P.A.; Sastry. M. Synthesis of gold nanotriangles and silver nanoparticles using aloe vera plant extract. *Biotechnol. Prog.* **22**, 577-583 (2006).
16) Dauthal, P.; Mukhopadhyay M. Noble metal nanoparticles: Plant-mediated synthesis, mechanistic aspects of

synthesis, and applications. *Ind. Eng. Chem. Res.* **55**, 9557-9577(2016).
17) Panda, K.K.; Acharya, V.M.M.; Krishnaveni, R.; Padhi, B.K.; Sarangi, S.N.; Sahu, S.N. *In vitro* biosynthesis and genotoxicity bioassay of Ag-NPs using plants. *Toxicol. In Vitro* **25**, 1097-1105(2011).
18) Plants, I. Screening of Indian plants. 1968. *Indian J. Exp. Biol.* **6**, 232-247(2011).
19) Mohanta, Y.K.; Panda, S.K.; Jayabalan, R.; Sharma, N.; Bastia A.K.; Mohanta, T.K. Antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles synthesized by leaf extract of *Erythrina suberosa*(Roxb.). *Front. Mol. Biosci.* **4**, 14(2017).
20) Dhar, M.L.; Dhar, M.M.; Dhawan, M.B.N.; Mehrotra B. N.; Ray, C. Screening of Indian plants for biological activity: Part I. *Indian J. Exp. Biol.* **6**, 232-247(1968).
21) Agrawal, S.K.; Agrawal, P.R.M.; Sharma B.D.; Arora, G.S.; Saxena, A.K. Induction of apoptosis in human promyelocytic leukemia HL60 cells by an extract from *Erythrina suberosa* stem bark. *Nutr. Cancer* **63**, 802-813(2011).
22) Osuntokun, O.T.; Ajayi, O.A.; Adeoye, M.I.; Odufunwa, A.E. Assessment of antimicrobial and phytochemical properties of crude leaf and bark extracts of *Ceiba pentandra* on selected clinical isolates found in Nigerian teaching hospital. *J. Bacteriol. Mycol.* **4**, 17-23 (2017).
23) Obi, C.F.; Nzeakor, T.A.; Okpala, M.I.; Ezeha, I.O.; Nwobi, L.G. *et al.* Evaluation of antitrypanosomal activity of *Pterocarpus santalinoides* L'H'erit DC hydroethanol leaf extract in rats experimentally infected with *Trypanosoma brucei. J. Ethnopharmacol.* **243**, 112085(2019).
24) Liaqat, I.; Sumbal, F.; Sabri, A.N. Tetracycline and chloramphenicol efficiency against selected biofilm forming bacteria. *Curr. Microbiol.* **59**, 212-220(2009).
25) Afreen, A.; Ahmed, R.; Mehboob, S.; Tariq, M.; Alghamdi, H.A. *et al.* Phytochemical-assisted biosynthesis of silver nanoparticles from *Ajuga bracteosa* for biomedical applications. *Mater. Res. Express* **7**, 075404(2020).
26) Ejikeme, C.M.; Ezeonu, C.S.; Eboatu. A.N. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. *Eur. Sci. J.* **10**(18), 247-270(2014).
27) Karim, A.; Sohail, M.N.; Munir, S.; Sattar. S. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatement of diabetes. *Int. J. Pharmacol.* **7**, 419-439(2011).
28) Harborne, J.B.; Harborne, A.J. Phytochemical methods: A guide to modern techniques of plant analysis. 3: 2, Kluwer Academic Publishers, London, UK. pp. 255-256(1998).
29) Cliffe, S.; Fawer, M.; Maier, G.; Takata K.; Ritter, G. Enzyme assays for the phenolic content of natural juices. *J. Agric. Food Chem.* **42**, 1824-1828(1994).
30) Dewanto, V.; Wu, X.; Adom K.K.; Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **50**, 3010-3014(2002).
31) Ghafoori, S.M.; Entezari, M.; Taghva, A. Biosynthesis and evaluation of the characteristics of silver nanoparticles using Cassia fistula fruit aqueous extract and its antibacterial activity. *Adv. Nat. Sci. Nanosci. Nanotechnol.* **8**, 045019(2017).
32) Oyaizu, M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Jpn. J. Nutr.* **44**, 307-315(1986).
33) Liaqat, I.; Hussain, T.; Qurashi, A.W.; Saleem, G.; Bibi, A. *et al.* Antibiofilm activity of proteolytic enzymes against *Salmonella gallinarum* isolates from commercial broiler chickens. *Pak. J. Zool.* **53**, 1111 (2021).
34) Liaqat, I.; Bachmann, R.T.; Sabri, A.N.; Edyvean, R.G.; Biggs, C.A. Investigating the effect of patulin, penicillic acid and EDTA on biofilm formation of isolates from dental unit water lines. *Appl. Microbiol. Biotechnol.* **81**, 349-358(2008).
35) Kulkarni, N.; Muddapur, U. Biosynthesis of metal nanoparticles: A review. *J. Nanotech.* **2**, 1-8(2014).
36) Gurunathan, S.; Han, J.W.; Kwon, D.N.; Kim, J.H. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale Res. Lett.* **9**, 373(2014).
37) Mohanty, S.; Mishra, S.; Jena, P.; Jacob, B.; Sarkar, B.; Sonawane, A. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. *Nanomed. Nanotechnol. Biol. Med.* **8**, 916-924(2012).
38) Dhotre, R.; Kare, M. Phytochemical studies on stem bark of *Ceiba pentandra* L. *Juni Khyat UGC Care Group I Listed J.* **10**, 2278-4632(2020).
39) Meenakshi, S.C.; Basavaraj S.B.; Ramesh, L.L. Friendly approach for synthesising silver nanoparticles(SNPs) from an exceptional medicinal plant *Bombax Ceiba* bark extract and its anti-bacterial activity. *Int. J. Chemtech. Res.* **10**, 331-336(2017).
40) Mandal, P. Green synthesis and characterization of polyvinyl alcohol embedded silver nanoparticles using Simul(*Bombax ceiba*)flower extract. *Int. J. Eng. Sci. Inv.* **9**(14), 3(2018).
41) Wan Mat Khalir, W.K.A.; Shameli, S.D.K.; Jazayeri, N.A.; Othman, N.W.; Jusoh C.; Hassan, N.M. *In-situ* biofabrication of silver nanoparticles in *Ceiba pentandra* natural fiber using *Entada spiralis* extract with their antibacterial and catalytic dye reduction properties. *Nanomaterials* **10**, 1104(2020).

42) Kumar, P.V.; Pammi, S.V.N.; Kollu, P.; Satyanarayana, K.V.V.; Shameem, U. Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their antibacterial activity. *Ind. Crop Prod.* **52**, 562-566 (2014).
43) Tanase, C.; Berta, L.; Coman, N.A.; Roşca, I.; Man, A. *et al.* Antibacterial and antioxidant potential of silver nanoparticles biosynthesized using the Spruce bark extract. *Nanomaterials* **9**, 1541 (2019).
44) Yousaf, H.; Mehmood, A.; Ahmad, K.S.; Raffi, M. Green synthesis of silver nanoparticles and their applications as an alternative antibacterial and antioxidant agents. *Mater. Sci. Eng. C* **112**, 110901 (2020).
45) Ahmed, Z.; Aziz, S.; Alauddin, S.; Mohiuddin, S.G.; Javed, A. *et al. In vitro* cytotoxic and antimicrobial activities of *Erythrina suberosa* (Roxb) bark. *J. Pharm. Bioallied Sci.* **12**, 210-216 (2020).
46) Phull, A.R.; Abbas, Q.; Ali, A.; Raza, H.; Zia, M.; Haq, I.U. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of *Bergenia ciliate*. *Future J. Pharm. Sci.* **2**, 31-36 (2016).
47) Ahmad, A.; Kaleem, M.; Ahmed Z.; Shafiq, H. Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections—A review. *Food Res. Int.* **77**, 221-235 (2015).
48) Javid, T.; Adnan, M.; Tariq, A.; Akhtar, B.; Ullah, R.; AbdELsalam N.M. Antimicrobial activity of three medicinal plants (*Artemisia indica*, *Medicago falcata* and *Tecoma stans*). *Afr. J. Tradit. Complement Altern. Med.* **12** (3), 91-96 (2015).
49) Liaqat, I. Fourier transform infrared spectroscopy of dental unit water line biofilm acteria. *Spectroscopy* **23**, 175-189 (2009).
50) Liaqat, I.; Liaqat, M.; Tahir, H.M.; Ali, N.M.; Arshad, M.; Arshad, N. Motility effects biofilm formation in *Pseudomonas aeruginosa* and *Enterobacter cloacae*. *Pak. J. Pharm. Sci.* **32**, 927-932 (2019).