

Green mediated synthesis of silver nanoparticles using *Ipomoea quamoclit* to explore the potential antimicrobial activity against human pathogens

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Abstract

Ipomoea quamoclit, a medicinal plant, known as a common medication for stomach ache, hemorrhoids and rheumatoid arthritis, has been employed for the biosynthesis of silver nanoparticles in the current study. The phytochemical analysis of this plant revealed that it is rich in phenolics, flavonoids, steroids, saponins, carbohydrates, triterpenes compounds. The present study has demonstrated an ecofriendly and safer method for developing a simple and cost effective production of silver nanoparticles using *Ipomoea quamoclit* flowers, leaves and seeds. The formation of nanoparticles was supported by color change and UV-Vis spectroscopy, showed maximum absorption peak at the range of 400-450 nm. X-ray diffraction, Fourier transform-Infrared spectroscopy and Scanning electron microscope, demonstrated spherical and oval shaped nanoparticles in the sizes ranging between 25 and 45 nm. The phytosynthesized silver nanoparticles have shown a remarkable antimicrobial activity against tested gram negative and gram positive pathogenic organisms such as *Pseudomonas aeruginosa* PAO1, *Salmonella typhimurium*, *Serratia marcescens* and *Micrococcus luteus* by conventional agar well diffusion in a dose dependent manner. The observations of the present investigation appear to be promising and offer a valuable agent for antimicrobial and antibiofilm activities. Among the various nanoparticles, the silver nanoparticles synthesized using the flower extract of *Ipomoea quamoclit* had a notably marked reduction in biofilm formation against *Salmonella typhimurium*. This paves the way in designing a new alternative therapeutic management of biofilm associated infections in future.

Keywords Nanoparticles; *Ipomoea quamoclit*; Antibiofilm; Antimicrobial; Human pathogens.

Introduction

Two major approaches were used for the synthesis of nanoparticles, namely, top-down and bottom-up. The chemicals used for nanoparticle synthesis are highly toxic, more expensive and non-ecofriendly that restrict their use, predominantly in medical applications (1). The major advantages of using biological methods over chemical methods are their robustness, economic viability no use of toxic chemicals, no toxic byproducts and environment friendly nature (2-4). Nanoparticles are synthesized by utilizing the sources such as microorganisms, which include bacteria, fungi,

mushroom, seaweed, or plant extracts, when compared with conventional chemical synthesis methods (5-10). Though microorganisms are exploited to synthesize nanoparticles, the rate of production is slow in comparison with the phyto-mediated synthesis. The importance of plant sources for the production of nanoparticles is still underrated (11-17). Several plants including the extracts of *Tagetes* sp.(marigold), *Ziziphora tenuior*, *Abutilon indicum*, *Solanum tricobatum*, *Erythrina indica*, *Garcinia mangostana*, *Ocimum tenuiflorum*, *Spirogyra varians*, *Melia dubia*, *Acalypha indica* and *Sesuvium portulacastrum* have been identified for the production of silver nanoparticles (AgNPs). The compounds present in the plant extract can act as reducing agents, converting Ag^+ to Ag^0 and as capping agents of the AgNPs (18). AgNPs possess medicinal properties including antibacterial and antibiofilm activities, antifungal, antiviral, anticancer, anti-inflammatory, anti-angiogenesis and anti-platelet activities (19, 20). AgNPs exert their antimicrobial activity by disturbing the permeability, cell wall integrity, penetrating inside the bacterial cell wall, interacting with DNA, proteins and enzymes of the cell and blocking the activity of the enzymes (20, 21).

Ipomoea quamoclit, a perennial plant also known as Mayil Mannikkam, cypress vine or star glory has been used for decades as a conventional medicine to treat different types of diseases (22, 23), such as hemorrhoids, rheumatoid arthritis and stomach aches (24). The bioactive compounds present in the plant extract such as flavonoids, alkaloids, glycosides, tannins, terpenoids, saponins, proteins, carbohydrates and other phenolic compounds exhibit therapeutic properties. It possesses antioxidant activity, antiviral activity and neuroprotective activity, which are well known but its ability to produce AgNPs is not known. AgNPs are extensively used in medicine due to their large surface areas and high reactivity and are being successfully used in cancer diagnosis and treatment as well (18). Hence, in the present study, the synthesis of AgNPs was carried out using the natural aqueous extracts of *Ipomoea quamoclit* leaves, seeds and flowers to study the bactericidal and antibiofilm activities against *Salmonella typhimurium*, *Pseudomonas aeruginosa* PAO1, *Serratia marcescens* and *Micrococcus luteus*. These pathogenic microorganisms are generally found on polluted water and food causing diarrhoea,

fever and gastric enteritis, and on medical devices, forming biofilm on the surfaces causing pulmonary infections. Hence, the present study illustrated Ipomoea quamoclit as a nontoxic and ecofriendly bioresource for synthesizing AgNPs and evaluating the effects of the synthesized nanoparticles against various pathogenic microorganisms. The study also focused on determining the shape and size of the biosynthesized AgNPs. This could be a promising tool for minimizing the colonization of microbes on the surfaces and medical associated devices, which subsequently leads to infections; therefore, it is interesting to carry out this investigation while exploring the potential antimicrobial and antibiofilm properties using the phytosynthesized nanoparticles to overcome biofilm associated infections.

Materials and Methods

Collection of sample and preparation of plant extract

Fresh and healthy flowers/leaves/seeds of the perennial plant Ipomoea quamoclit were collected from the herbal garden of Sri Kaliswari College, Sivakasi, washed with water, air dried and powdered. About 10 g of Ipomoea quamoclit flower/leaf/seed powder was weighed respectively and mixed in 100 mL of sterile double distilled water. Ipomoea quamoclit (flowers/leaves/seeds) extracts were incubated at 70 °C for 15 min. The boiled plant extracts were filtered using Whatmann paper. Then, 90 mL of 1 mM silver nitrate was mixed with 10 mL of each boiled plant extract, respectively, incubated for 24 h and the color change was observed (19,20).

Synthesis and characterization of silver nanoparticles using different parts of plant extracts

Different concentrations of aqueous silver nitrate were mixed with different ratios of phytoextracts (flowers/leaves/seeds), to improve the efficiency and yield of AgNPs. The mixed aqueous solutions were monitored at regular time intervals for the reduction of Ag by taking small aliquots (1.0 mL) and measuring absorbance in a UV-Visible spectrophotometer in the wavelength range of 200 to 800 nm. The maximum absorbance of the synthesized nanoparticles was recorded using UV-Vis spectra. The colloidal solutions were spun at 1000 rpm for 1 h and the pellets were air dried and dispersed in 1 mL of deionized water/DMSO. Finally, the purified phytosynthesized AgNPs were examined using analyses such as FTIR, SEM and X-ray diffraction (XRD) (25).

Analysis of antimicrobial activity by well diffusion

The AgNPs synthesized using Ipomoea quamoclit flower, leaf and seed extracts were tested for their antimicrobial activity against pathogenic organisms such as Pseudomonas aeruginosa PAO1, Salmonella typhimurium, Serratia marcescens and

Micrococcus luteus by agar well diffusion method. The pure cultures of the pathogenic organisms were initially subcultured on Muller Hinton broth at 37°C for overnight, followed by adding 10⁹ cells of each strain onto the Muller Hinton agar plates and wells were made by using gel puncture. Different concentrations of phytosynthesized AgNPs dissolved in DMSO were added onto the wells, incubated at 37°C for 18-24 h and the bactericidal activities were measured via zone of inhibition in mm (25).

Analysis of antibiofilm activity

The antibiofilm activity of the phytosynthesized AgNPs was studied using a 96 well microtiter plate. Biofilm production by clinical pathogenic strains grown in Luria broth (LB) and treated/untreated with nanoparticles was studied using a semi-quantitative adherence assay. Briefly, for control experiment, an equal number of cells of overnight grown culture were seeded in a 96-well microtiter plate and after incubation at 37°C for 24-48 h, the biofilm plates were washed thrice with phosphate buffered saline to remove the free floating cells. The adhered cells were stained with 1 % crystal violet for 15 min, washed and air dried and resuspended in 200 µL of 33 % glacial acetic acid or ethanol. Biofilm formation was quantified by measuring the absorbance at 570 nm. In another set, 100 µg of AgNPs solution was added to the culture inoculum and the culture was grown overnight. The 200 µL of treated sample was then seeded onto the microtiter plate and the same procedure was followed as mentioned above. The assays were performed in triplicates to test the biofilm inhibition (9,34).

Results and discussion

Ipomoea quamoclit, a medicinal plant is used for various bioactivities around the world for various illnesses, which include antioxidant, antimicrobial, anticancer, anti-diabetic activities as well as insecticidal activity (24). Hence, this medicinal plant parts (flowers, leaves and seeds) were used for bioreduction, capping and stabilization of AgNPs.

Confirmation of synthesis of silver nanoparticles



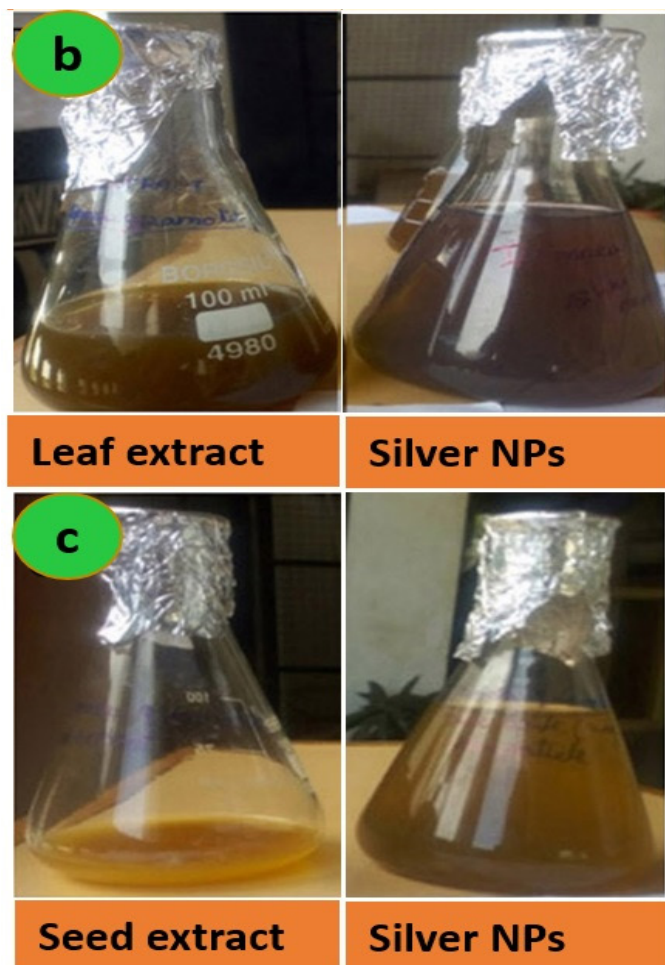


Fig.1 Visual observation of color change upon formation of silver nanoparticles (a) Flower; (b) Leaf; (c) Seed AgNPs

Nanoparticles such as silver (Ag), gold (Au), osmium (Os), iridium (Ir), platinum (Pt) have been recognized to have the property of Surface Plasmon Resonance (SPR) due to their optical nature (26). The synthesis of AgNPs was observed by visual color change followed by UV-Vis spectroscopy. The colloidal solution changed color to dark reddish brown after 1 h, indicating the production of AgNPs. The active molecules present in the flower extract aided in the reduction of Ag^+ into Ag^0 (Fig.1a) and the AgNPs synthesized using *Ipomoea quamoclit* extracts were confirmed using UV-Vis spectrophotometer at various wavelengths. The UV-Vis spectrum of AgNPs synthesized using the flower extract of *I. quamoclit* had a maximum absorbance at 450 nm, which signified the occurrence of AgNPs (Fig.2a). Similarly, the generation of AgNPs with the extract of leaf, showed that the color of the reaction mixture changed to light purple within 10 min and to dark purple after 3 h, indicating the reduction of silver ions via the bioactive molecules present in the leaf extract (Fig.1b). The UV-Vis spectrum of the AgNPs synthesized using *I. quamoclit* had the maximum absorbance at 400 nm, which specified the presence of silver nanoparticles (Fig.2b). The synthesis of AgNPs using the seed

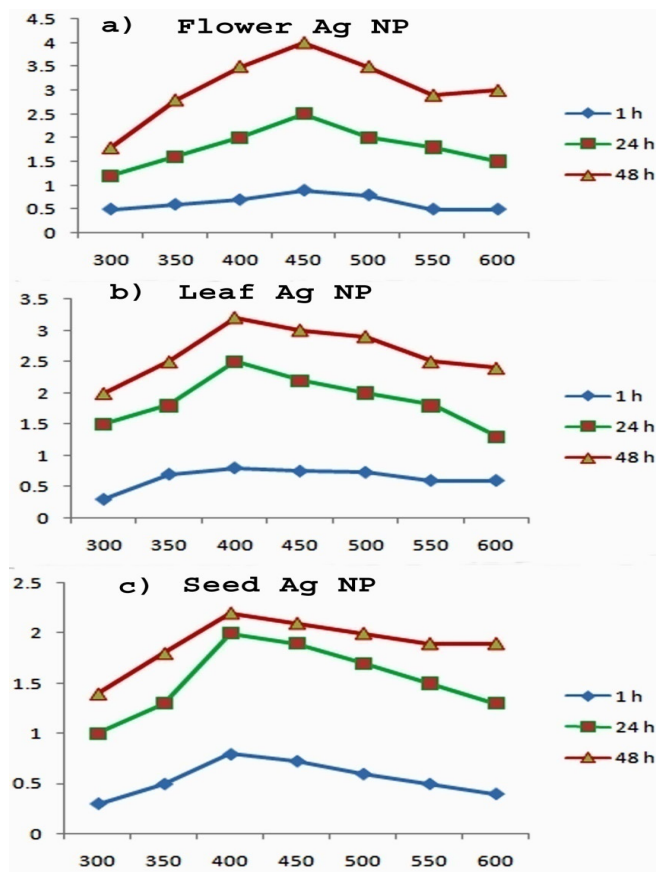


Fig.2 UV Visible absorption spectra of the synthesized silver nanoparticles using (a) Flower; (b) Leaf (c) Seeds of *Ipomoea quamoclit*

Table 1: Phytochemical analysis of *Ipomoea quamoclit* flower, leaf and seed extracts

S.No	Phytochemical test	Flower extract	Leaf extract	Seed extract
1.	Alkaloids	+	+	+
2.	Flavonoids	+	+	+
3.	Terpenoids	-	-	+
4.	Cardiac glycosides	+	+	+
5.	Saponins	+	-	+
6.	Phenols	+	+	-
7.	Tannins	-	-	-
8.	Carbohydrates	+	+	-
9.	Proteins	-	+	+
10.	Steroids	+	-	+

extract showed that the colour of the reaction mixture changed from yellowish brown and to dark brown after 1 h, showing the production of AgNPs (Fig.1c). Further, the AgNPs had a maximum absorbance at 400 nm, which confirmed the presence of silver nanoparticles (Fig.2c). Similar kind of results were observed in the reduction of $AgNO_3$ to AgNPs using the *Ipomoea digitata* flower extract, the leaf extract of *Calliandra haematocephala* and the seed coat of *Vigna mungo*, which showed maximum absorption peak nearer at λ_{max} of 412 nm, 414 nm and 421 nm respectively because of surface plasmon resonance (SPR) (27-29).

Phytochemical analysis

Plant source contains copious natural bioactive compounds such as steroids, alkaloids, flavonoids, saponins, tannins, and other nourishing compounds derived from various parts of the plant such as flowers, barks, seeds, leaves, stems, roots, shoots and the compounds acts as reducing and stabilizing agents for the synthesis of AgNPs (19). The analysis of phytochemicals in *Ipomoea quamoclit* (Table 1) showed the presence of phenolic compounds and other metabolites that aided in the reduction and stabilization of AgNPs.

Morphology of silver nanoparticles

The biosynthesized AgNPs were analyzed using SEM to envision the size and shape. The typical SEM images showed that the product mainly consisted of particles like silver nanoclusters with panoramic view and the size ranged from 150 to 200 nm. However, further observations at higher magnifications revealed that the silver nanoclusters were assembled by smaller nanoparticles, which exhibited good uniformity and the average size of the nanoparticles synthesized from flower, leaf and seed extracts of *Ipomoea quamoclit* were about 43 to 45 nm, 16 to 25 nm and 20 to 25 nm respectively (Fig.3 a-c).

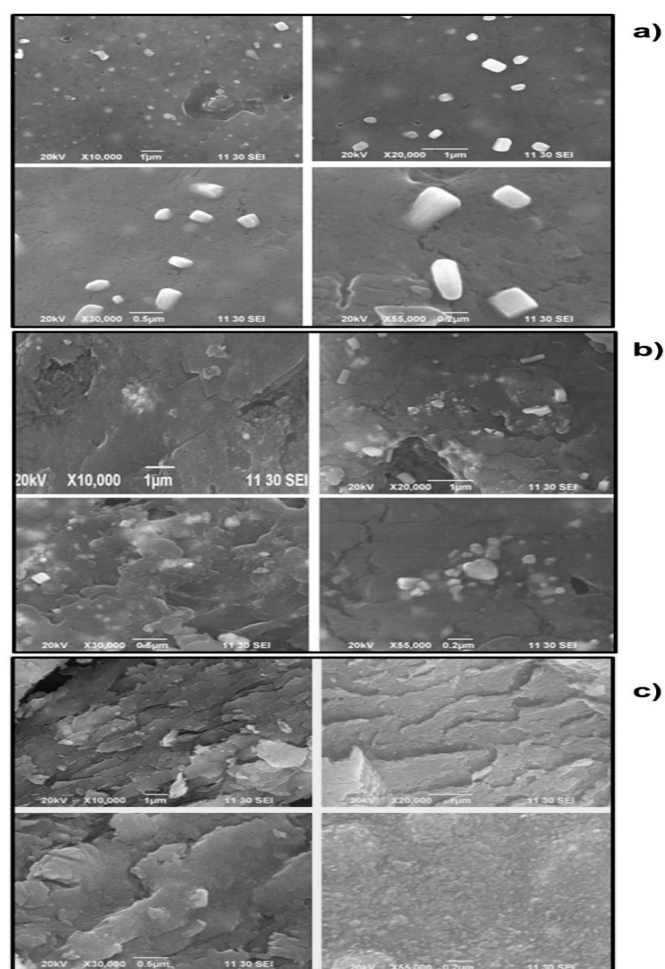


Fig.3 SEM micrograph of the silver nanoparticles synthesized using (a) Flower; (b) Leaf; C) Seeds of *Ipomoea quamoclit*

X-ray diffraction of silver nanoparticles

XRD patterns of AgNPs showed atleast three main characteristic peaks observed at $2\theta = 28.24^\circ, 32.46^\circ, 46.44^\circ$ for the flower extract, $28.20^\circ, 32.07^\circ, 38.02^\circ$ for the leaf extract and at $37.82^\circ, 44.12^\circ, 77.70^\circ$ for the seed extract (Table 2), which confirmed the formation of AgNPs (Fig.4a-c), which were synthesized by treating the *Ipomoea quamoclit* flower, leaf and seed extracts with 1 mM aqueous AgNO_3 solution. Similar kind of observation has been reported in (30,31) for the synthesis of AgNPs from fruit extract of the plant *Bridelia retusa*.

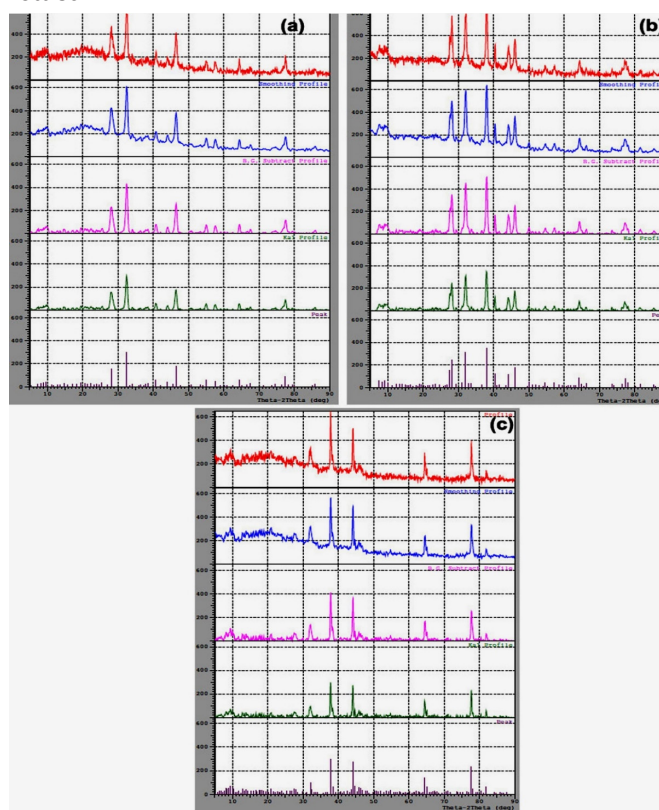


Fig.4 X-ray Diffraction pattern images of silver nanoparticles synthesized using (a) Flower; (b) Leaf; (c) Seed extracts of *Ipomoea quamoclit*

Table 2: X-ray diffraction of silver nanoparticles (Flower/Leaf/Seed Ag) showing the strongest 3 peaks

Peak	2 θ (deg)	D (Å)	I/II	FWHM (deg)	Intensity (counts)	Integrated Int (counts)
Flower Ag NPs						
24	32.4655	2.75560	100	0.65110	178	1267
36	46.4412	1.95373	59	0.64020	105	719
23	28.2425	3.15729	52	0.97380	93	962
Leaf Ag NPs						
36	38.0222	2.36469	100	0.52220	209	1247
32	32.0720	2.78850	89	0.60470	187	1129
29	28.2000	3.16196	69	0.43140	144	730
Seed Ag NPs						
57	37.8204	2.37684	100	0.31880	179	648
67	44.1220	2.05089	92	0.31370	164	515
127	77.7038	1.22794	78	0.30350	140	417

FTIR analysis of silver nanoparticles

The AgNPs synthesized using *Ipomoea quamoclit* flower/leaf/seed extracts were assessed by FTIR measurements to identify the functional groups responsible for capping and reduction. FTIR spectroscopy of the AgNPs showed major peaks at 3423 cm⁻¹, 1600 cm⁻¹, 1458 cm⁻¹, 1345 cm⁻¹, 1268 cm⁻¹ and 370 cm⁻¹ which corresponded to -NH, C-C Stretch, amines, hydroxyl, S-S stretch and ether groups (Table 3), representing the occurrence of proteins and polyphenolics, which acted as reducing and capping agents for AgNPs, as inferred in the FTIR spectrum (Fig.5 a-c). These results suggested that the biomolecules present in *I. quamoclit* aided in the formation and stabilization of AgNPs in a colloidal medium (31).

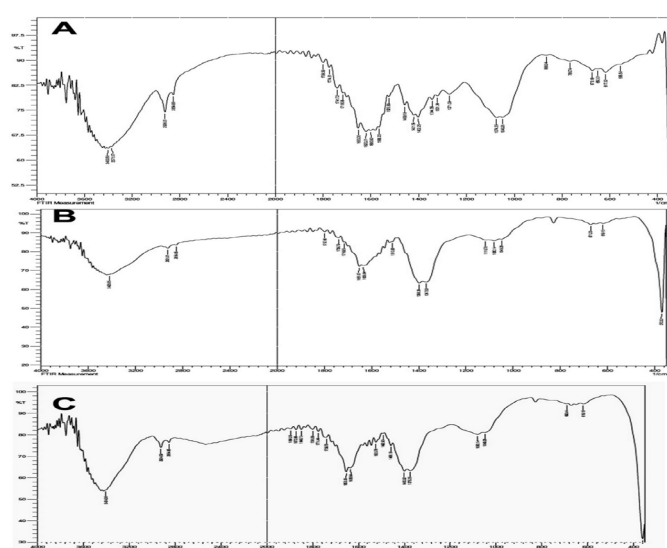


Fig.5 FTIR spectra of silver nanoparticles using (a) Flower; (b) Leaf; (c) Seed of *Ipomoea quamoclit*

Antibacterial activity of silver nanoparticles

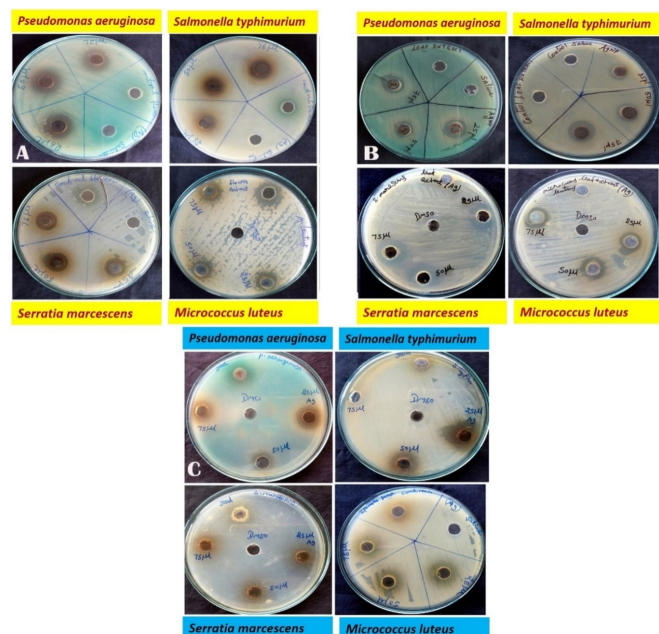


Fig.6 Antimicrobial activity of silver nanoparticles using (a) Flower; (b) Leaf; (c) Seed of *Ipomoea quamoclit* against the tested pathogens using agar well diffusion assay

Table 3: FTIR spectrum of the silver nanoparticles synthesized by using *Ipomoea quamoclit* Flower/Leaf/Seed extracts

Group frequency wave number (cm ⁻¹)	Functional group
3400.50/3404.36/3423.65	NH stretch
3371.57	NH stretch
2924.09/2928.01/2926.01	Methylene C – H asym /sym .stretch
2854.65	Methylene C – H asym /sym .stretch
1716.65/1739.79/1799.50/1849.73	Ester
1774.51/1896.03/1872.88	Ester
1635.64/1651.07/1653.00	Alkyl C- C stretch
1600.92/1620.21/1799.59/1776.44	Alkyl C- C stretch
1510.26/1525.69/1566.20	Secondary amine , NH bend
1523.76	Aromatic nitro compounds
1458.18	Aromatic ring stretch/ Carbonate ion
1421.54 /1492.90	Carbonate ion
1400.32 /1402.25	Carboxylate (carboxylic acid salt)
1344.38/1367.53/1375.25/1398.39	Nitrate ion
1268.16 /1271.09	Primary or secondary OH-plane bend
1116.78	Secondary amine CN stretch
1074.35 /1080.14	Organic silicone or silicone(Si-o-Si)
1049.28 /1090.14	Organic silicone or silicone(Si-o-Si)
866.04	Peroxides , C-O-O-strech
765.74	Aliphatic chloro compounds C-C1 stretch
671.23 /673.16 /697.44	Aryl thioethers C-S stretch
619.15	Aliphatic bromo compounds(Br-strech)
650.01	Aliphatic bromo compounds C-Br stretch
617.22	Aliphatic bromo compounds C-Br stretch
555.50	Disulfides C-C stretch
370.33	Aryl disulfides (S-S stretch)

Antibacterial property of the synthesized AgNPs was tested against gram negative and gram positive bacteria namely *Pseudomonas aeruginosa* PAO1, *Salmonella typhimurium*, *Serratia marcescens* and *Micrococcus luteus* by conventional agar well diffusion. The results indicated that the nanoparticles synthesized from flower/leaf/seed extracts of *Ipomoea quamoclit* have an antimicrobial activity against the tested pathogenic microorganisms. As the concentration of the nanoparticles increased, the zone of inhibition also increased (Fig.6 a-c; Table 4). Similar kind of dosage dependent zone of inhibition results were observed in the bactericidal action of AgNPs synthesized from leaf extract of *Spondias dulcis*, against gram negative human pathogens *S. typhimurium* and *E. coli* (32). Thus, it can be concluded that the antimicrobial activity of the synthesized nanoparticles increased in a dose dependent manner. Hence, the bactericidal activity of the AgNPs against the tested bacteria was most likely due to the attachment and interaction of the AgNPs to the cell membrane, DNA, RNA, proteins, enzymes, ribosomes, causing the production of free radicals and blocking the protein machinery leading to cell death (1, 20, 21).

Antibiofilm activity of silver nanoparticles

Table 4: Antibacterial activity of the silver nanoparticles synthesized using *Ipomoea quamoclit* Flower/Leaf/Seed extracts by agar well diffusion

S.No	Microorganisms	Zone of inhibition (mM)				
		Control (Plant extract)	Negative control (DMSO)	25 μ L	50 μ L	75 μ L
Flower AgNPs						
1.	<i>P. aeruginosa</i>	-	-	14	12	10
2.	<i>S. typhimurium</i>	7	-	7	10	12
3.	<i>S. marcescens</i>	-	-	10	12	14
4.	<i>M. luteus</i>	12	-	6	8	9
Leaf AgNPs						
1.	<i>P. aeruginosa</i>	-	-	10	5	9
2.	<i>S. typhimurium</i>	-	-	3	6	12
3.	<i>S. marcescens</i>	-	-	10	12	14
4.	<i>M. luteus</i>	-	-	6	8	9
Seed AgNPs						
1.	<i>P. aeruginosa</i>	6	-	10	8	7
2.	<i>S. typhimurium</i>	2	-	12	9	-
3.	<i>S. marcescens</i>	-	-	5	5	10
4.	<i>M. luteus</i>	6	-	10	10	8

It is well known that biofilm is one of the major important virulence factors of the pathogenic organisms (33,34). Several investigations have been made to report the antibiofilm activity of the AgNPs synthesized using phytomediated processes (34-38).

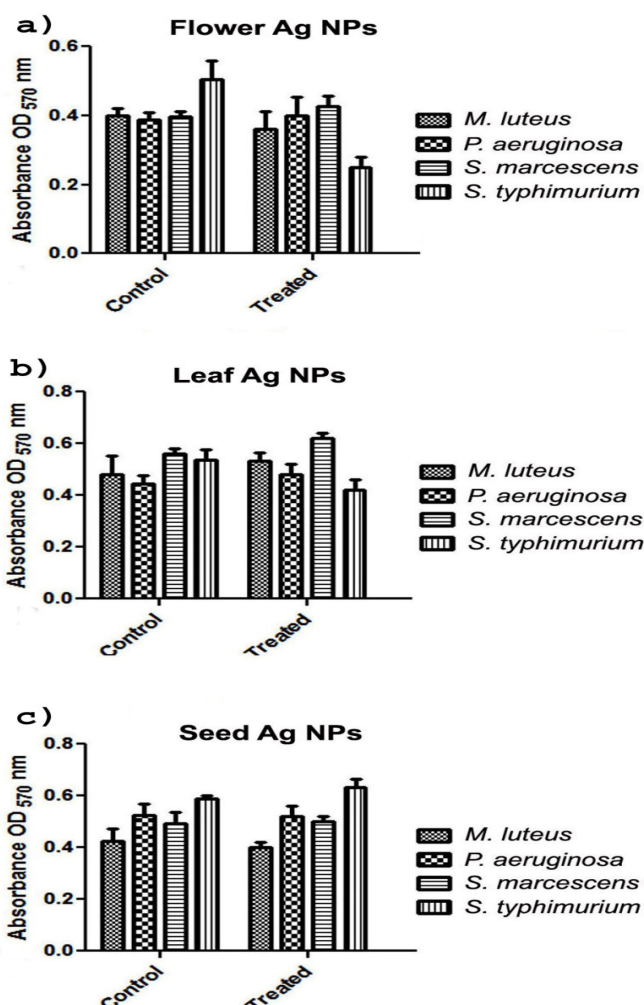


Fig.7 Antibiofilm activity of silver nanoparticles using (a) Flower; (b) Leaf; (c) Seed of *Ipomoea quamoclit* against the tested pathogens

Antibiofilm activity of the AgNPs synthesized using the flower, leaf and seed extracts of *Ipomoea quamoclit* were studied against the human pathogenic strains, *Pseudomonas aeruginosa* PAO1, *Salmonella typhimurium*, *Serratia marcescens* and *Micrococcus luteus*. Biofilm formation of the control organisms (untreated) and test (treated with nanoparticles) were studied in microtiter plates and stained with dye, dissolved and the absorbances were measured. Each experiment was carried out in four replicates. Among the nanoparticles, the AgNPs synthesized using the flower extract of *Ipomoea quamoclit* have notably marked reduction in biofilm formation against the pathogenic strain, *Salmonella typhimurium* (Fig.7 a-c). Based on the obtained results, it could be concluded that the AgNPs synthesized using *I. quamoclit* can be alone or in formulation with drug molecules be used as an effective antimicrobial agent or drug delivery agent. AgNPs produced using the biological source, *I. quamoclit* will have potentially less problems in applications compared to the chemical agents that are not ecofriendly, harmful with side effects and costlier and silver are more biocompatible and biodegradable and safer to use in the field of medicine.

Conclusion

The major threat posed by the antibiotic treatment is that the growing pathogenic bacteria become resistant to the given antibiotics and develop new mechanisms to evade the treatment. Therefore, there is an urgent need to find alternative and effective antibacterial agents against the pathogenic organisms. The antibacterial activity of the AgNPs has been extensively documented since they exhibit good physical, optical and natural properties, in medical applications. Nanoparticles biosynthesized using plant compounds are safe to use as biomedicine. AgNPs synthesized using the flower/leaf/seed extracts of *Ipomoea quamoclit*, were capped with phenolic compounds, which would damage the bacterial cell wall, and in addition, generated free radicals and reactive oxygen species, posing damage and toxicity to the bacterial cells. The present study demonstrated that the AgNPs synthesized using *Ipomoea quamoclit* would be capable of providing a high antibacterial activity against the various tested pathogens and can have great potential for the preparation of antibacterial drugs. However, their bio-distribution and pharmacokinetics in clinical applications need to be investigated.

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