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Biofabrication and optimization of silver nanoparticles using *Campsis* sp. to explore their antimicrobial properties

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Plant mediated green synthesis of silver nanoparticles (AgNPs) holds promising applications in the field of Biomedicine, Food packaging and Wound healing. In the present investigation, biofabrication of AgNPs was performed using the aqueous extracts of *Campsis* sp. (Family Bignoniaceae) leaves and flowers growing in the premises of Kirori Mal College, University of Delhi, Delhi. Optimization of AgNPs was performed to analyse the varying effect of pH (6.0, 8.0, 10.0) and silver salt concentration (2 mM, 4 Mm and 6 Mm) in controlling the shape and size of AgNPs which in turn governs their further applications. Interestingly, change in colour of the reaction mixture from pale yellow to reddish brown indicated the formation of AgNPs. These AgNPs were further characterized by UV-Visible spectroscopy and showed peak in the range of 400-450 nm which confirmed the synthesis of silver nanoparticles. Dynamic light scattering and zeta potential analysis (DLS-Zeta) confirmed the size of AgNPs around 200-300 nm. A significant zone of inhibition was observed for both *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacterial strains which revealed the antimicrobial potential of *Campsis* sp. AgNPs. Therefore, *Campsis* AgNPs may provide a green, eco-sustainable alternate method for sustainable production of nanomaterials for biomedical applications. These AgNPs may also show tremendous applications in food packaging, wound healing and biomedical fields.

Keywords: Biofabrication, *Campsis* sp., DLS, Silver Nanoparticles, Zeta potential and ZOI (Zone of Inhibition)

Over the last century, nanotechnology has emerged as an innovative subject of research, and represents an important area of modern science dealing with the synthesis, manipulation, and use of nanoparticles. The potential of nanomaterials in various fields, including biomedical, chemical, cosmetics, drug-gene delivery, electronics, environment, food and feed, health care, mechanical, optical, and space sectors, is growing by the day[1]. Ever, recently the research has been shifted towards green nanotechnology which involves synthesis of nanoparticles using plant extracts as reducing elements for synthesis of nanoparticles. Owing to their increased catalytic, optical, and electrical capabilities, metallic nanoparticles including AgNPs are regarded as the most promising. Silver nanoparticles (AgNPs) belongs to the category of metallic nanoparticles with wide range of applications in different fields such as sensors[2], photonics, catalysis and photocatalysis[3]. These particles have also been reported for their wider applications in biological fields such as cosmetic products, food industries, antiseptic sprays, cancer diagnosis, treatment, topical creams and many more. From ancient times till date various physical and chemical methods including chemical reduction, electrochemical, radiation and microwave assisted methods are being used for production of silver NPs. Although, these methods provided us high amount of yield however the nature of reagents could have detrimental effects on the environment. Moreover, these methods also demand additional toxic capping agents and specific instruments for completion of a single reaction. On the contrary, green synthesis involves plants, enzymes, microorganisms which are cheaper in cost, causes no degradation of the environment, and doesn't involve use of toxic chemicals and high temperature, pressure. Across the wide range of biofabrication methods, plant extracts are considered as best for nanoparticles synthesis as the phytochemicals acts asstrong reducing agents and has the capacity to reduce metal ions to elemental metals[4]. Being a single step process the method it also reduces the time of reaction, requires no processing like microbial cultures and have an added advantage of possessing antimicrobial activity[5]. Plant phytochemicals such as saponins, flavonoids, coumarins, proteins, carbohydrates, tannins and terpenoids play a key role in synthesis as well as capping of NPs. Researchers have

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shownthat various plants parts were used for silver NPs production including Pomegranate peel[6], *Azadirachta indica* leaf[7], *Ziziphora tenuior* leaf[8], *Salvia officinalis* leaf extract[9], *Mirabilis jalapa* flower[10] and seed extracts of *Coriandrum sativum*.

Campsis radicans L. (Family: Bignoniaceae; common name: Trumpet vine) is considered as one of the beautiful flowering plants showing cosmopolitan distribution and is widespread in United States, Canada, China, and South Asia. The flowers of this plant are trumpet-shaped and are found to be very attractive for humming bird species. This plant is traditionally used for a variety of human ailments including wound healing, treatment of infectious disease caused by fungal species like *Hemophyllus*, *Candida* and as anti-itching medication[11]. Chinese people also considered the plant as anticoagulant drug which is frequently used for irregular dysmenorrhea and abnormal menstruation. *Campsis radicans* is shown to possess various phytoconstituents and coumarins including 8-methoxy furanocoumarin, pabulenone, pereflorin B and 17-methyl bothrioclinin, flavonoids such as luteolin, quercetin 3-methyl ether, apigenin and chrysoeriol[11]. Islam *et al*. 2020 revealed the significant pharmacological potential of *C. radicans* leaf *via in vitro* antioxidant, thrombolytic, membrane-stabilizing, *in vivo* analgesic, hypoglycemic, anti-diarrheal, and CNS anti-depressant activities[11]. Likewise, *Campsis* sp. leaf extract has been reported to possess antimicrobial activity against *E. coli* strain as indicated by significant Zone of Inhibition (4.6 ± 0.44 mm)[12]. Moreover, the essential oil obtained from *Campsis* sp. is also found to be effective against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*[13]. Despite showing significant pharmacological potential, literature search reveals that the scientific reports on *Campsis* sp. biological studies are very scanty. Therefore, the present investigation was done to study the cost-effective, safe, and eco-friendly biosynthesis of AgNPs using *Campsis* sp. leaf and flower extracts and to evaluate their antimicrobial activity against gram positive and gram-negative bacterial strains (Table 1).

Materials and Methods

Chemical requirements

Silver nitrate ($AgNO_3$, 99.99 %) was purchased from Merck, USA. LB media (Nutrient Broth), Hard agar, soft agar, Gentamycin were purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). Whatman filter paper I and ethanol were of analytical grade. Deionized water was used throughout the experiment.

Plant collection and preparation of extracts

Campsis sp. Figure 1 belongs to family Bignoniaceae. The leaves and flowers of *Campsis* sp. were collected from Department of Botany, Kirori Mal College, University of Delhi, Delhi, India. These samples were thoroughly washed with tap water followed by distilled water to remove the surface contaminants and left for air-drying. For preparation of aqueous extracts fresh leaves and flowers (5 g) were weighed, ground to fine powder using mortar pestle and the slurry was prepared by adding 50 mL of deionised water in the ratio 1:10. The slurry was filtered using Whatman filter paper 1 and the aqueous extracts obtained in the form of supernatant were kept at 4°C for further use. The original pH of leaves and flowers extract was observed as 5 and 6, respectively, using pH paper.

Table 1 — Showing DLS of AgNPs synthesized using *Campsis* sp. flower and leaf extracts

Different pH of 6, 8 and 10				
Sr no.	AgNPs	pH variation	Size (nm)	PDI
1.	Flower extract	pH 6	256.0±176.5	0.325
2.		pH 8	301.7±170.2	0.445
3.		pH 10	233.7±366.6	0.415
4.	Leaf extract	pH 6	157.6±76.65	0.341
5.		pH 8	174.4±106.7	0.385
6.		pH 8	213.1±274.4	.470
Different salt concentrations of 2 mM, 4 mM and 6 mM				
Sr no.	AgNPs	Concentration of $AgNO_3$(mM)	Size (nm)	PDI
1.	Flower extract	2	206.7±64.67	0.249
2.		4	216.4±166.3	0.306
3.		6	227.9±94.34	0.235
4.	Leaf extract	2	290.8±79.70	0.292
5.		4	315.1±101.8	0.282
6.		6	308.6±155.8	0.286

Fig. 1 — Plant used in the present study. *Campsis* sp. (Trumpet vine) with flower and leaves

Synthesis of silver NPs

For the green synthesis of AgNPs, leaves and flower extracts (1 mL)were added to 2 mM silver salt solution (9mL). Within few minutes, the colour change was observed from colourless to reddish brown thus indicating the formation of AgNPs[14].

Characterisation of AgNPs

UV-Visible spectroscopy

After the initial change in colour of the reaction mixture from colourless to reddish-brown, the synthesis of AgNPs was further confirmed using UV-visible spectroscopy. Therefore, the shift in AgNPs absorption spectra owing to their surface plasmon resonance was monitored using UV-Spectrophotometer (Cary-60, Agilent Technologies, USA). Using deionised water as blank, the shift in absorption spectra of different samples were observed in the range of 200-800 nm.

Dynamic light scattering (DLS) and ZETA characterisation

Hydrodynamic radii of different AgNPs were determined using Dynamic light Scattering (DLS) using Zeta-sizer (Nano-ZS, Malvern USA). Initially, the silver samples were diluted with deionised water to segregate all individual nanoparticle from their aggregate forms to assess distribution size and charge on the surface of NPs. For the sampling, migration voltage used was 100 mV[15].

Antimicrobial disc diffusion assay

The antimicrobial disc diffusion assay was performed according to Mittal *et al*. 2019. Briefly,for raising the fresh stock of bacterial strains, 1.4 g of LB media was dissolved in 50 mL of deionised water. This nutrition broth was autoclaved at high temperature (120°C) and high pressure (15 psi) for 1 h. For the fresh revival of bacterial stocks, inoculum was used in 1:100 ratio and added in sterile nutrient LB media which was kept on orbital shaker for overnight at 37°C. Within 24 h, clear media turned into turbid media which confirmed the growth of bacterial colonies[16]. The optical density and absorbance were recorded with the use of UV-visible spectroscopy and CFU/mL, 1O.D = 0.8×10^9 CFU/mL was calculated. The effectiveness of AgNPs were determined using disc diffusion assay via Kirby Bauer method[16]. Nutrient agar plates were used as bedding for the bacterial inoculum mixed with soft agar. The plates were divided into different sections and the autoclaved sterile disc of 5 mm diameter was placed in the allocated sections which was further loaded with Gentamycin (5 mg/mL; positive control), Milli-Q water (negative control), flower and leaf extracts as well as corresponding AgNPs. For proper incubation of bacterial cultures, the plates were kept overnight in BOD incubator at 37°C. The zone of inhibition (ZOI) was measured after 24 h[17].

Statistical analysis

The data was illustrated depicting mean value ± standard deviation for n=3. Further comparison between different groups was analysed with one-way analysis of variance (ANOVA) under Turkey's test using Prism software (Prism software Inc. CA). Significance level was accepted at $P \leq 0.05$ level. For Antimicrobial activity (*)denotes the significant variation of negative control *vs* AgNPs flower and leaf extract with pH variations for *E. coli* and (+)denotes the significant variation of negative control *vs* AgNPs flower and leaf extract with pH variations for *S. aureus* where $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

Results and Discussion

Campsis sp. leaves and flower aqueous extract showed pH values of 6 and 5 respectively. As both flower and leaves extracts lie in acidic range therefore, optimization of AgNPs was done using the extracts maintained in the alkaline ranges of pH 6.0, 8.0 and 10 NaOH was used for maintaining the alkaline pH as higher pH displays reducing nature which promotes the reduction of silver salts to elemental silver. Higher concentration of phytochemicals was observed in leaf extract which also corresponds to their darker colouras compared to flower extracts. *C. radicans* possess numerous phytochemicals like phenolics, coumarins including 8-methoxy furanocoumarin, pabulenone, pereflorin B and 17-methyl bothrioclinin, and flavonoids such as luteolin, quercetin 3-methyl ether, apigenin and chrysoeriol[11]. Phenolics and Flavonoids possess high antioxidant activity and redox potential due to the presence of hydroxyl groups which act as reducing agents. UV-visible spectroscopy of flower extracts showed peaks in the range of 200-300 nm whereas higher intensity of peaks was observed for leaf extracts in the range of 200-400 nm corresponding to phenolics, tannins and flavonoids. Interestingly, these results suggested higher reducing potential of leaf extracts in comparison with flower extracts.

Addition of leaf and flower extracts to colourless silver nitrate solution led to change in color of solution into reddish brownish thus indicating the formation of silver nanoparticles due to the reduction of silver ions (Ag^{3+}) to AgNPs (Ag^0) (Fig. 2). The color of AgNPs arises due to the resulting in surface Plasmon resonance (SPR). Relatively, faster bioreduction due to higher

reducing potential of leaf was observed using *Campsis* leaves extract in comparison with flower extracts. The duration of reaction of silver nanoparticles varies from plants to plants, as in this case, the reaction took only few minutes (5-10 min) to get completed. *Dioscorea*, *Nerium indicum* and *Euphorbia hirta* leaf extract showed complete reduction reaction within two-three h whereas *Syzygium cumini* leaf extract at normal temperature requires 24 h to complete the reaction[5]. The proposed mechanism of reaction involves intermediary complex formation with phenolic component (-OH group) found in tannins which are of hydrolysable nature. The phenolic group follows quinine oxidation which reduces silver salt into elemental silver nanoparticles.

The optical absorption spectra of leaf and flower AgNPs were observed using UV-Vis spectrophotometer (Fig. 3). The reddish-brown solution was the resultant of surface plasmon resonance excitation wavelength falling in the range of 400-460 nm. Optical absorption spectra for both leaves and flower AgNPs showed that the particles were well dispersed with constant SPR peak.

AgNPs formed using flower and leaf at pH 6 and different salt concentrations of 2 mM, 4 mM and 6 mM showed peaks in the range of range of 410-430 nm for flower and leaf extracts (Fig. 4A & B). Comparatively, lesser amount of *Campsis* flower AgNPs with negligible peak in the range of 400-420 nm was observed at pH 6.0. However, the intensity of flower AgNPs increased with increase in pH showing a single peak of 405 nm at pH 8.0 and two peaks at 415 nm and 435 nm at pH 10.0. Similarly, AgNPs synthesis via leaf extract at pH 6.0 also showed negligible peak around 400-420nm, whereas at pH 8 a prominent leaves AgNPs peak was observed at 430 nm and multiple peaks in the range of 410 to 450 nmwere obtained at pH 10 (Fig. 4C & D). Similar result have been observed using *Nigella sativa* seed extract where AgNPs peak lied in the range of 350-450nm with prominent peak at 426 nm[17] and *Malva sylvestris* flower extract showing prominent peak at 430 nm[18].

The DLS (Dynamic Light Scattering) is one of the techniques to determine the nanoparticles size and their distribution profile in polymers or in suspension form (Table 2). This principle is used to evaluate the size distribution profile of the *Campsis* sp. AgNPs dissolved in deionised water. DLS equipment also facilitate the anlysis of the polydispersity index (PDI) which indicates the homogeneous nature of AgNPs. *Campsis* flower and

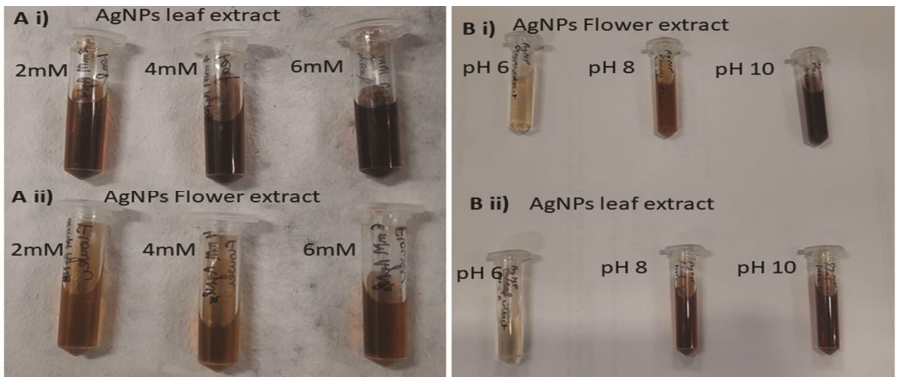

Fig. 2 — Optimization and synthesisof *Campsis* leaf and flower silver nanoparticles (A) using different concentrations of silver salt; and (B) at different pH of 6, 8 and 10 showing variations in color intensity

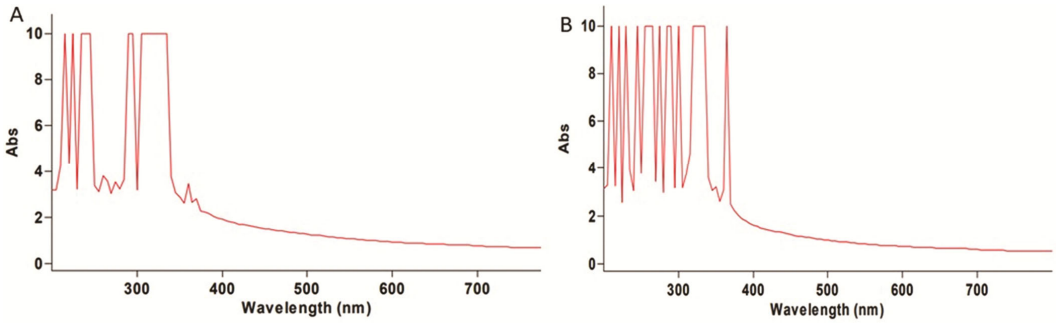

Fig. 3 — (A) UV-visible spectroscopy of flower extract showing prominent phytochemical peaks in the range of 200-400 nm; and (B) UV-visible spectroscopy of leaf extract showing numerous peaks in the range of 200-400 nm

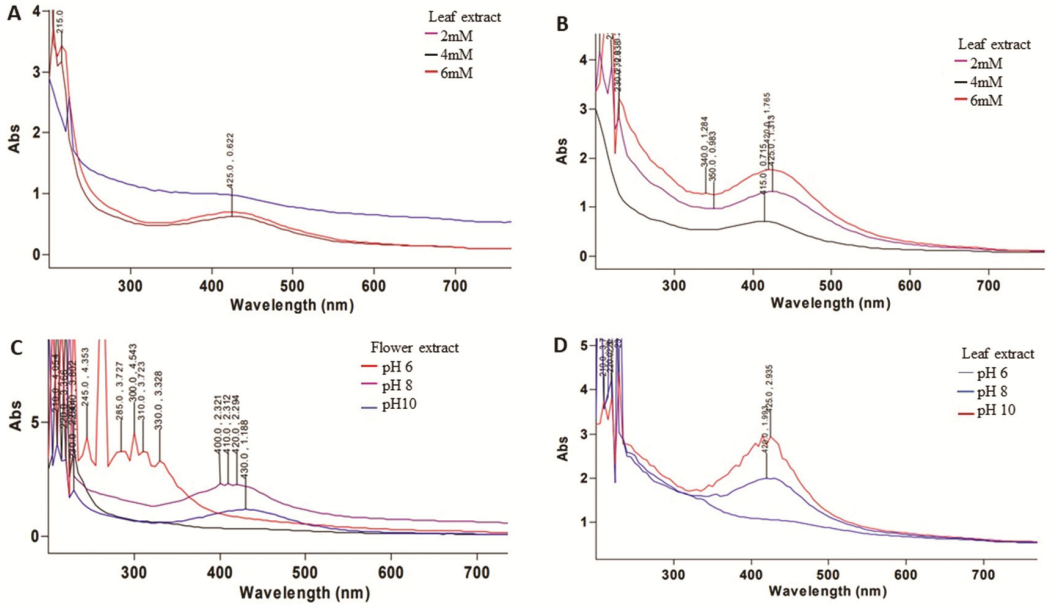

Fig. 4 — (A & B) UV-visible spectroscopy of silver nanoparticles absorption spectra using different salt concentration (A) flower extract; and (B) leaf extract. (C & D) UV-visible spectroscopy of silver nanoparticles absorption spectra at different pH of 6, 8 and 10 (C) flower extract; and (D) leaf extract

Table 2 — Showing ZETA potential of AgNPs formed using flower and leaf extracts formed

Different pH - 6, 8 and 10

Sr no.	AgNPs	pH	Zeta potential(mV)
1.	Flower extract	pH 6	–26.6±8.88
2.		pH 8	–28.1±8.59
3.		pH 10	–27.2±6.11
4.	Leaf extract	pH 6	-18.1±5.67
5.		pH 8	–25.2±11.8
6.		pH 10	–18.7±8.42

Different silver salt concentration of 2, 4 and 6 mM

Sr no.	AgNPs	$AgNO_3$(mM)	Zeta potential(mV)
1.	Flower extract	2	–23.5±8.36
2.		4	–26.6±7.22
3.		6	–26.2±8.78
4.	Leaf extract	2	–21.9±11.0
5.		4	–20.5±8.36
6.		6	–21.9±6.70

leaf AgNPs at pH 6, 8 and 10 showed size and PDI of 256, 301.7, 233, 157, 174.4, 213.1 nm, and 0.325, 0.445, 0.415, 0.341, 0.385, 0.470, respectively. On the other hand, increasing the concentration of salt from 2 mM to 4 mM, and 6 mM led to increase in size of *Campsis* flower AgNPs 206.7, 216.4, 227 nm, with respective PDI values of 0.249, 0.306, 0.235 suggesting the limitation of reducing agent *i.e.*, extract for reducing higher concentration of salt (Fig. 5). Similar increase in the size of *Campsis* leaf AgNPs from 290, to 315, 308 nm and corresponding PDI value of 0.292, 0.282, 0.286 was observed with increasing concentration of salt solution[19].

Due to the smaller size of nanoparticles, they are very unstable in nature which form aggregates in turn to gain stability. Also, the particles have some potential charges on their external surface which make these unstable leading to aggregation. In the present study, to find out the stability of biofabricated AgNPs, zeta potential was calculated for *Campsis* AgNPs which showed the negative values in the range of –26.6, –28.1, –27.2 for flower extract at pH of 6, 8 and 10, respectively (Table 3). Likewise, for leaf extract at pH 6, 8 and 10 the values were –18.1, –25.2, –18.7, respectively (Fig. 6). On varying the silver salts concentrations (2, 4 and 6 mM) flower AgNPs showed zeta potential values of –23.5, –26.6, –26.2, respectively. Similar results were observed for leafAgNPs showing zeta potential of –21.9, 20.5, –21.9, respectively. All the AgNPs showed negative zeta potential which indicated their higher stability (Fig. 7).

Disc diffusion assay

After 24 h of incubation of microbial disc plates, area of inhibition was measured. The ZOI is circular because of the radial disbursement of sample loaded on the disc. The inhibition of bacterial strains occurred in two ways *viz*. bactericidal and bacteriostatic. In the initial step, all the bacterial colonies were killed by the drug and a clear zone of inhibition was obtained whereas in the

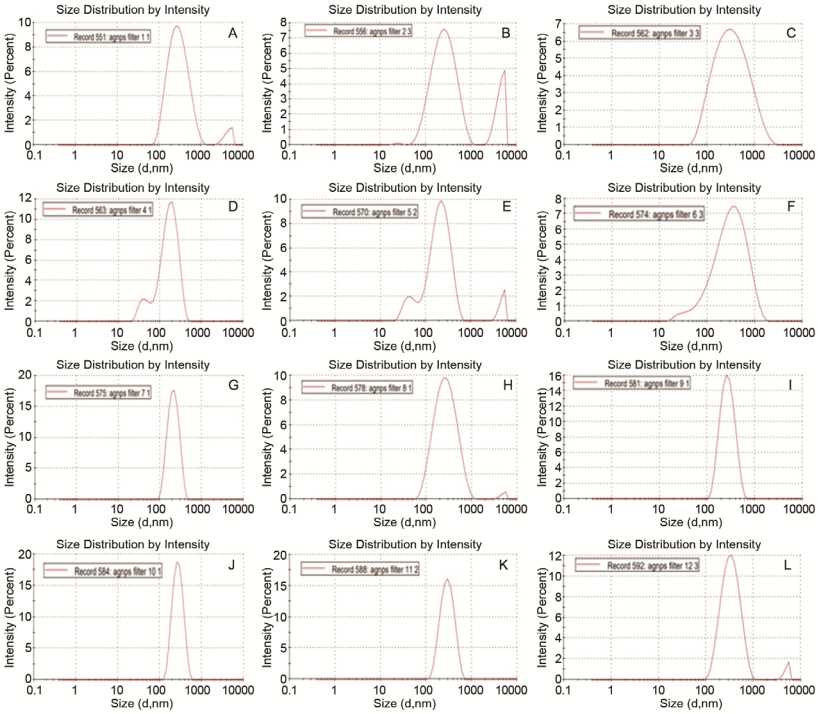

Fig. 5 — Diagram showing Diameter of nanoparticle (DLS): (A-C) Silver nanoparticles prepared with flower extract at pH-6, 8 and 10 respectively; (D-F) Silver nanoparticles prepared with leaf extract at pH-6, 8 and 10 respectively; (G-I) Silver nanoparticles prepared with flower extract using salt concentration of 2, 4, and 6 mM respectively; (J-L) Silver nanoparticles prepared with leaf extract using salt concentration of 2, 4, and 6 mM, respectively

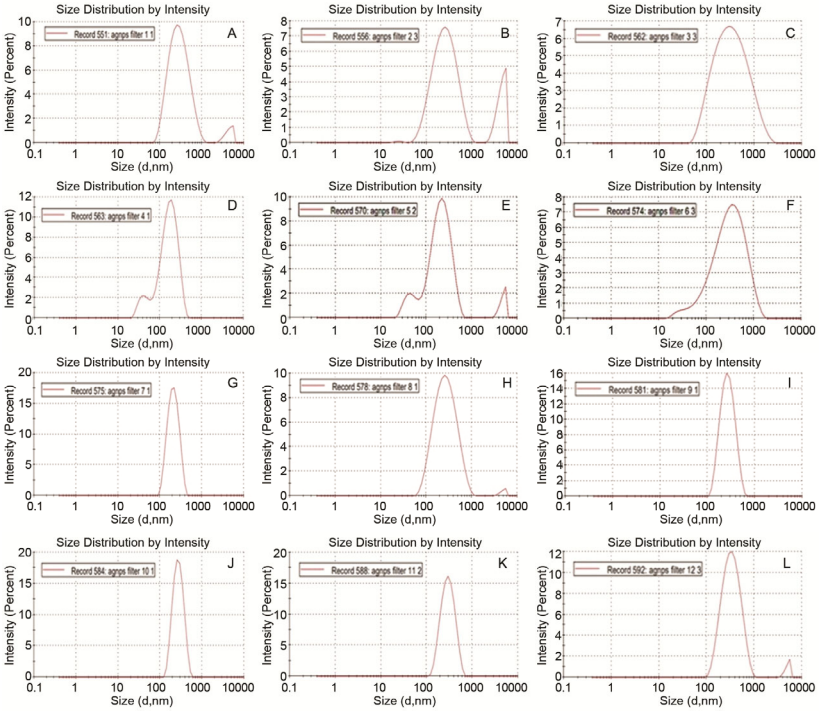

Fig. 6 — Diagram showing surface charge of the nanoparticle (ZETA potential): (A-C) Silver nanoparticles prepared with flower extract at pH-6, 8 and 10 respectively; (D-F) Silver nanoparticles prepared with leaf extract at pH-6, 8 and 10 respectively; (G-I) Silver nanoparticles prepared with flower extract using salt concentration of 2, 4, and 6 mM respectively; (J-L) Silver nanoparticles prepared with leaf extract using salt concentration of 2, 4, and 6 mM, respectively

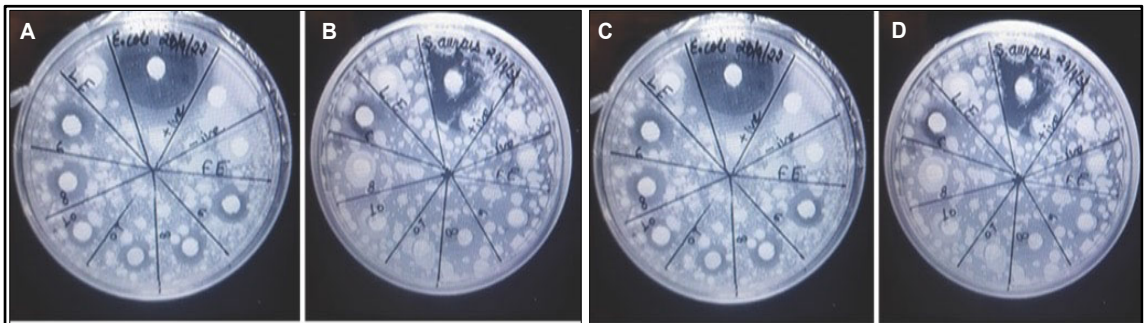

Fig. 7 — Analysis of AgNPs antimicrobial activity against *Staphylococuccus aureus* (gram-positive) and *E.coli* (gram-negative)bacterial strains using disc-diffusion assay. A. *E. coli* disc diffusion plate showing effect of AgNPs formed at pH 6.0, 8.0 and 10.0. B. *S. aureus* disc diffusion plate showing effect of AgNPs formed at different pH. C. *E. coli* disc diffusion plate showing effect of AgNPs formed at different silver salt concentration. D. *S. aureus* disc diffusion plate showing effect of AgNPs formed at different silver salt concentration

Table 3 — Showing antimicrobial activity of AgNPs formed

Different pH - 6, 8 and 10			
Sample		*E. coli* (ZOI*, mm)	*S. aureus* (ZOI*, mm)
Positive control		$12\pm0.152^{***}$	$9\pm0.1^{+++}$
Negative control		0	0
Flower Extract		0	0
Leaf Extract		0	0
AgNPs Flower Extract	pH 6	$6\pm0.153^{***}$	$3\pm0.2^{+++}$
	pH 8	$6\pm0.1^{***}$	$5\pm0.1528^{+++}$
	pH 10	$6\pm0.1523^{***}$	$6\pm0.2516^{+++}$
AgNPs Leaf Extract	pH 6	$6\pm0.1^{***}$	$2\pm0.2^{+++}$
	pH 8	$6\pm0.208^{***}$	$3\pm0.2082^{+++}$
	pH 10	$6\pm0.0577^{***}$	$4\pm0.2082^{+++}$
Different salt concentration of 2, 4 and 6mM			
Sample		*E. coli* (ZOI*, mm)	*S. aureus* (ZOI*, mm)
Positive control		$12\pm0.115^{***}$	$9\pm0.1^{+++}$
Negative control		0	0
Flower Extract		0	0
Leaf Extract		0	0
AgNPs Flower Extract	2	$3\pm0.152^{***}$	$2\pm0.1527^{+++}$
Silver salt(mM)	4	$5\pm0.115^{***}$	$3\pm0.2081^{+++}$
	6	$7\pm0.0577^{***}$	$5\pm0.0577^{+++}$
AgNPs Leaf Extract	2	$3\pm0.2645^{***}$	$1\pm0.2645^{+++}$
silver salt(mM)	4	$4\pm0.1527^{***}$	$2\pm0.2081^{+++}$
	6	$5\pm0.2516^{***}$	$4\pm0.1^{+++}$

*Data of ZOI are represented as mean± standard deviation for n=3,***P<0.001

second step, the bacterial growth was inhibited, and translucent zones were obtained. Our experimental studies showed that AgNPs exhibited maximum ZOI for *E. coli* and comparatively lesser ZOI in case of *S. aureus* because of the more cell wall layer found in gram-positive strain. Similar results have been observed for other NPs[20,21].

Conclusion

The present investigation showed, green synthesis of AgNPs using *Campsis* leaf and flower extracts. Faster bioreduction of silver nitrate salt into AgNPs was obtained as indicated by the change of reaction mixture from pale colour to reddish within few minutes. Synthesis of smaller size, uniform and well dispersed AgNPs was achieved by controlled synthesis and optimisation of synthesis parameters. Higher intensity of flower AgNPs was observed at pH 10 showing corresponding peaks at 415and 435nm whereas, leaf AgNPs showed maximum intensity at pH 8 with prominent peak at 430nm. The different salt concentrations at pH 6 for both flower and leaf extract shows intensity gradient which implies intensity of AgNPs is directly proportional to pH and salt concentrations. These AgNPs showed promising antibacterial potential against both Gram positive and negative bacteria as indicated by their higher antimicrobial efficacy. Hence, may provide an alternative against the multi-drug resistant bacteria in future. Thus, the silver NPs synthesized using a green, eco-sustainable process from flower and leaf extract of *Campsis* species shows an alternate method for sustainable production of nanomaterials. These AgNPs holds tremendous application in food packaging, wound healing and biomedical fields.

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Conflict of interest

All authors declare no conflict of interest.

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