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Antibacterial potential of silver nanoparticles synthesized using *oxalis corniculata* leaf extract

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Abstract
Nanoparticles have a wide range of applications in the field of medical science and can be synthesized using a variety of physical, chemical and biological methods. The present study focuses on the green synthesis of silver nanoparticles using *Oxalis corniculata* leaf extract and evaluation of their antibacterial activity. The extract which revealed the presence of alkaloids, glycosides, flavonoids and diterpenes on phytochemical analysis was used for the synthesis of nanoparticles using three different concentrations of silver nitrate i.e., 1 mM, 3 mM and 5 mM. Colour change of the solution from yellow to dark brown due to surface Plasmon resonance confirmed the formation of nanoparticles which were characterized by UV-visible spectroscopy, Fourier transform-infrared spectroscopy, X-ray diffraction and field emission scanning electron microscopy. The synthesized nanoparticles were found to possess excellent antibacterial activity against Gram positive *Staphylococcus aureus* and Gram negative *Escherichia Coli*. The nanoparticles formed of 1 mM silver nitrate were found to be more effective against Gram negative bacteria while those made of 3 mM silver nitrate were more active against Gram positive bacteria. It could be observed that the minimum inhibitory concentration and minimum bactericidal concentration of the nanoparticles were 20µg/mL against both *E Coli* and *S Aureus*.

Keywords: *Oxalis corniculata*, green synthesis, silver nanoparticles, antimicrobial activity

Introduction
Nanotechnology, which deals with materials of size between 1 to 100 nm, focuses on multiple areas of science such as dentistry, pharmaceuticals and biotechnology. A variety of physical, chemical and biological methods are relied upon for the production of a variety of nanoparticles. However, most of the chemical techniques employed are costly and require the use of toxic chemicals that pose different biological hazards. Hence alternative methods that are less toxic, environmentally friendly and inexpensive are being explored. New eco-friendly "green" methods of synthesis are being discovered to meet the increasing demand for commercialized nanoparticles. The biological methods for the synthesis nanoparticles involve the utilization of various microorganisms and plants. Because of their easy availability, eco-friendliness and safety index, the use of plant extracts in the synthesis of nanoparticles is becoming more and more popular (Ahmad *et al*., 2003) [1]. A variety of metals like zinc, copper, silver platinum, gold, iron etc. are utilized to synthesize nanoparticles. Many plant components aid to reduce these metal ions faster than microbes because of the phytochemicals contained in them (Torresday *et al*., 2002) [22]. Different techniques like UV-visible (UV-Vis) spectrophotometry, transmission electron microscopy (TEM) and X-ray diffraction (XRD) aids in the characterisation of the synthesised nanoparticles.
Multidrug resistance among bacteria is emerging as a global health threat to both humans and animals which make them impervious to almost all commonly used antibiotics. Nano medicine offers a very promising system to counter the hazards posed by such organisms. Different mechanisms of action have been proposed for the antibacterial property of nanoparticles including disruption of cell membrane, inhibition of formation of biofilm, stimulation of host immune responses and generation of free radicals. The size of the nanoparticles has been found to be a critical determinant of their antimicrobial activity with the activity decreasing with increase in size.
Oxalis corniculata, commonly referred to as the creeping wood sorrel, belonging to the Oxalidaceae family, is a small procumbent herb well known for its effect as a good appetiser and is used in traditional system of medicine to treat dysentery, diarrhoea and skin diseases. This plant has been reported for hypoglycemic, antihypertensive, chronotropic, antipsychotic, CNS-stimulant, inotropic and smooth muscle relaxing activities (Badwaik *et al*., 2011) [2].

The present study has focused on the green synthesis of silver nanoparticles (AgNPs) using aqueous extract of *O. corniculata* and the *in vitro* evaluation of antibacterial potential of these nanoparticles.

Materials and Methods

The leaves of *O. corniculata* were collected from the campus of College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India. The plant materials were identified and authenticated at the Department of Botany, St. Thomas College, Thrissur (Fig 1). The collected leaves were shade dried, pulverized and subjected to aqueous extraction.

Fig 1: Leaves of *Oxalis corniculata*

For phytochemical analysis, the leaves were shade-dried and finely pulverized. About 10 g of the dried powder was soaked in 100mL distilled water for 48 h with intermittent shaking. The mixture was then filtered through Whatman No.1 filter paper (Duraipandiyan *et al*., 2006) [6]. The filtrate was concentrated to near dryness using rotatory vacuum flash evaporator under reduced pressure at 30ºC and stored under refrigeration conditions. The aqueous extract thus isolated was tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins (Harborne, 1991) [8].

For the green synthesis of silver nanoparticles, the *O. corniculata* leaves were washed with double distilled water, dried at room temperature and then stored till further use. About 10 g of dried leaves were taken in a 250 mL flask along with 100 mL of distilled water and kept in a water bath at 80°C for 15 min (Salisu *et al*. 2014) [20]. The resulting solution was filtered using Whatman No.1 filter paper to obtain a golden yellow extract and stored at 4 °C for further use. Silver nitrate ($AgNO_3$) solutions of varying molarities (1.0, 3.0 and 5.0 *mM*) were prepared and stored in amber coloured bottles. The nanoparticles were synthesized according to the protocol of Moosa *et al*. (2015) [14] with slight modifications. About 10mL plant extract was added to 90 mL of each of the $AgNO_3$ solution and mixed well. The mixtures thus prepared were then exposed to sunlight for about 5-10 min and incubated overnight in darkness. Reduction of silver ions (Ag^+) to metallic silver (Ag^0) was confirmed by the colour change of solution from colorless to brown. The resultant solutions were purified by repeated centrifugation at 8000 rpm for 20 min followed by re-dispersion of the pellet in de-ionized water. The purified solutions obtained were lyophilized, vacuum sealed and stored at room temperature till further use.

The formation of AgNPs by reduction of Ag^+ to Ag^0 using *O. Corniculata* leaves extract was initially confirmed by measuring the surface Plasmon resonance (SPR) peak of the samples using UV-Vis spectrophotometer (Perkin-Elmer, Lamda 25) by exposing to light of 300-700 nm wavelengths with 1nm as wavelength resolution. The chemical composition of the synthesized AgNPs was studied by using Fourier transform-infrared (FTIR) spectrometer **(**Perkin-Elmer Pte Ltd**)** with wave number in the range of 4000-450 cm^{-1}. The phase variety as well as grain size of the AgNPs was determined by XRD spectroscopy (Rigaku X-ray diffract meter, Miniflex, UK) with CUKα radiation at a voltage of 30 kV and a current of 20 mA with a scan rate of 10°2θ/min (θ is the Braggs angle in radians). Different phases present in the samples were determined by X′ pert high score software with search and match. The morphological features of the lyophilized AgNPs were studied by field-emission scanning electron microscopy (FESEM) (Zeiss, Sigma).

The *in vitro* antibacterial potential of the synthesized nanoparticles were tested against *Escherichia coli* and *Staphylococcus aureus* procured from Microbial Type Culture Collection (MTCC), Chandigarh and propagated in selective media. The inocula were prepared by direct colony suspension method following CLSI guidelines (2015) [3]. Well diffusion method (Ibrahim, 2015) [9] was used to compare the antibacterial activity of the nanoparticles of varying concentrations. Two nutrient agar plates were prepared, one for *E. coli* and the other for *S. aureus* respectively. Four wells of 6 *mM* diameter were made in the agar to which 15µL each of the nanoparticle solutions (1.0, 3.0 and 5.0 *mM*) as well as 15µL of a standard antibiotic solution (Ampicillin) were added. The plates were then incubated at 37 °C for 24 h after which the diameter of zones of inhibition developed around each well was measured. Among the three samples of AgNPs synthesized using different concentrations of $AgNO_3$, the sample that produced the widest zone of inhibition for each bacterium was selected to perform broth micro dilution test to determine the minimum inhibitory concentration (MIC) as per CLSI guidelines.

A volume of 10 mL nutrient broth supplemented with the selected molarity AgNPs in varying concentrations (20, 10, 5, 2.5 and 1.25 µg/mL) were inoculated with 0.1 mL inoculum containing 1×10^6 cells of *E Coli*. The tubes were then incubated in an orbital shaking incubator at 150 rpm for 24 h at 37 °C. Silver nanoparticle-free broth inoculated with the organism was used as negative control while 10mL nutrient broth supplemented with 20 µg ampicillin inoculated with the bacteria served as positive control. The microbial growth was indexed by measuring the optical density (O.D. 600) of all the tubes using UV-Vis spectrophotometer (Perkin-Elmer, Lamda 25). The whole procedure was repeated with *S Aureus*. The lowest concentration of AgNPs that prevented the visible growth of bacteria was considered as the MIC against both the organisms (Wiegeand *et al*., 2008) [23]. The concentrations showing no turbidity were further subjected to test for assessment of minimum bactericidal concentration (MBC) by sub-culturing with the concentrations having MIC or above on to nutrient agar plates that did not contain the test agent.

Results and Discussion

The phytochemical analysis of *O Corniculata* leaves revealed

the presence of alkaloids, glycosides, flavonoids and diterpenes. A colour change from golden yellow to dark or reddish brown was observed visually when 10 mL leaf extracts were mixed with 90mL $AgNO_3$ solution of varying molarities (1 *mM*, 3 *mM* and 5 *mM*) and incubated under sunlight for 5-10 min (Fig 2).

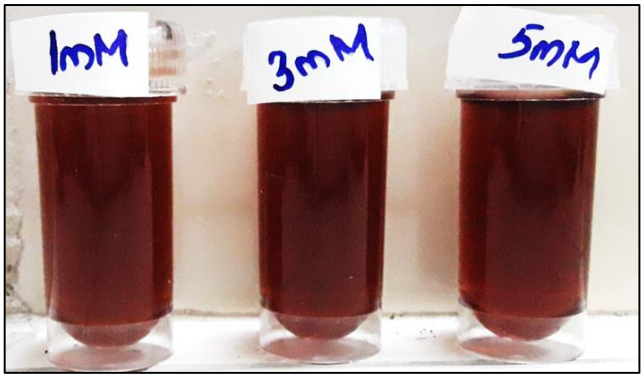

Fig 2: Aqueous extract of *O Corniculata* treated $AgNO_3$ solution after exposure to sunlight

In order to complete the reduction process, the mixtures were further incubated at room temperature for 24 h in darkness. The UV-Vis absorption spectra of the AgNPs synthesized with 1 *mM*, 3 *mM* and 5 *mM* $AgNO_3$ solutions revealed surface Plasmon resonance (SPR) peaks at 438, 440nm and 445nm respectively. Kumar *et al*. (2006) [11] reported that the aqueous and methanolic extract of *O. corniculata* revealed the presence alkaloids, flavonoids, glycosides, saponins and tannins. The presence of flavonoids and alkaloids in the extract was confirmed by thin layer chromatography. Raghavendra *et al*. (2006) [17] produced extracts from powdered leaves of *O. corniculata* with various solvents such as petroleum ether, benzene, chloroform, methanol and ethanol and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins.

The formation of AgNPs using *O Corniculata* leaf extracts was confirmed by measuring the SPR peak of the samples at wavelengths ranging from 300 to 700 nm. Results obtained were compatible with many previous reports. Dubey *et al.* (2009) [5] used *Eucalyptus hybrid* leaf extracts to synthesize AgNPs. The change of yellow colour to brown due to SPR band revealed the presence of AgNPs. Onion (*Allium cepa*) extract was used to synthesize AgNPs by Saxena *et al.* (2010) [19] who concluded that the extract of onion increases the rate of reduction reaction and is therefore a convenient method to synthesize nanoparticles. Extracts from different plants like *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* were used AgNPs synthesis (Logeswari *et al*., 2013) [12]. The UV-Vis spectral analysis confirmed the formation of AgNPs in solution where the nanoparticles showed a peak at 420nm. Moosa *et al.* (2015)[14] reported the synthesis of AgNPs using *Aloe vera* plant extract and tea leaves extract which act as a stabilising and reducing agent. The formation of AgNPs was confirmed by change in their colour to dark brown due to SPR phenomena. Packiyam and Uthappa (2017) [15] synthesized stable AgNPs using *Cynodon dactylon* leaf extract. The UV-Vis spectrum analysis of AgNPs showed SPR peak around 450 nm.

Analysis of the AgNPs by means of FTIR spectroscopy was conducted to identify the possible functional groups in them that could be responsible for the reduction of Ag^+ to Ag^0 as well as capping or stabilization of the nanoparticles. The intensive bands obtained were compared to the standard values to demarcate the corresponding functional groups. The 1 *mM*, 3 *mM* and 5 *mM* AgNPs showed three peaks in their spectra. Out of the three peaks of 1 *mM* AgNPs, a strong peak was seen at 3436.03 cm^{-1} which was found to be due to the hydroxyl (-OH) group stretching in alcohol and water. A peak was observed around 2054.79 cm^{-1} which corresponded to the methylene group and methoxy group. The peak at 1634.06 cm^{-1} attributed to N-H bond, aromatic and alkene groups. In 3 *mM* AgNPs, a strong peak was observed at 3448.87 cm^{-1} which was attributed to –OH group of water and alcohol, the second peak at 2059.31 cm^{-1} indicated the methylene and methoxy groups while the third peak at 1640.62 cm^{-1} denoted the N-H bond, aromatic and alkene groups. The 5 *mM* AgNPs exhibited a peak at 3467.93cm^{-1} which was assigned to -OH group stretching of alcohol and water. The peak observed at 2063. 48 cm^{-1} was indicative of methylene and methoxy groups. The peak at 1654.02 cm^{-1} corresponded to stretching vibration of N-H bond, aromatic and alkene groups. The results of this study are similar and analogous to the reports of Jaidev and Narasimha, (2010) [24] who revealed the formation of stable nanoparticles from their FTIR spectrum consisting of three separate peaks i.e. 3347.85, 1636.17 and 548.38 cm^{-1}, respectively. The peak at 3347.85 cm^{-1} referred to primary amine stretching vibrations, whereas the one at 1636.17 cm^{-1} was due to the stretch of carbonyl group. Logeswari *et al.* (2015) [25] reported that the FTIR spectra analysis of the green synthesized AgNPs showed peaks corresponding to -OH alcohol stretch and N-H amine group bond ensuring that nanoparticles are capped with these functional groups. Moosa *et al.* (2015) [14] synthesized nanoparticles using *A Vera* plant extract which upon FTIR spectroscopy analysis exhibited peaks corresponding to -OH, carbonyl and secondary amine groups.

The crystalline nature of the AgNPs was studied by analyzing their X-ray diffraction pattern (Fig 4.7, 4.8, 4.9) which showed different diffraction peaks at 2θ value of 38.15, 44.30, 64.75 and 78.05 which were indexed to the (1 1 1), (2 0 0), (2 2 2) and (3 1 1) planes respectively. By comparing the JCPDS (file no: 04-0783), the green-synthesized AgNPs were found to possess a face centred cubic structure. The XRD patterns obtained are similar and compatible with the previous reports. Prema and Raju (2009) [16] reported similar pattern of XRD in AgNPs synthesised chemically which showed sharp reflections at (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes at 2θ angles of 38.28°, 44.38°, 64.54°, and 77.64° respectively. The leaf extract of *Datura stramonium* was used by Gomathi *et al.* (2017) [7] to synthesize AgNPs. The crystalline nature of AgNPs as face centre cubic structure and oriented along (1 1 1) plane with an average 18 nm size of the crystallite on XRD analysis was reported. Silver nanoparticles were successfully synthesized using *Calliandra haematocephala* leaf extract by Raja *et al*. (2017) [18] and XRD analysis showed the presence of (1 1 1) and (2 2 0) lattice planes of the face cantered cubic structure of metallic silver.

The FESEM analysis was employed to understand the size and shape of the AgNPs. The size of AgNPs synthesized with different concentrations of $AgNO_3$ ranged between 23-45nm and were of spherical shape (Fig 3, 4 and 5). The result of this study is in agreement with the morphology of the AgNPs synthesised using banana peel extract (Ibrahim, 2015) [9] were

studied using FESEM. The particles were found to be of spherical shape having an average size of 23.7 nm. Jyoti *et al.* (2016) [10] reported the size of AgNPs synthesised with *C Dactylon* and *U Dioica Linn.* Leaves as between 20-30 nm and they were spherical in shape. Packiyam and Uthappa (2017) [15] synthesized AgNPs using *C Dactylon* leaf extract. The particles were found by SEM to be of 30 to 50 nm size and spherical shape.

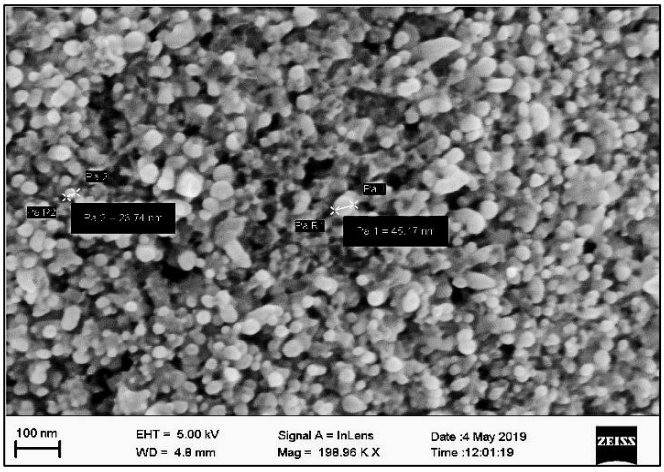

Fig 3: FESEM micrograph of the silver nanoparticles (1mM)

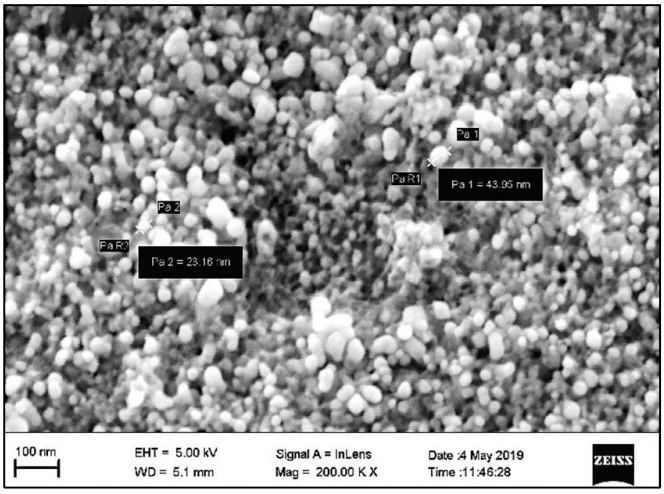

Fig 4: FESEM micrograph of the silver nanoparticles (3 mM)

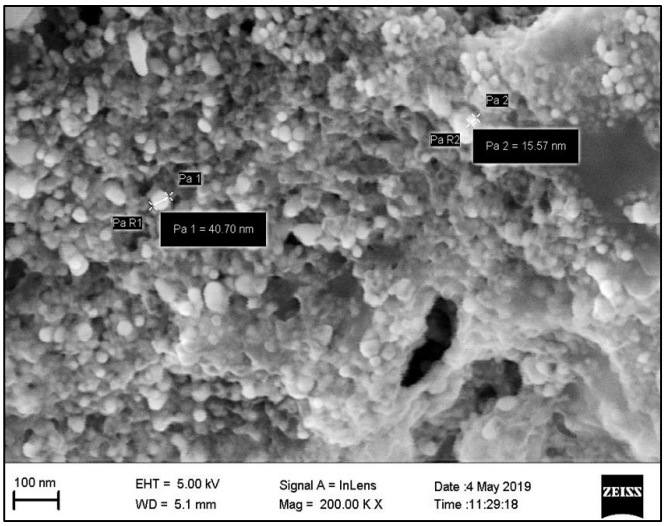

Fig 5: FESEM micrograph of the silver nanoparticles (5 mM)

For assessing the *in vitro* antibacterial activity, well diffusion method was performed for both *E. coli* and *S. aureus* using 20 µL of the different (1.00, 3.00 and 5 *mM*) AgNPs as well as 20 µL ampicillin solution which served as control (Plates 4.9 and 4.10). The diameter of the zone of inhibition obtained for ampicillin was 22 *mM* against *S Aureus*, while that for 1 *mM* and 3 *mM* were of diameter 15 *mM* and 20 *mM* respectively. No zone of inhibition was found around the well containing 5 *mM* AgNPs. In *E Coli* grown medium, the zone of inhibition for Ampicillin was of 18 *mM* diameter while those for 1 *mM* and 3 *mM* AgNPs were 19 *mM* and 15 *mM* respectively. Here also no zone of inhibition was developed around the well with 5 *mM* AgNPs. The AgNPs showing maximum zone of inhibition for *E Coli* and *S Aureus*, i.e. 1 *mM* and 3 *mM* respectively were selected for calculating their minimum bactericidal concentration (MBC) by broth dilution test. Tippayawat *et al.* (2016) [21] prepared AgNPs by a hydrothermal method using *A Vera* plant extract and tested their antibacterial activity against the pathogenic bacteria, *S Epidermidis* and *P Aeruginosa* by well diffusion assay. It was found that AgNPs possess excellent antibacterial activities which were dependent on their methods and conditions of synthesis.

In the present study, a concentration of 20 µg/mL of AgNPs depicted a clear solution indicating a complete inhibition of bacterial growth when read by unaided eye similar to their positive control, 20 µg ampicillin suspension, with both *E. coli* and *S Aureus*,. The positive control for *E. coli* showed an optical density (OD) of 0.496 whereas that of *S. aureus* showed an OD of 0.508. An OD of 0.046 was observed with 1 *mM* AgNPs against *E Coli* and an OD of 0.050 with 3 *mM* AgNPs against *S Aureus* both at the concentration of 20 µg/mL. These results showed that the MIC of 1 *mM* and 3 *mM* AgNPs against *E. coli* and *S. aureus* respectively were 20 µg/mL. No bacterial growth could be detected in both the samples when sub-cultured on nutrient agar plates which indicated that the MBC of 1 *mM* AgNPs for *E. coli* and that of 3 *mM* AgNPs for *S. aureus* was 20 µg/mL. The methodology followed and the results obtained were compatible with the many previous reports (Ibrahim (2015) [9]; Gomathi *et al.* (2017) [7]; Loo *et al.* (2018) [13], Choi *et al.* (2021) [4] who demonstrated that the green synthesised AgNPs possess excellent antibacterial activity against representative bacterial and yeast pathogens.

Nanoparticles have been in lime-light for the past few decades as an alternative to antibiotics. Many nanoparticle coated implant devices and medical devices are already in use that can prevent bacterial infection and augment wound healing. Nanocarrier-based vaccines against bacterial infections is one of the frontier areas in focus these days as many of the properties of these particles are believed to protect the antigen from degradation as well as bring about targeted delivery of the antigen. There are many limitations in the current research on nanoparticles including the lack of clarity about their mechanism of action as well as the dearth of standards to study the mechanisms of different types of nanoparticles. In spite of their drawbacks, nanoparticles promise a better strategy to curb the menace of the multidrug resistant organisms that inevitably endanger the human and animal populations alike.

Conclusion

Chronic bacterial infections in humans and animals often tend

to be grave since the causative pathogens display resistance to multiple antibiotics that are commonly in use. The widespread use of antibiotics has led to the development of the so called superbugs or super-bacteria that have acquired resistance against almost all the routinely used antibacterial drugs. Nanoparticles offer an alternative strategy to deal with such persistent bacterial infections. In the present study the green synthesized nanoparticles formed of 1 mM $AgNO_3$ promises to be more effective against Gram negative bacteria while those made of 3 mM $AgNO_3$ are more against Gram positive bacteria.

References

1. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, *et al.* Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids. Surf. B: Bio interfaces. 2003;28:313-318.
2. Badwaik H, Singh MK, Thakur D, Giri TK, Tripathi DK. The botany, chemistry, pharmacological and therapeutic application of *Oxalis corniculata* Linn - A review. International Journal of Phytomedicine. 2011 Jan 1;3(1):1-8.
3. Clinical and Laboratory Standards Institute. Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—10th Edition. CLSI Document M07-A10, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania; c2015.
4. Choi JS, Jung HC, Baek YJ, Kim BY, Lee MW, Kim HD, *et al.* Antibacterial activity of green-synthesized silver nanoparticles using Areca catechu extract against antibiotic-resistant bacteria. Nanomaterials (Basel). 2021 Jan 14;11(1):205.
5. Dubey M, Bhadauria S, Kushwah BS. Green synthesis of nanosilver particles from extract of *Eucalyptus hybrid* (Safeda) leaf. Dig. J Nanomater. Bio structures. 2009;4:537-543.
6. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethno medicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine. 2006 Dec;6(1):01-07.
7. Gomathi M, Rajkumar PV, Prakasam A, Ravichandran K. Green synthesis of silver nanoparticles using *Datura stramonium* leaf extract and assessment of their antibacterial activity. Resource - Efficient Technologies. 2017 Sep 1 ;3:280-284.
8. Harbone JB. *Phytochemical methods:* A Guide to modern techniques of plant analysis (3rd Ed.), Springer (India) private limited, India; c1991. p 302.
9. Ibrahim HM. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative micro-organisms. Journal of Radiation Research and Applied Sciences. 2015 Jul 1;8:265-275.
10. Jyoti K, Baunthiyal M, Singh A. Characterization of silver nanoparticles synthesized using Urtica dioica Linn. leaves and their synergistic effects with antibiotics. Journal of Radiation Research and Applied Sciences. 2016;9(3):217-227.
11. Kumar A, Chaudhary P, Verma NK, Kumar P. TLC based phytochemical and antioxidant analysis of *Oxalis corniculata* Linn. Int. Res. J. Pharm. App. Sci. 2006;3:6-12.
12. Logeswari P, Silambarasan S, Abraham J. Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties. Scientia Iranica. 2013 Jun 1;20(3):1049-1054.
13. Loo YY, Rukayadi Y, Nor-Khaizura MA, Kuan CH, Chieng BW, Nishibuchi M, *et al*. *In vitro* antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Front. Microbiol. 2018;16(9):1555.
14. Moosa AA, Ridha AM, Allawi MH. Green synthesis of silver nanoparticles using spent tea leaves extract with atomic force microscopy. International Journal of Current Engineering and Technology. 2015;5(5):3233-3241.
15. Packiyam JE, Uthappa BBC. Synthesis of silver nanoparticles from *Cynodon dactylon* leaf extract. Int. J Curr. Biotechnology. 2017;5:9-12.
16. Prema P, Raju R. Fabrication and characterization of silver nanoparticle and its potential antibacterial activity. Biotechnol. Bioprocess. Eng. 2009 Dec;14(6):842-847.
17. Raghavendra MP, Satish S, Raveesha KA. Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. My science. 2006;1(1):72-78.
18. Raja S, Ramesh V, Thivaharan V. Green biosynthesis of silver nanoparticles using *Calliandra haematocephala* leaf extract, their antibacterial activity and hydrogen peroxide sensing capability. Arabian journal of chemistry. 2017 Feb 1;10(2):253-261.
19. Saxena A, Tripathi RM, Singh RP. Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial activity. Dig. J. Nanomater. Bios. 2010 Jun 1;5(2):427-432.
20. Salisu IB, Abubakar AS, Said, MA. A Novel Biosynthesis, Characterization and antimicrobial activity of silver nanoparticles using leaves extract of *Aloe vera* Plant. Int. J Sci. Res. 2014;3(6):311-314.
21. Tippayawat P, Phromviyo N, Boueroy P, Chompoosor A. Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. Peer J. 2016 Oct 19;4:e2589.
22. Torresdey JL, Parsons JG, Gomez E, Peralta-Videa J, Troiani HE, Santiago P, *et al*. Formation and growth of Au nanoparticles inside live alfalfa plants. Nano letters. 2002 Apr 10;2(4):397-401.
23. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008 Feb;3(2):163-175.
24. Jaidev LR, Narasimha G. Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. Colloids and surfaces B: Bio interfaces. 2010 Dec 1;81(2):430-3.
25. Logeswari P, Silambarasan S, Abraham J. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society. 2015 May 1;19(3):311-7.