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Green synthesis of silver nanoparticles using *Caltropis procera* and *Amaranthus ascendens* stem extracts and evaluation of their antimicrobial activity

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ABSTRACT

The importance of metal nanoparticles in various fields has led to the consideration of various methods for their synthesis in the present using physical and chemical techniques. However, both of these techniques are time-consuming, costly, and hazardous to the environment. Due to their non-toxic nature and extensive synthesis, biological methods are very advantageous. In the current study, aqueous stem extracts of *Caltropis procera* and *Amaranthusa scendens* were used for synthesis of silver nanoparticles (SNPs) from silver nitrate. The plant extract functions as a capping and reducing agent. The quantitative formation of AgNPs was observed using UV-visible spectroscopy. The reduction of Ag^+ to AgNPs by plant extract capping material was confirmed by Fourier transform infrared spectroscopy. The silver nanoparticles had a strong signal, as indicated by the EDX spectrum. SEM analysis revealed the irregular shape of the bioengineered silver nanoparticles. The synthesis of AgNPs using *C. procera* and *A. ascendens*, with average sizes of 14 nm and 25 nm, respectively, was visible in images taken using a transmission electron microscope. Utilizing the well diffusion method, these biosynthesized silver nanoparticles were evaluated for their ability to inhibit the growth of bacteria and fungi. The AgNPs produced from extracts of *C. procera* and *A. ascendens* exhibited the highest antimicrobial activity against *Bacillus cereus* and *Aspergillus niger*.

Keywords: *Caltropis procera*, *Amaranthus ascendens***,** silver nanoparticles, spectroscopy analyses, antimicrobial activity.

INTRODUCTION

Nanotechnology is a fast-growing field of science due to the increasing interest in nanostructures (Eckhardt *et al*,. 2013). Nanoparticles have new characteristics such as particle shape and size range 1–100 nm as well as they have large surface area to volume ratio (Kaviya *et al.*, 2011; Edelstein *et al.*, 2000) which provides them with various unique characteristics that their macroscopic counterparts lack, allowing them to be used in fields such as electronics (Lee *et al.*, 2008), bioremediation (Cropek *et al.*, 2008), biological markers, catalysts (Astruc *et al.*, 2005) and antimicrobials(Lara *et al.*, 2011).

Metal nanoparticles have been widely used in scientific research due to their tremendous usage in various fields of applications (Abbasi *et al.*, 2012, Kayalvizhi *et al.*, 2016). Silver nanoparticles (AgNPs) are the most widely synthesized nanomaterials and have been extensively utilized in a wide range of biomedical applications, including therapy, catalysis, plasmonics, biological sensors, diagnosis, health care products and medication delivery (Kayalvizhi *et al.*, 2016). The pharmaceutical industry and researchers are searching for new antibacterial agents in response to the outbreak of diseases caused by various pathogenic bacteria and the development of resistance to antibiotics. AgNPs are considered as antimicrobial agent nanodrugs because of their therapeutic efficacy at low doses and toxic effect against antibiotic-resistant microbes. It takes advantage of the oligo-dynamic effect of silver on microbes, in which silver ions bind to reactive groups in bacterial cells, precipitating and inactivating them (Jena *et al.*, 2015; Parashar *et al.,* 2009).

Metallic nanoparticles have been synthesized using three different methods: chemical, physical and biological. Although chemical synthesis allows the production of large amounts of nanoparticles in a short time, this process needs the use of capping agents to keep the nanoparticles sizes stable. The chemicals used in this method of nanoparticle synthesis and stabilization are toxic and the process produces non- ecofriendly byproducts. Green synthesis is preferred over traditional synthesis because it is an environmentally friend, non-cost-effective, single-step method that can easily be scaled up for large-scale synthesis and does not require high pressure, temperature, energy or toxic chemicals (Saha *et al*,. 2017). For the synthesis of silver nanoparticles, many researchers have used plant leaf extract, root, stem, bark, leaf, fruit, bud and latex (Mariselvam *et al.*, 2014), fungi (Bhainsa and D'souza, 2006), bacteria (Saifuddin *et al.*, 2009) and enzymes (Willner *et al.*, 2007). Plant extracts are the most effective capping material for stabilizing silver nanoparticles (Ahmed *et al.*, 2016) . Furthermore, plant extracts decreases the cost of microorganism isolation and growth, improving the cost-effectiveness of nanoparticle production (Singhal *et al.*, 2011). Several studies have focused on the biosynthesis of AgNPs using plant extracts such as *Azadirachta indica* (Mankad *et al.*, 2020), *Limonia acidissima* (Annavaram *et al.*, 2015), *Acacia nilotica* (Magesh *et al*,. 2019), *Canarium ovatum* (Arya *et al.*, 2017) and *Matricaria recutita* (Uddin *et al.*, 2017).

Calotropis procera (Asclepiadaceae) is a wild xerophytic shrub that grows in Africa, Asia, and South America. It produces milky white latex with a variety of medicinal properties (Iqbal *et al.*, 2005; Ramos *et al.*, 2007; Saadabi *et al.*, 2012). Latex is found in special branching tubes known as latex tubes (Pandey, 2001; Mahajan and Badgujar, 2008) and has been the subject of interest due to its biological activities such as antibacterial (Ishnava and Thakkar, 2012), antifungal (de Freitas *et al.*, 2011), antiviral (Oliveira *et al.*, 2010), anticandidal (Sehgal *et al.*, 2005) and anticarcinogenic (Silva *et al.,* 2010; Samy *et al.*, 2012) activity. Rubber represents more than 80% of the dry mass of crude latex, while the remaining 20% is made up of soluble protein fractions such as antioxidant enzymes, cysteine protease with free thiol group and tryptophan (Pal and Sinha, 1980; Freitas *et al.* 2007).

Amaranthus is a genus in the Caryophyllales order, family Amaranthaceae, and subfamily Amaranthoideae (Sauer, 1967). *Amaranthus* extracts from all plant parts appear to have medicinal properties, so recent research has focused on identifying therapeutic elements of *Amaranthus* from various bio parts. Most *Amaranthus* species have high antioxidant activity as well as anti-inflammatory properties, which has sparked interest in studying their nutraceutical and therapeutic potential as functional foods. Active substances such as alkaloids, flavonoids, glycosides, phenolic acids, steroids, saponins, amino acids, vitamins, minerals, terpenoids, lipids, betaine, catechuic tannins, and carotenoids have been found in aerial sections of several *Amaranthus* species. (Akubugwo *et al.*, 2007; Clemente and Desai, 2011; Nana *et al.*, 2012; Sharma *et al.*, 2012; Ahmed *et al.* 2016).

The present work aims to reliable, simple, reproducible, and ecofriendly green approach for synthesis of Ag nanoparticles using aqueous stem extract of *C. procera* and *A. ascendens* and test the antimicrobial activity of the produced nanoparticles against some selected bacterial and fungal isolates.

MATERIALS AND METHODS

1. Collection and preparation of plant material

Fresh stem of medicinal plant of *Caltropis procera* and *Amaranthus ascendens* were collected from greenhouse Minia University. They were washed three times with tap water then distilled water and dried for 10 days by using a hot air oven at 40^0C. The dried stems were grinded into a fine powder. In 250 mL Erlenmeyer flasks, five grams of stem powder were mixed with 50 mL of distilled water. The mixture was boiled for 15 min. The extract was filtered through muslin cloth and then centrifuged at 10,000 rpm for 10 min. The filtered extract was kept at 4°C in the refrigerator for further use (Escarcega-Gonzalez *et al.* 2018).

2. Titrimetric analysis

A potassium permanganate back-titration method was used to study the reducing properties of the various stock solutions quantitatively. Three milliliters of extract were acidified with 2 mL of sulfuric acid (3N). The mixture was then oxidized with an excess solution of potassium permanganate (0.1N), heated at 80^0C and then the unreacted permanganate was titrated against oxalic acid solutions (0.2N). When changing color from violet to pink, the endpoint was determined and faded into colorless. The concentration of reducing agents in different samples was calculated using the volume and normality of the permanganate solution used for the oxidation of the samples and reported as mg of $KMnO_4$ per mg of the extract (Ramezani *et al.*, 2008).

3. Preparation of 3mM Silver Nitrate (AgNO3) Solution

Silver Nitrate ($AgNO_3$) was purchased from Sigma-Aldrich (purity 99%). To prepare a 3 mM aqueous solution of $AgNO_3$, 0.0509 g of $AgNO_3$was dissolved in 100 ml distilled water and stored in an amber-colored bottle.

4. Green Synthesis plant Silver Nanoparticles

Ten milliliters of extract were mixed with 90 ml of silver nitrate solution and the pH was adjusted to 10 using 0.1 N NaOH. To avoid photo-activation of silver nitrate, the mixture was incubated in the dark at room temperature for 72 h. The color changed from colorless to brown that confirmed the reduction of Ag^+ to Ag^0. The pellet was washed with distilled water 3 times and centrifuged at 10,000 rpm for 15 min for isolation. The silver nanoparticles were collected and dried in a hot air oven at 50 °C (Ahmed *et al.*, 2016).

5. Characterization of Silver Nanoparticles

The synthesized silver nanoparticles were characterized using the following techniques:

a. UV-VISIBLE Spectroscopy

Detection of silver nanoparticles was done using UV–visible spectroscopy. Using distilled water as a reference, a small aliquot of sample was placed in a quartz cuvette and scanned at wavelengths between 200 and 800 nanometers. UV Visible spectra were done using a Shimadzu UV- 2600 spectrophotometer (Singh *et al.*, 2014).

b. Energy-Dispersive X-ray (EDX) Analysis

The detection and confirmation of elemental silver was done using EDX analysis. A small amount of the sample was drop coated onto carbon film and the composition of the synthesized nanoparticles was determined (El-Agamy, 2014).

c. FTIR (Fourier Transform Infra-Red)

In the present investigation, FTIR spectroscopy was carried out to help identify the chemical composition and nano silver particles of the test samples. A portion (1-2 mg) of test samples was mixed with 100-mg portion of Spec Pure dry KBr and ground in an agate mortar. The resulting powder was placed in a die and compressed into a thin disk (1 cm in diameter). The spectra were taken from the resulting disk over the frequency range at 4000-400 cm-1 at the resolution of 4 cm-1 by means of a Genesis-II Fourier-transform infrared spectrometer (Mattson/U.S. A) (Deepa *et al.*, 2013).

d. SEM (Scanning Electron Microscope) analysis

A scanning electron microscope (JEOL JSM-IT200) was used to evaluate the morphology of the samples containing AgNPs of *C. procera* and *A. ascendens* by preparing thin films of the sample on a carbon coated copper grid (Saravana Kumar *et al.*, 2015).

e. Transmission Electron Microscopy (TEM).

Transmission electron microscopy (TEM) was performed with JEOL JEM-100CX II. This measurement was important for determining the particles size and shape. The samples were evenly distributed and supported on a copper grid before being dried at room temperature (Devi and Joshi, 2015).

6. Determination of the Antimicrobial Activity of AgNPs

Using the well diffusion method, the antibacterial assays of biosynthesized AgNPs were determined against *E. coli, Pseudomonas aeruginosa* (Gram negative bacteria), *Staphylococcus aureus* and *Bacillus cereus* (Gram positive bacteria). L.B (Luria Broth) and L.A (Luria Agar) media were prepared. Solid plates of L.A were prepared. Suspension of tested isolates was prepared L.B medium.350 µl of suspension were spread on the solid LB plates. Wells of 10 mm were made in the solid plates and 60 µl of silver nanoparticle suspension was added to wells. Plates were incubated for 24 to 48 h at 37°C. Three replicates were prepared for each treatment. Plant extracts and $AgNO_3$ solution were used as controls. Antifungal activity was tested against the following fungal species: *Fusarium oxysporum, Rhizopus sp., Aspergillus niger and Aspergillus flavus.* Potato dextrose agar medium was poured into Petri plates and then fungal suspension was inoculated into each plate. After solidification, 60 µl of AgNPs solution was added to each well and incubated for 5 days at 28°C. The inhibitory zone was measured. Plant extracts and $AgNO_3$solution were used as controls.

RESULTS AND DISCUSSION

1. Screening the reducing potential of plant extracts

One of the most popular techniques for creating silver colloids is chemical reduction of the aqueous solution of silver nitrate ($AgNO_3$). In this experiment, an oxidation-reduction titrimetric assay with $KMnO_4$ was used to evaluate the reducing properties of various extracts. *A. ascendens* extract showed the highest reducing capacity followed by *C. procera*. Aqueous extract of *A. ascendens* showed higher reducing potential than that of *A. indica* (positive control), but that for *C. procera* was less than control. However, extracts for both plants showed higher optical density (at around 440 nm) (Table 1) than control plant using UV/vis spectrophotometer. Both plants were selected for further experimentation for AgNPs synthesis.

2. Color change and UV–Visible spectra analysis

Within 72 h of incubation at room temperature, the synthesized AgNPs shows dark brown color (Fig. 1), and the UV–Visible absorption spectrum of AgNPs is shown in Table (1) and Figure (2). The maximum absorption to the surface plasmon resonance of the formed AgNPs was observed at peaks 440 and 442 nm from *C. procera* and *A. ascendens*, respectively. The excitation of color change due to silver nanoparticles synthesis was reported by Narayanan and Sakthivel (2008) and Song *et al.* (2009). This was similar to the results from the bioreduction of silver nanoparticles with *Spirulina platensis*, which revealed the presence of an SPR silver band at 400–480 nm (Kasthuri *et al.*, 2009; Narayanan and Sakthivel, 2008),Geranium leaf extract (Lavhale and Mishra, 2007) aqueous extract of Areca nut and pomegranate peel extract (Mathur *et al.*, 2014).

The reaction of mixture of *C. procera* and *A. ascendens* bark extract and $AgNO_3$ was subjected to different pH using diluted NaOH solutions. At basic pH, AgNPs was formed faster than at acidic conditions. At higher pH, larger number of functional groups, available for silver binding, is binding to greater number of silver nanoparticles, resulting in a larger number of nanoparticles with smaller diameters. At room temperature, the optimal pH of *C. procera* and *A. ascendens* bark extract was 10.0. AgNPs of *C. procera* and *A. ascendens* showed a sharp SPR bands at wavelengths of 420 and 406 nm, respectively (Fig. 2).

3. EDX Analysis

The EDX analysis revealed both the qualitative and quantitative status of the nanoparticle's constituents (Dada *et al.*, 2014; Dada *et al.*, 2016). Figure (3) reveals the chemical composition of AgNPs synthesized using *C. procera* extract. The EDX elemental percentage composition of AgNPs synthesized with *C. procera* stem extract revealed the highest proportion of silver through weight (57.99%) in nanoparticles, followed by carbon (23.13%) and oxygen (16.31%). The EDX profile revealed a strong silver signal together with a weak oxygen peak, which could be from biomolecules bound on the surface of AgNPs, indicating that silver ions were reduced to elemental silver. Other EDX peaks for Cl and Si were determined, indicating that they are mixed with precipitates present in the plant extract. The presence of metallic silver was indicated by the sharp peak at 3 keV. The presence of other phytochemical elements from the EDX spectrum, which may arise from the *C. procera* extract serving as the capping agents, is one of the advantages of green synthesis. This was reported by other researchers (Das *et al.*, 2011; Prathna *et al.*, 2011; Babu and Prabu, 2011; Singh *et al.*, 2013; Banerjee *et al.*, 2014; Logeswari *et al.*, 2015; Ramesh *et al.*, 2015; Oluwaniyi *et al.*, 2016; Ahmed *et al.*, 2016).

Figure (4) shows the EDX spectra of silver nanoparticles of *A. ascendens.* According to EDX spectra, weight percentages of silver, carbon, and oxygen were measured as 35.93, 39.24 and 13.50%, respectively, in silver nanoparticles reduced by *A. ascendens* stem extract. The high carbon content corresponds to the carbon-coated copper grid that was used to mount nanoparticles.

4. FTIR (Fourier Transform Infra-Red)

The obtained FTIR spectra for *C. procera* and *A. ascendens* are shown in Figure (5). The corresponding IR spectrum of *A. ascendens* (Fig. 5) monitors absorption bands assignable to Caroboxlate and amids. Furthermore, the spectrum of *A. ascendens* samples displays bands due to the extract and silver nitrate (at 1384 cm^{-1}) (due to incomplete washing). Correspondingly, the FTIR spectrum of *C.*

procera is shown (Fig 5) to be void of any detectable absorption, thus confirming the reduction of all of the silver nitrate species into nanoparticle silver metal (both plant spectra showed flat pattern due to the presence of silver metal nanoparticles and this agrees with the high concentration of them in the EDX spectra (Figs. 3 & 4).

5. SEM analysis

Scanning electron microscopy was used to examine the surface morphology and topography of the AgNPs (Fig. 6). When different stem extracts were used as reducing and capping agents, AgNPs of various shapes were obtained. *C. procera* and *A. ascendens* extracts formed irregular-shaped AgNPs. The reason for the synthesis of silver nanoparticles with plant extract is due to interactions with active groups such as hydrogen bonds and electrostatic interactions between the bio-organic capping molecules (Priya *et al.,* 2011). The presence of a weak capping agent, which moderately stabilized the nanoparticles, caused agglomeration of the particles in the majority of cases (Nethra and Renganathan, 2012).

6. TEM Analysis

The TEM images show the morphology and size of the particles. As shown in TEM image (Fig. 7.A), the silver nanoparticles obtained by reducing Ag^+ with the *C. procera* stem extract were predominantly spherical in shape, with sizes ranging from 1nm to 32nm, and the average mean size of AgNPs was 14nm. The majority of the Ag nanoparticles using *A. ascendens* stem extract were spherical. Their average particle size was estimated to be 25 nm, with particle sizes ranging from 8 to 42 nm (Fig. 7.B).

7. Antimicrobial Activity of AgNPs

The antimicrobial activity of silver nanoparticles synthesized by plant extracts were tested against some pathogenic bacterial isolates such as *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* and against some pathogenic fungi; *F. oxysporum, Rhizopus sp, A. niger* and *A. flavus* using well diffusion method. The diameter of inhibition zone (mm) around each well with silver nanoparticles solutions is represented in Table (2) and Figures (8 & 9). The the highest antibacterial activity of AgNPs of *C. procera* was found against *B. cereus* followed by *P. aeuginosa* and *E. coli* species and antifungal activity was observed against *A. niger*. On the other hand, *A. ascendens* showed the highest antibacterial activity against *B. cereus* followed by *P. aeuginosa* and *E.coli* and antifungal activity against *A. niger* and *A. flavus* followed by *Rhizopus sp*.

The AgNPs for both plants showed stronger antibacterial activity against gram positive bacteria than gram negative ones and this may be due to the complicated structure of their cell wall. The AgNPs showed high antimicrobial property due to their extremely large surface area, which provides better contact with microbial cells and their ability to attach to the cellular membrane and penetrated inside the bacterial cells. Also, AgNPs interact with phosphorus containing proteins in the cell membrane and with phosphorus present in the DNA which leads to denaturation of part cellular membrane and cellular DNA. Besides, AgNPs preferably attached to the respiratory chains and cause cell death (Sondi and Salopek-Sondi, 2004; Morones *et al.*, 2005). The reason of fungal death could be explained by the findings of Farrag and Mohamed (2016) and Van Long *et al.* (2016) where the AgNPs may accumulate in the cytoplasmic membrane of fungi causing increase in the permeability of fungal cell and causing fungal death.

Conclusion

AgNPs were synthesized from *Caltropis procera* and *Amaranthus ascendens* stem extract as reducing agent. They were characterized by different spectroscopic analyses. According to SEM studies, *C. procera* and *A. ascendens* stem extract synthesized AgNPs with irregular shapes with an EDX potential of 3 keV and a silver percentage of 57.99% and 35.93%, respectively. The AgNPs obtained from these two plant species varied in size due to the differences in their properties. The AgNPs obtained from a stem extract of *C. procera* were smaller in size than those obtained from a stem extract of *A. ascendens*. The synthesized AgNPs showed strong antimicrobial activity against some microorganisms like *B. cereus* and *A. niger*. Because of their larger surface area to volume ratio, nanoparticles provide more chemical, catalytic, physical, and thermal activities than conventional antibiotics. The present results could be useful in the fields of health and environment.

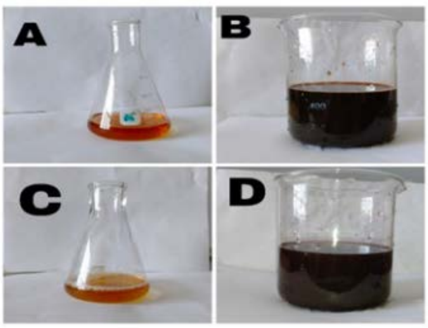
Table 1: The reducing ability of some medicinal plant extracts and UV/Vis characteristics of the silver nitrate solution treated with these extracts.

Plant name	Spectrum characteristics		Amount of $KMnO_4$ (mg) used for oxidation of dried plant extracts
	λmax (nm)	Optical density	
C. procera	440	1.21	1.62
A. ascendens	442	1.2	2.62
***A. indica* (positive control)**	440	0.533	1.79

Table 2 :Antimicrobial activity of *C. procera* and *A. ascendens* medicinal plants

Bacterial species	Inhibition zone (mm)				
	AgNPs		Plant extract		$AgNO_3$(3mM)
	C. procera	*A. ascendens*	*C. procera*	*A. ascendens*	
P. aeruginosa	19	16	ne	ne	20
E. coli	12	12	ne	ne	ne
B. cereus	29	22	ne	ne	20
S. aureus	ne	ne	ne	ne	16
Fungal species					
A. flavus	ne	15	ne	ne	15
A. niger	14	15	ne	ne	ne
F. oxysporum	ne	ne	ne	ne	17
***Rhizopus*.sp**	ne	14	ne	ne	19

Note: "ne" indicates no effect



Fig, 1. Color change of plant extracts after addition of silver nitrate: (A*) C. procera* stem extract, (B) AgNPs synthesized using extract of *C. procera* at pH=10 after incubation, (C) *A. ascendens* stem extract, (D) AgNPs synthesized using extract *A. ascendens* at pH 10 after incubation.

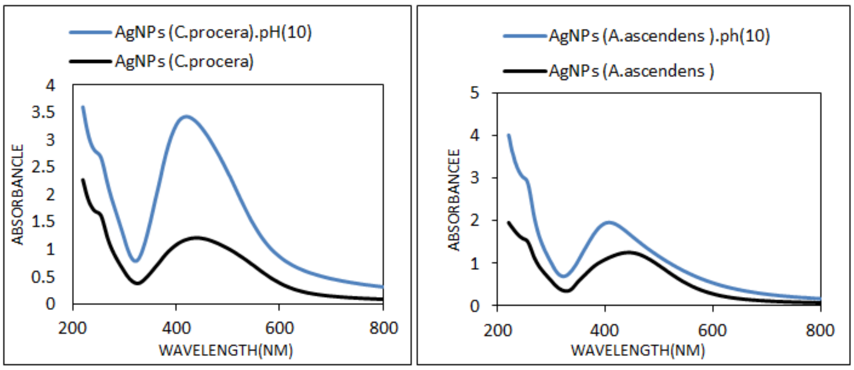


Fig. 2. UV–vis absorption spectrum of silver nanoparticles.

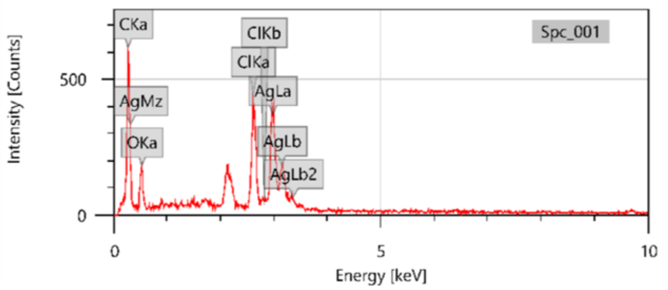


Fig. 2.EDX Spectrum of silver nanoparticles using stem extract of *C. procera.*

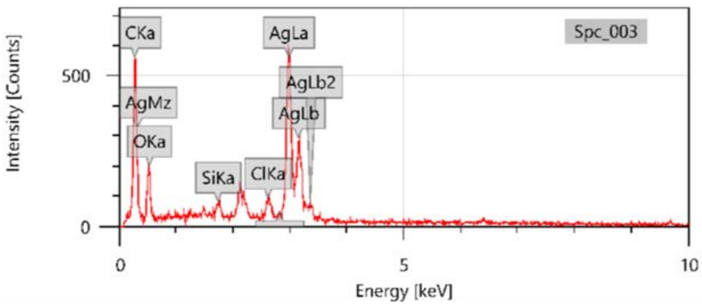


Fig. 3. EDX Spectrum of silver nanoparticles using stem extract of *A. ascendens*.

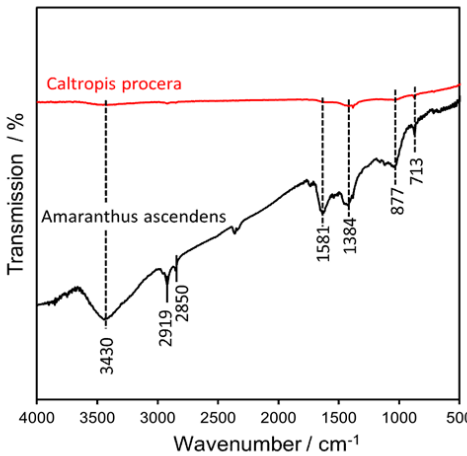


Fig. 4.Fourier transform infrared spectroscopy (FTIR) analysis of silver nanoparticles using stem extract for *C. procera*. and *A. ascendens.*

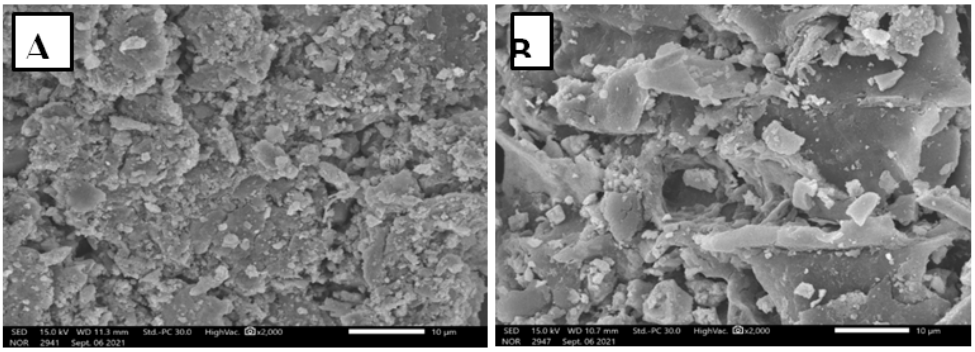


Fig. 5: SEM images of silver nanoparticles using stem extract: (A) *C. procera,* (B) *A. ascendens.*

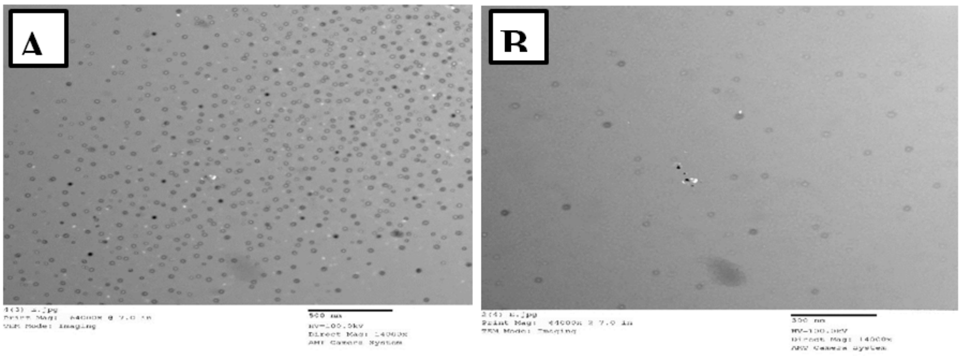


Fig. 6. TEM images of silver nanoparticles using stem extract: (A) *C. procera,* (B) *A. ascendens.*

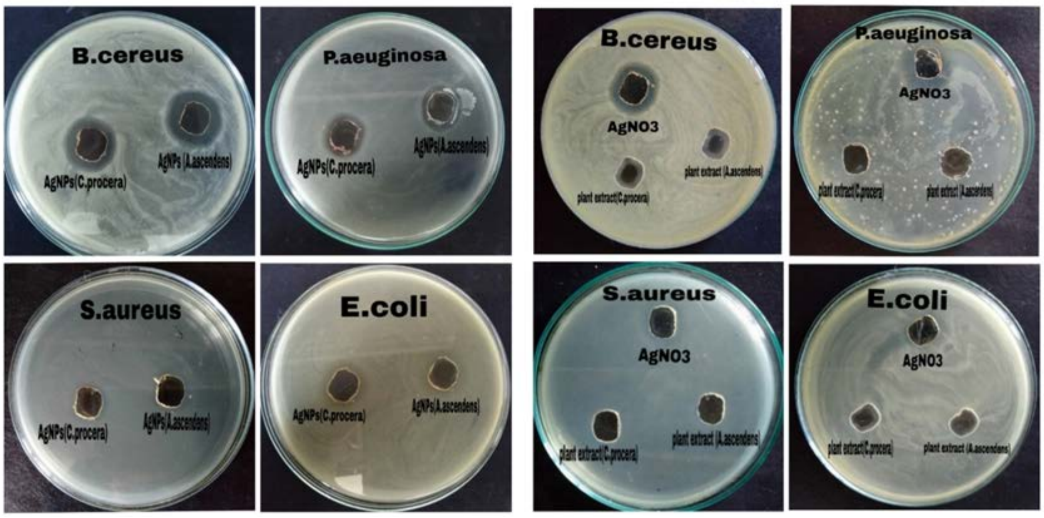


Fig. 8. Effect of silver nanoparticles synthesized by stem extract of *C. procera* and *A. ascendens* on *B. cereus, P. aeruginosa, E. coli* and *S. aureus*.

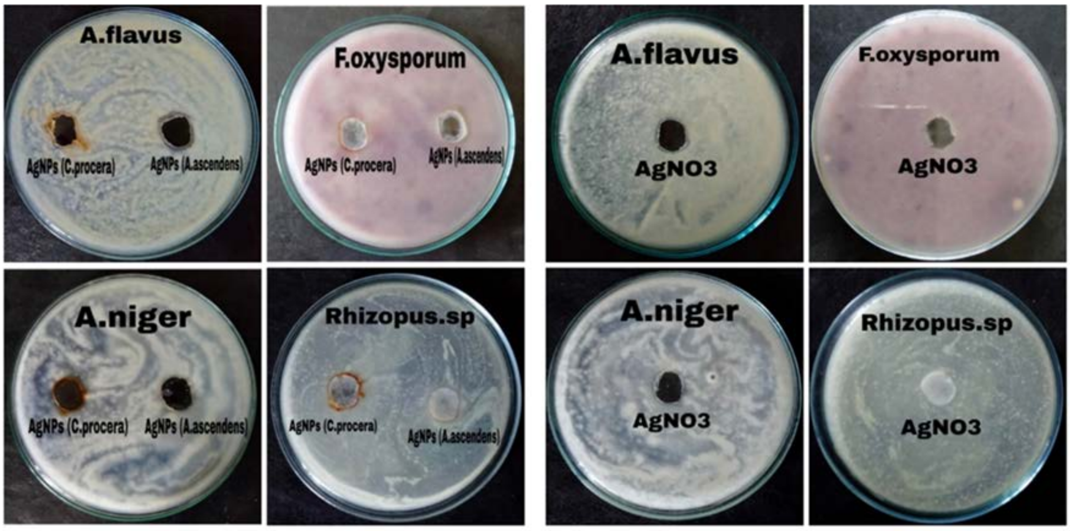


Fig. 9. Effect of silver nanoparticles synthesized by stem extract of *C. procera* and *A. ascendens* on *A. flavus* , *A. niger* , *F. oxysporum* and *Rhizopus sp.*

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التخليق الأخضر لجزيئات الفضة النانومترية باستخدام مستخلص السيقان لنبات *Caltropis procera* و نبات *Amaranthus ascendens* مع تقييم نشاطها المضاد للميكروبات

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المستخلص

لأهمية الجزيئات النانومترية فى الوقت الحاضر فى مجالات مختلفة تطلبت الحاجة الى طرق لتخليقها. من هذه الطرق تشمل الطرق الفيزيائية و الكيميائية، و لكن كلاهما مستنزف للوقت ومكلف وضار للبيئة. و لميزتها وعدم ضررها ووفرة انتاجها تعتبر الطرق البيولوجية ذات مميزات اكثر. فى الدراسة الحالية تم بنجاح تخليق جزيئات الفضة النانومترية باستخدام المستخلص المائى لسيقان نبات *Caltropis* procera و نبات *Amaranthus ascendens* من محلول نترات الفضة، حيث استخدم المستخلص كعامل مختزل. تم تقدير كمية الجزيئات النانومترية باستخدام مطياف الاشعة فوق البنفسجية. تم كذلك تأكيد تكون الجزيئات باستخدام مطياف الاشعة تحت الحمراء، و قد اظهرت الجزيئات اشارة قوية باستخدام مطياف اشعة اكس. باستخدام الميكروسكوب الالكترونى الماسح ظهرت أشكال غير منتظمة للجزيئات النانومترية لكلا النباتين وباستخدام الميكروسكوب الالكترونى النافذ كان متوسط حجم الجزيئات ١٤ ، ٢٥ نانوميتر لنباتى .C procera و *A. ascendens* على التوالى. و باستخدام طريقة الثقوب فى الاجار تم اختبار قدرة الجزيئات المخلقة على تثبيط نمو البكتريا والفطريات، حيث كان للجزيئات المخلقة بواسطة النباتين نشاطا قويا ضد *Bacillus cereus* وكذلك *Aspergillus niger* .