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Green synthesis of silver nanoparticles from ethanol and ethyl acetate extracts of brachypterum scandens for antimicrobial applications

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Due to their environmentally friendly manufacturing process, silver nanoparticles (AgNPs) have attracted a lot of attention from chemists and researchers in recent years. The antibacterial activity of the synthesized AgNPs was thoroughly analyzed and characterized. For the purpose of characterization, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR) were utilized. The ethanol extract's UV analysis wavelengths were identified as 428 nm and 414 nm, respectively. The produced AgNPs had significantly greater antibacterial activity against a variety of bacteria, including E. coli, B. subtilis, S. aureus, K. pneumoniae, when compared to $AgNO_3$ and untreated extracts.

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1. Introduction

The development of environmentally friendly methods for the synthesis of nanoparticles is an essential component of nanotechnology (Raveendran et al., 2006; Armendariz and others, 2002). Throughout the course of recent many years, the area of nanotechnology has seen critical development attributable to the assorted utilizations of engineered nanomaterials in material science, science, science, and medication (Melody and Kim, 2008).Several strategies exist for the creation of silver nanoparticles, remembering synthetic decrease of silver particles for watery arrangements regardless of balancing out specialists (Liz-Marzan and Lado-Tourino, 1996), warm decay in natural solvents (Esumiet al., 1990), photoreduction and chemical reduction in reverse micelles Sun and Co., 2001), and chemical reduction of radiation (Henglein, 1993; 1998; 2001).The greater part of customary nanoparticle combination techniques include the utilization of unsafe and harmful synthetic compounds, which posture dangers to both the climate and human wellbeing, as well as being exorbitant. However, eco-friendly reducing and capping agents and environmentally friendly plant extracts have enabled the development of green nanoparticle production methods. Plants and microorganisms are currently utilized as sources for the production of nanoparticles. According to Huang et al., the biosynthesis of nanoparticles using plants is a quick, cost-effective, and environmentally friendly procedure that only requires one step. According to Kumar and Yadav (2009), plant-mediated nanoparticle synthesis is preferred over other methods of synthesis because it is cost-effective, safe for the environment, and suitable for human therapeutic applications.

Silver nanoparticles have numerous biological applications and are widely used in a variety of nanosystems. Silver nanoparticles have superior medicinal and non-medical properties and applications when compared to nanoparticles made from other metals (Ge et al., 2014). Due to its low toxicity, the environmentally friendly method of producing nanoparticles made from plant and herbal extracts (Ahmed et al., 2016). The synthesis of silver nanoparticles has garnered significant attention due to their multifaceted properties, including catalysis (Shiraishi and

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Toshima, 2000), magnetic and optical polarizability (Shiraishi and Toshima, 2000), electrical conductivity (Chang and Yen, 1995), and antimicrobial activity (Sharverdiet al., 2000). Many secondary metabolites present in plant extracts act as reducing or capping agents during nanoparticle 2007) and surface-enhanced Raman scattering (SERS) 1992).

Utilizing microorganisms, biological methods for the production of nanoparticles (Klaus et al., 1999; 2007 by Konishi and Uruga; Willner et al., 2006), chemicals (Willner et al., 2006), and spores (Vigneshwaran et al., 2007), as well as plants or extracts from plants (Shankar et al., 2004; Chandran and other, 2006; Jae and Beom, 2009; Ahmad and co., 2011; Dubey et al., 2009), have arisen as promising eco-accommodating options in contrast to synthetic and actual techniques. The use of plants or plant parts for nanoparticle combination can some of the time be profitable over other natural strategies by working on the mindboggling techniques expected to keep up with microbial societies (Shankar et al., 2004).

Silver's ability to inhibit microorganisms found in industrial and medical processes has long been recognized (Lok et al., 2007; Dubey and other, 2009). Silver and silver nanoparticles are frequently used in topical ointments in the medical field to treat burns, close wounds, and prevent infections (Ip et al., 2006). In addition, it has been demonstrated that these biologically synthesized nanoparticles have potent activity against a variety of multidrug-resistant human diseases.

The focal plant in this investigation, Brachypterum scandens, is a member of the Fabaceae family. It is described by liana development, arriving at levels of roughly 30 meters (1.5-). The plant has leaves that are 9-11(-19)-foliate and juvenile stems that are puberulent and glabrescent. The sessile fruit has a wing width of 1 to 5 mm and a length of 5.5-8 cm, a diameter of 9-13 mm, typically containing 1 to 5 seeds. The reniform, dark brown, 9-10 mm-long seeds are reniform. The axial racemose inflorescences have a length of 25–45 cm. In biomedical research, extensive screening of plants for novel bioactive compounds is common. The phytochemical components of B. scandens were qualitatively and quantitatively examined in this study using a crude extract.

2. Materials and methods

2.1. Collection of plant materials

Fresh root samples of B. scandens were selected at random from the Veeramalai hills in Pochampalli, Krishnagiri, Tamil Nadu. The samples were thoroughly washed with running water, air-dried and subsequently homogenized into a fine powder. The powdered samples were then stored in airtight containers in a refrigerator.

2.2. Preparation of extracts

Soxhlet extraction was employed to prepare the crude sample. A uniform 20 grams of powdered sample material was packed into a thimble, with 250 ml of ethanol and ethyl acetate used for each extraction. The extraction process was continued for 24 hours or until the solvent in the syphon tube of the extractor became colourless. Subsequently, the extract was transferred to a beaker and heated on a hot plate at a temperature range of 30 to 40°C until the solvent completely evaporated. The dried extract was then stored in a refrigerator at 4°C until further use.

2.3. Synthesis of silver nano-particles

By diluting 1 mM of silver nitrate into 100 ml of solvent, a solution of 1 mM silver nitrate was made. Plant extract was mixed with 9 ml of the prepared 1 mM silver nitrate solution for each reaction. All through the examination, the fluid root concentrates of ethanol, ethyl acetic acid derivation, and silver nitrate arrangements were used as controls (Smetana et al., 2005). Following the reaction, 200 milliliters of the final solution were centrifuged for 25 minutes at 18,000 rpm. The pellets that were produced were carefully collected and kept at 4°C. In the meantime, the supernatant was heated to between 50 and 95°C. As the temperature of the solution rose, a noticeable color shift was observed.

2.4. Screening of antibacterial activity

The near antibacterial viability of the restorative concentrates and the silver nanoparticles orchestrated from the comparing removes against E. coli, B. subtilis, S. aureus, K. pneumoniae, and E. faecalis was effectively assessed. According to Bauer et al.'s description, the disc diffusion method (1966), was used to evaluate the antibacterial movement. For in vitro bactericidal movement appraisal (directed in Mumbai), Muller Hinton Agar (MHA) got from Greetings media was utilized. MHA plates were ready by pouring 15 ml of liquid medium onto sterile petri dishes. A 0.1% inoculum suspension was evenly swabbed onto the plates and left to dry for an additional five minutes after the plates had been allowed to solidify for five minutes. On sterile discs measuring 6 mm, the extracts were applied at a concentration of 40 mg per disc. Following the arrangement of the stacked circle onto the outer layer of the medium, the concentrates were permitted to diffuse for five minutes and the plates were then hatched for 24 hours at 37°C.

2.5. Characterization of synthesized silver nanoparticles

UV-Vis range examination was led utilizing a Shimadzu UV-noticeable spectrophotometer (UV-1800, Japan). This spectrophotometer can measure wavelengths from 200 to 800 nm and has a resolution of 1 nm. A milliliter of the substance was pipetted into a test tube and analyzed at room temperature for the purpose of analysis. Using a Spectroscatter 201 instrument, dynamic light scattering was used to determine the average size of the synthetically produced silver nanoparticles. FTIR spectra were gotten utilizing the KBr pellet strategy on a Perkin Elmer 4000-400 cm-1 stage. SEM, which operates at an accelerated voltage of 20 kV, was used to examine the surface morphology and particle size. Transmission electron microscopy (TEM) was used to observe the morphology of the silver nanoparticles. The JEOL JEM 2100 HR with EELS, a 200 kV ultra-high-resolution transmission electron microscope, was used for the analysis.

3. Results and discussion

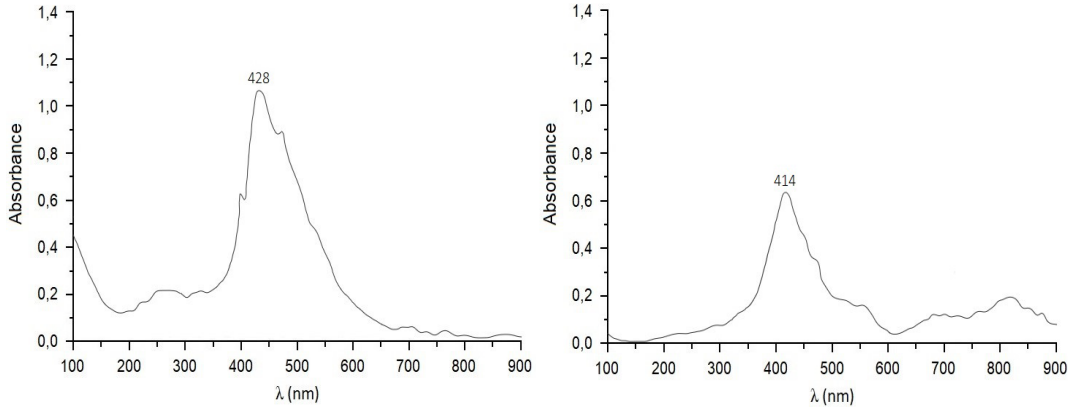
Silver nanoparticles were synthesized using extracts of Brachypterum scandens that were extracted using either ethanol or ethyl acetate. Silver ions can undergo reduction to form silver particles when exposed to plant extracts, often accompanied by a noticeable change in color. This peculiarity is credited to surface plasmon reverberation, giving a dull yellowish-earthy colored tone to the silver nanoparticles in watery arrangement. The production of nanoparticles is a major focus of current nanotechnology, with biosynthesis methods relying on plant extracts. A significant part of nanotechnology includes the advancement of trial conventions roused by organic cycles for nanoparticle amalgamation. The goal of this study is to use medicinal herbs to make silver nanoparticles with powerful antiplasmodial properties.

3.1. Characterization studies

3.1.1. UV analysis

Silver nanoparticles (AgNPs) have a yellowish-brown color in aqueous media because of vibrations caused by surface plasmons (Krishnaraj et al., 2010). When a variety of leaf extracts were added to the aqueous silver nitrate solution, the color gradually changed from a light yellow to a reddish brown, then a colloidal brown, indicating that AgNPs were being produced.

The affirmation of the response finishing between leaf separate and $AgNO_3$ was approved by noticing comparable variety changes as revealed in past examinations (Shukla et al., 2009; 2013 by Namratha and Monica; Lalitha and other, 2013; Singhal and others, 2011; (2011) (Philip and Unni). The UV-vis spectra that were taken 15, 30, 45, 60, and 24 hours after the reaction started are shown in Figure 1A. Figure 1B shows that the surface plasmon resonance of AgNPs resulted in absorption maxima around 428 nm for the sample made with ethanol and 414 nm for the sample made with ethyl acetate in the absorption spectra of AgNPs formed in the reaction medium.



1A - Silver synthesized ethanol *1B - Silver synthesized Ethyl acetate*

Fig. 1. UV Analysis of B. scandens Ethanol and Ethyl acetate Silver synthesized nanoparticles.

Broadening peaks indicating slow reduction rates leading to the formation of polydisperse large nanoparticles were observed in the UV-vis spectra, indicating that the Brachypterum scandens root extract facilitated the fastest bio reduction (Singhal et al., 2011; (2011) (Philip and Unni). Moreover, the UV-vis spectra uncovered that the blend of AgNPs happened quickly inside the initial 15 minutes and that the AgNPs stayed stable in arrangement even 24 hours after the consummation of the response.

3.1.2. FTIR analysis

The results of the FTIR analysis used to characterize the AgNPs produced by the ethanol and ethyl acetate extracts of Brachypterum scandens are shown in Figure 2. The absorption bands at 3473.16 cm^{-1} in the ethanolic extract of B. scandens silver-synthesized nanoparticles indicated stretching of primary and secondary amines and amides; 3287.99 cm^{-1}, which is ROH/PhOH (OH that is hydrogen bonded); 2935.27 cm^{-1}, which is equivalent to stretching R-H; 2413.82 cm^{-1}, which suggests COOH; 1678.47 cm^{-1}, which corresponds to imides, oximes, and 1602.34 cm^{-1}, which is linked to bending and primary and secondary amines and amides; 1105.14 cm-1 and 1013.39 cm^{-1}, which represent the ratio of any functional group-containing O/N; and 874.05 cm^{-1}, which suggests that it is aromatic (a bend out of plane).

These bands, as indicated by Siddiqui et al., (2000) and Huang et al., (2007), represent stretching vibrational bands associated with substances such as flavonoids and terpenoids, suggesting their potential role in effectively capping and stabilizing the produced AgNPs. According to Gilaki (2010), varying factors such as pH, elemental strength, plant sources, duration, incubation temperature, and reaction mixture can result in the production of a variety of distinct nanoparticles. FTIR studies suggest that the bio-reduction of Ag+ ions to silver nanoparticles is facilitated by the reduction and capping materials present in the plant extract. According to Gole et al., (2001), proteins in the extract may bind to silver nanoparticles via either free amino or carboxyl groups.

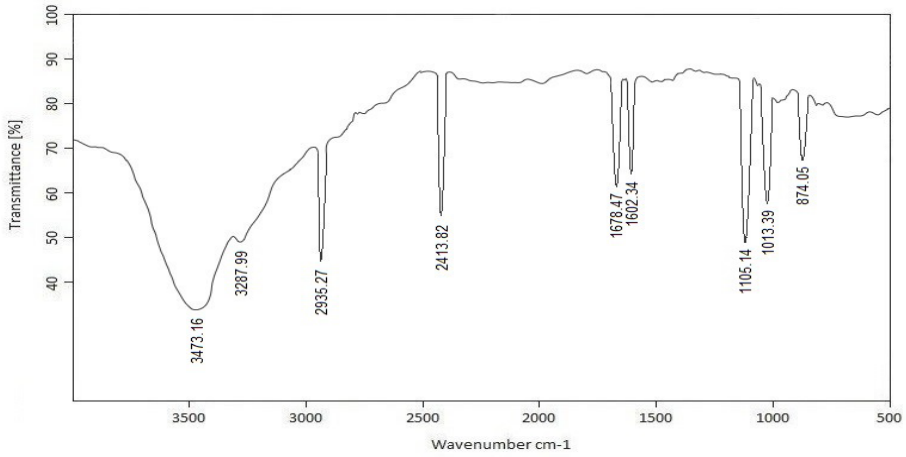


Fig. 2. FTIR Analysis of Ethanolic Silver synthesized nanoparticles of B. scandens.

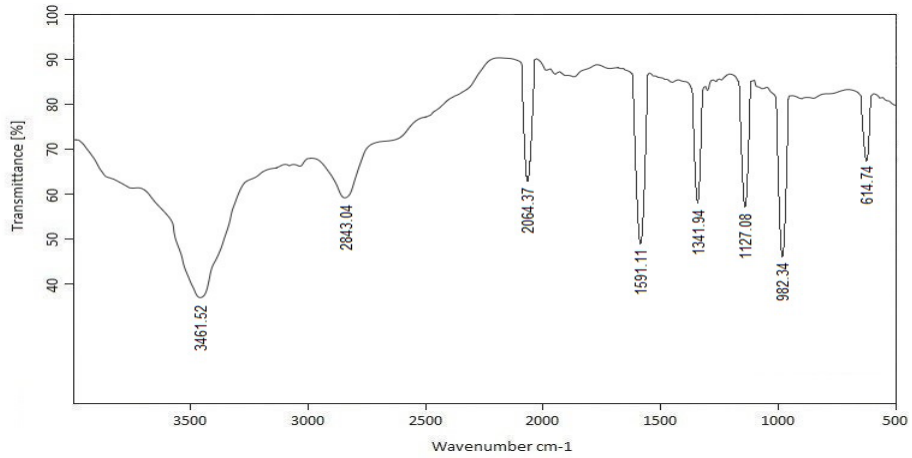


Fig. 3. FTIR analysis of ethyl acetate silver synthesized nanoparticles of B. scandens.

3.1.3. SEM analysis

The AgNPs are depicted in SEM images in Figure 4. The images show that AgNPs of various shapes were produced when root extracts were used as reducing and capping agents. In particular, approximately spherical, triangular, and cuboidal AgNPs were found when Brachypterum scandens extracts were synthesized into ethanol and ethyl acetate extracts, respectively. The observed changes and differences in peak regions identified by the FTIR analysis support the possibility that this variation is due to differences in the amounts and types of capping agents present in the various leaf extracts. A type of electron microscope known as a SEM allows for the visualization of the size and shape of silver nanoparticles by raster-scanning a sample with a high-energy electron beam.

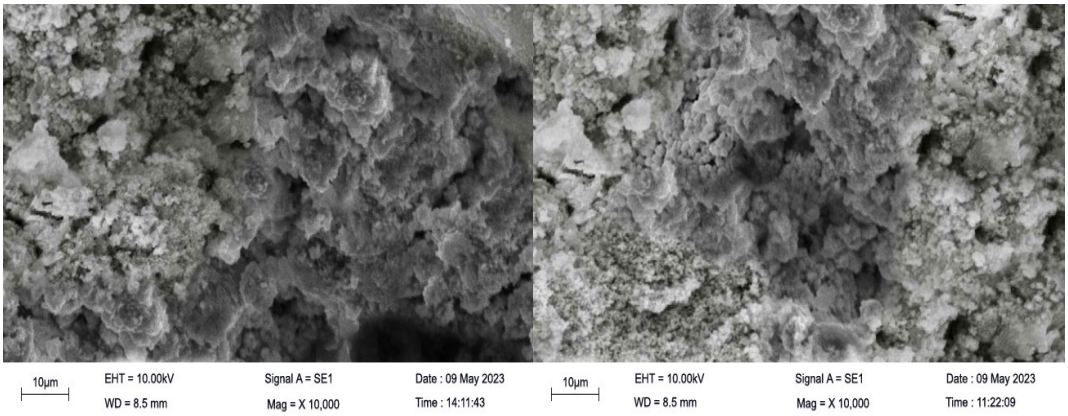


Fig. 4. SEM analysis of B. scandens ethanol and ethyl acetate silver synthesized nanoparticles.

3.1.4. TEM analysis

Figure 5 depicts TEM images produced by the reaction of individual solutions of 1 mM silver nitrate with 5% Brachypterum scandens ethanol and ethyl acetate extracts. Plates (triangles, pentagons, and hexagons) and spheres were also observed, although the majority of the shapes were spherical. This example is reliable with the SEM pictures portrayed in Figure 4. With dimensions of up to 50 nm, the triangles, pentagons, and hexagons are clearly plate for formations.

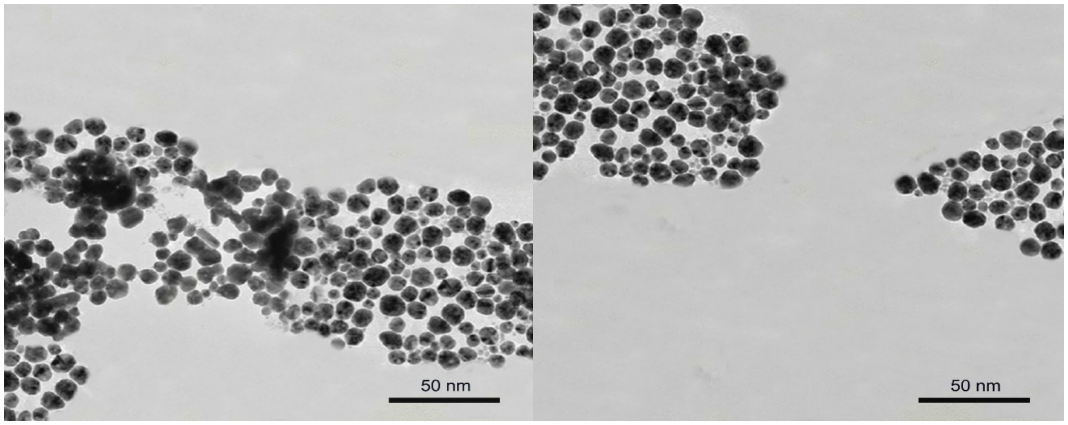


Fig. 5. TEM Analysis of B. scandens Ethanol and Ethyl acetate Silver synthesized nanoparticles.

3.2. Antibacterial activity

The well diffusion method was used to evaluate the antibacterial activity of silver nanoparticles derived from Brachypterum scandens extracts in ethanol and ethyl acetate. Adjusted to a McFarland turbidity of 0.5 (approximately 110-8 CFU/mL), Mueller Hinton agar (MHA, Oxoid, Hampshire, England) was inoculated with bacterial suspensions of the test and control species. Wells with a diameter of 12 mm were made in the agar after 30 minutes at room temperature, and molten MHA was used to seal the bottoms. Antibacterial movement was assessed for both ethanol and ethyl acetic acid derivation concentrates of Brachypterum scandens-orchestrated silver nanoparticles against five organic entities: Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, and Enterococcus faecalis. Analyses showed that nanoparticles made with ethanol and ethyl acetate had lower activity than those made with ethanol. More specifically, the ethanol-synthesized sample was more effective against Bacillus subtilis (16 mm) and Klebsiella pneumoniae (18 mm) (see Figure 6 and Table 1).

Kim et al. conducted research (2007), Rogers et al., (2008), Galdiero and coworkers, 2011), Oves et al., (Bhuyan et al., (2013a), 2015), Aziz and coworkers, 2016), as well as Zhang et

al., 2016) demonstrates that biologically synthesized silver nanoparticles (AgNPs) possess significant antibacterial and antiviral properties, making them promising therapeutic agents. Nanoscience research continues to primarily focus on the search for novel nanoparticles with precise biological, physical, and chemical properties, despite the successful synthesis of numerous nanoparticles using plants and microbes.

Table 1. Antibacterial activity of B. scandens ethanol and ethyl acetate silver synthesized nanoparticles.

Organism	Control	E_{30}	E_{60}	$E.A_{30}$	$E.A_{60}$
Escherichia coli	18	15	18	9	14
Bacillus subtilis	20	16	23	12	18
Staphylococcus aureus	24	15	21	13	19
Klebsiella pneumoniae	23	18	24	14	20
Enterococcus Faecalis	21	14	21	12	19

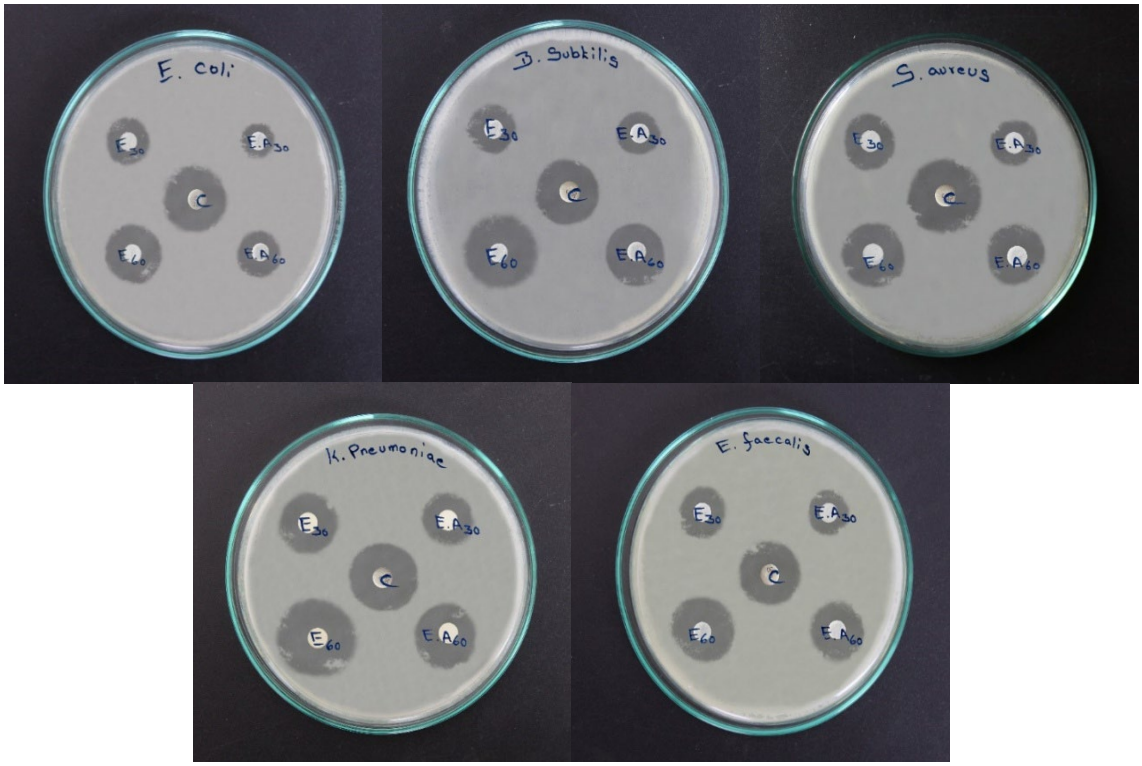


Fig. 6. Antibacterial activity of escherichia coli, bacillus subtilis, staphylococcus aureus, klebsiella pneumonia, enterococcus faecalis were shown to be susceptible to antibacterial action by b. scandens ethanol and ethyl acetate silver synthesized nanoparticles.

According to Savoia (2012), the bioactivity of plant extracts is due to secondary metabolites, paving the way for the potential creation of new antibiotics. Silver nanoparticles created through biosynthetic techniques have shown action against pathogenic microorganisms, albeit the exact components hidden their antibacterial activity are still being scrutinized. Silver ions may accumulate within membranes and penetrate cells, causing membrane damage, or they may interact with proteins and nucleosides through complexes formed between nucleic acids and Ag+ ions. Additionally, denaturation of the DNA molecule could result from interactions between silver ions and purine and pyrimidine base pairs disrupting hydrogen bonds (Calderone and Fonzi, 2001; Dakal and other, 2016).

4. Conclusion

Due to their widespread availability, safety, and variety of metabolites, plants are regarded as ideal candidates for green synthesis with ethanol and ethyl acetate extracts. Silver nanoparticles (AgNPs) stand out for their widespread application across a variety of fields, despite extensive research on antibacterial materials made from various organic and inorganic compounds. However, despite their potential, AgNPs' beneficial applications outside of laboratory settings have not been thoroughly investigated. Despite the fact that their chemical synthesis presents environmental risks, AgNPs are frequently utilized for disease control in plants. As a result, the biosynthesis of AgNPs with biomolecules that are good for the environment is getting more and more attention. For nanoparticle synthesis, plants have an advantage over other biological entities because they don't need to keep microbial cultures, which can take a long time and limit their potential. Using plant extracts to make nanoparticles has a lot of potential for use in the future, and it promises to be a more sustainable and effective way to make AgNPs. In the years to come, this method could have a significant impact on a variety of fields.

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