EVALUATION OF *In-vitro* ANTIBACTERIAL ACTIVITY AGAINST GRAM-NEGATIVE BACTERIA USING SILVER NANOPARTICLES SYNTHESIZED FROM *Dypsis lutescens* LEAF EXTRACT

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ABSTRACT

Recent advances in nanotechnology and the synthesis of nanoparticles through biosynthesis have increased the urge in scientists than for chemical or physical methods. The biosynthesis method is the most significant method than a conventional method because of its eco-friendly, low cost and rapid synthesizing process. The present study describes the antibacterial activity of silver nanoparticles (AgNPs) synthesized from leaf extracts of Dypsis lutescens. The synthesis of AgNPs was confirmed by colour change from light yellow to brown colour. Further, the morphology of the biosynthesized nanoparticles, average size and presence of functional groups were characterized by UV – Visible spectroscopy (UV-Vis), X-Ray diffraction (XRD) and Fourier transform infrared spectroscopy, respectively. The UV spectra results show a strong resonance center and surface of silver nanoparticles at 450 nm. XRD studies revealed that the synthesized AgNPs show crystalline in shape. The FT-IR spectrum described the biological molecules which stabilize and form the silver nanoparticles in the aqueous medium. The average AgNPs size was found to be 31 nm by using the Debye-Scherrer formula. The antimicrobial property of AgNPs was tested against Escherichia coli (MTCC 443) and Vibrio cholerae (MTCC 3906) pathogen, which showed maximum zones of inhibition of 22 mm at a concentration of 100 µL. Therefore, the biosynthesized AgNPs proved to have significant antibacterial activity.

Keywords: *Dypsis lutescens*, silver nanoparticles, XRD, *Escherichia coli*, *Vibrio cholerae*.

1. INTRODUCTION

Over the past few decades, the field of nanotechnology has a strong impact on many scientific applications. Nanotechnology deals with the smaller size particles called nanoparticles which contain tens or hundreds of atoms ranging in size 1 to 100 nm (Madkour, 2019). Nanoparticles (NPs) exhibit specific features such the large surface area to mass ratio, ultra-small size and high reactivity that show a lot of modified properties (Khan et al., 2019; Jamkhande et al., 2019). Extensive research has been undertaken due to the metal nanoparticles involved in various novel applications in catalysis (Astruc, 2020), medical (Rudramurthy and Swamy, 2018), pharmaceutical (Mitchell et al., 2021) and agricultures (Alomar et al., 2020). A wide variety of nanoparticles such as inorganic/metal nanoparticles, polymeric nanoparticles, nanotubes, nanocrystal and solid lipid nanoparticles are available and reported (Begines et al., 2020; Shimizu et al., 2020; Calvino et al., 2020; Paliwal et al., 2020). Recently, lipid nanoparticles have been involved in the COVID-19 vaccine (Anselmo and Miltragotri, 2021). Day by day increasing the demands in different fields, the development of novel metal NPs has great potential and benefits to human health, the environment and industries (Krishnan et al., 2020; Saravanan et al., 2020; Magalhaes-Ghiotto et al., 2021).

Various literature depicts that have many ways to synthesis metal nanoparticles which include physical, chemical and biological methods. A top-down approach is followed in the physical approach for synthesizing nanoparticles in which the material is reduced in size by various physical approaches like electrochemical methods (George et al., 2018). Metallic precursors, stabilizing agents and reducing agents are the main components and energy-consuming in the chemical approach (Jamkhande et al., 2019). The biological method is more effective for the synthesis of nanoparticles with the advantage of fewer chances of failure, eco-friendly, cost-effective and ease of characterization than physical and chemical approaches, as they have posed several stresses on the environment due to their toxic metabolites and stringent techniques (Gour and Jain, 2019; Kanchi and Ahmed, 2018). Green synthesis involves the use of vitamins (Hou et al., 2020), amino acids (Xue et al., 2020), plant extracts (EI Shafey, 2020), enzymes (Ovais et al., 2018), bacteria (Iravani and Varma, 2020), fungus (Halkai et al., 2017), honey (Balasooriya et al., 2017), yeast (Bolbanabad et al., 2020) and algae (Fatima et al., 2020). Among this, plant extract is easy to get and safe to handle where the reduction of metal is rapid and the procedure itself requires no specific conditions, unlike physical and chemical methods. This method of synthesis of nanoparticles appears to be reproducible and the particles produced through this environmentally friendly approach are highly stable (Krishnaraj et al., 2014).

The synthesis of nanoparticles from plant extracts involves three phases: 1) the activation phase during which the reduction of metal ions and nucleation of the reduced metal atoms occur; 2) the growth phase during which the small adjacent nanoparticles spontaneously coalesce into particles of a larger size; and 3) the termination phase determining the final shape of the nanoparticles (Punjabi et al., 2015). The various metabolites accumulated in the plant, including terpenoids, flavonoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins, play an important role in the bioreduction of metal ions, yielding nanoparticles (Makarov et al., 2014). Silver nanoparticles (AgNPs) have paid special attention among other NPs owing to their potential use in biological applications include therapeutics, biomolecular detection and diagnostics, drug delivery, food production, agriculture and waste treatment (Yaqoob et al., 2020). Silver nanoparticles have also been used as antibacterial/antifungal agents in diverse areas like air sanitizer sprays, face masks, wet wipes, vacuum cleaners, etc. (Balasubramaniam et al., 2020; Buzea et al., 2007). Recent studies also revealed that the AgNPs can control several plant diseases and can efficiently interact with various pathogens compare to other molecules (Lamsal et al., 2011). In the present scenario, due to their unique physicochemical properties and various applications, the green synthesis of silver nanoparticles has received considerable attention from young researchers. The plant (flower/leaf/root, etc.) extract for the synthesis of AgNPs has a high stability & antibacterial activity, easy to approach and is safe to handle (Sadeghi and Gholamhoseinpoor, 2015; Kathiravan et al., 2015).

Several green syntheses reports have been reviewed by Mousavi et al. (2018) for the synthesis of AgNPs using plant-extracts such as *Artemisia vulgaris*, *Justicia glauca*, *Ocimum gratissimum*, *Morus alba*, *Nigella sativa*, *Andrographis echioides*, *Alcea rosea*, etc. The variety of family plant extracts involved in the synthesis of silver nanoparticles are given in Table 1. Pirtarighat et al. (2019) studied the green synthesis of AgNPs using *Salvia spinosa* derived from the Lamiaceae family and the NPs proved to have antibacterial activity. Moteriya et al. (2020) evaluated the antimicrobial, cytotoxic and genotoxic activity on the synthesized AgNPs which was derived from *Caesalpinia pulcherrima* belongs to the Fabaceae family. From these different families, Arecacaea has been chosen for the synthesis of silver nanoparticles because of its medical application involves in antibacterial, antimicrobial, and anticancer activity. *Dypsis lutescens* is chosen from the Arecaceae family since the synthesis of silver nanoparticles from this plant has not been researched yet. *Dypsis lutescens* traditionally used in health benefits to removing acetone (nail polish, paints, detergents, adhesives and cleansers), xylene (paints and wooden furniture) and toluene (paints, cosmetics, etc.) which all causes central nervous system disorders, necrosis mainly in children and creating upper respiratory tract diseases (Kumar et al., 2012; Chiduruppa et al., 2018).

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Table 1. Synthesis of silver nanoparticles from a various families of plant extract.

FAMILY	PLANT	APPLICATION	REFERENCE
Lamiaceae	*Salvia spinosa*	Antimicrobial activity against *Bacillus subtilis*, *Bacillus vallismortis*, *Escherichia coli*.	Pirtarighat et al. (2019)
Arecacaea	*Phoenix dactylifera*	Antimicrobial action against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and one yeast fungi.	Zafar and Zafar (2019)
Euphorbiaceae	*Givotia moluccana*	Antimicrobial activity against both gram-positive and gram-negative bacteria	Sana et al. (2018)
Rosaceae	*Rosa brunonii Lindl*	Photocatalytic Characterizations	Bhagat et al. (2019)
Solanaceae	*Solanum lycopersicum*	Antibacterial activity	Santiago et al. (2019)
Fabaceae	*Caesalpinia pulcherrima*	Antimicrobial, Cytotoxic and Genotoxic Potential	Moteriya et al. (2020)
Grossulariaceae	*Ribes nigrum*	Antibacterial, electrochemical, and antioxidant activity	Vorobyova et al. (2020)
Apiaceae	*Eryngium bungei Boiss*	Antibacterial activity against *S. aureus*, *K. pneumonia* and *E. coli* bacteria	Mortazavi-Derazkola et al. (2021)
Asteraceae	*Anthemis atropatana*	Antimicrobial, anticancer and apoptotic effects on the cancer cell line.	Dehghanizade et al. (2018)
Arecacaea*	*Areca catechu*	Used as anticancer and antidiabetic agent.	Shwetha et al. (2020)

* Synthesis of zinc oxide nanoparticles.

Therefore, the present study aims to synthesize silver nanoparticles by a biological method using a leaf extract derived from *Dypsis lutescens* belongs to the Arecacaea family. The characterization of the synthesized nanoparticles was also analyzed by using UV-Visible spectroscopy, X-Ray Diffraction and Fourier Transform InfraRed spectroscopy. Besides, potential antibacterial activity against pathogenic gram-negative bacteria of *Escherichia coli* and Vibrio cholerae was investigated.

2. Materials and methods

2.1 Description of the study species

Dypsis lutescens is commonly known as bamboo palm/butterfly palm/yellow palm/Golden cane palm and the synonyms of *Areca lutescens* and *Chrysalidocarpus lutescens*. *Dypsis* are evergreen palms belonging to Arecaceae family and it grows 6-12 m in height. The fronds are arched 2-3 m long, pinnate with 40-60 pairs of leaflets and panicles of small yellow flowers in summer. It has also grown as an ornamental plant in gardens in tropical and subtropical regions. It can be found easily in many places, available in large quantities throughout the year.

It is widely distributed in regions of South Asia, India, Madagascar, China, Bangladesh, Myanmar, Malaysia, Thailand, Vietnam, and Philippines, etc. The scientific classification of bamboo palm is Kingdom: Plantae, Order Arecales, Family: Arecaceae, Genus: *Dypsis*, Species: *D. lutescens*, Binomial name: *Dypsis lutescens* and species authority is Harmann Wendland (1825).

Dypsis lutescens is one of the best tropical foliage plants, easy to grow and air purifying plants. It is traditionally involved in health benefits through healthy lungs, proper development of children and fetus and strengthens the central nervous system. The constituent Isovitexin which has the source from *Dypsis lutescens* possesses anticancer activity. The leaf extract of the same plant exhibited a suppressive effect on fat accumulation in 3T3-L1 cells and fat absorption in mice (Koyama et al., 2012). The antioxidant and anti-inflammatory properties of *D. lutescens* help in maintaining liver health and functions (El-Ghonemy et al., 2019).

Therefore, the fresh plant leaves of *Dypsis lutescens* have been selected for silver nanoparticles synthesis.

2.2 Biosynthesis of silver nanoparticles

2.2.1. Raw materials

The fresh and healthy leaves of *Dypsis lutescens* were collected from the Sri Venkateswara College of Engineering campus in January 2021 (Sriperumbudur, India). Silver Nitrate ($AgNO_3$>99.0%) of analytical grade was purchased from Sigma-Aldrich (Karnataka, India) and was used without any further purification. All of the solutions and chemicals were prepared in de-ionized water.

2.2. Preparation of leaf extract

Dypsis lutescens leaves were repeatedly washed with fresh water to remove dust and soil particles and then dried to remove moisture content by direct sun rays. Dried leaves were grained with the help of a mixer grinder mechanically to make a fine powder. In 250 mL of the conical flask, 5 g of powdered *Dypsis lutescens* were taken and added 100 mL of distilled water. The aqueous solution was mixed using a magnetic stirrer and then boiled for 10 min using a heating mantle. The leaf extract was filtered by a Whatman (No.1) filter paper and collected the filtrate, stored at 4°C for the biosynthesis of silver nanoparticles.

2.3. Green synthesis of silver nanoparticles

For the synthesis of AgNPs, 1 mM of silver nitrate solution was prepared and stored in an amber bottle. About 50 mL of 1 mM $AgNO_3$ were added dropwise with constant stirring in 5 mL of leaf extract taken in a conical flask. The aqueous solution was incubated at room temperature for 24 h in dark conditions to prevent photochemical reactions. The obtained solution was kept in an orbital shaker overnight to ensure the synthesis of silver nanoparticles. The light-yellow color of the leaf extract was changed to dark brown which indicates that the silver nanoparticles were formed from the leaves. The obtained mixture was added to deionized water and centrifuged (repeat three times) at 1000 rpm for 10 minutes to isolate pure AgNPs free from other bioorganic compounds. The synthesized NPs were kept in a hot air oven at 50°C for 3 hrs to get the powder form of AgNPs and then stored for further analysis (Lekshmanaswamy and Anusiyadevi, 2020).

2.4. Characterization of synthesized silver nanoparticles

The colour change observed in the aqueous solution indicates the formation of silver nanoparticles. To confirm the formation, the AgNPs were characterized by different analyses. Proper separation and purification are highly needed for the characterization of metal NPs (Srikar et al., 2016). Initially, the optical properties of AgNPs were explained by spectroscopic analysis which was performed using a UV-Visible spectrophotometer (UV-1650, SHIMADZU) at the wavelength range of 200 - 700 nm and path length of 1 cm, an important and most commonly used technique.

The AgNPs structure and size have been determined using X-Ray diffraction spectrum analysis (XRD, AERIS Metal edition). For this technique, the dried AgNPs were coated on the XRD grid and irradiating a material with incident X-rays and measuring the intensities with scattering angles of the X-rays between 10° and 90° was recorded in the 2θ range. The spectrum was operated at 40 kV with 40 mA of current. The average crystallite size of the silver nanoparticles formed in the bioreduction process was determined using the Debye-Scherrer equation (Shwetha et al., 2020),

$$D = \frac{k\,\lambda}{\beta.\cos\theta}$$

where D is a crystallite size (nm), k is the Scherrer constant (0.94), λ is the wavelength of the X-Ray source (0.15406 nm), β = is the full width half maximum (FWHM, radians) and θ is peak position (Bragg angle, radians).

Fourier Transform Infra-Red (FT-IR) spectroscopy (Nicolet is 5, Thermo Scientific) was used to identify the biologically active components for silver reduction, formation and capping via the KBr pellet method. FT-IR spectra were recorded with wavelength regions from 4000 to 500 cm^{-1} at 4 cm^{-1} resolutions (Zafar and Zafar, 2019).

2.5 *In vitro* inhibitory activity of silver nanoparticles against pathogenic bacteria

The antibacterial studies of biosynthesized silver nanoparticles were tested by a well diffusion method (Holder and Boyce, 1994; Gudikandula and Charya Maringanti, 2016) for the determination of antibacterial activity against two different pathogenic bacteria namely *Escherichia coli* (MTCC 443) and *Vibrio cholerae* (MTCC 3906). The selected pathogenic gram-negative bacterial strains were procured from Microbial Type Culture Collection and Genbank, IMTECH, Chandigarh. The gammaproteobacteria strains have been used and maintained in 4°C nutrient agar plates. About 25 mL of molten Mueller-Hinton agar was poured into a Petri plate. The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 µL of microorganisms were transferred onto the plate and made culture lawn by using a sterile L-rod spreader. After five minutes of setting of the pathogenic samples, a sterile cork borer was used to make 6 mm in size (well) are made on prepared plates. The synthesized silver nanoparticles were loaded into wells with various concentration of 25, 50, 75 and 100 µL. The solvent saline loaded served as negative control and azithromycin (30 µL/well) served as a positive control. The antibacterial activity was determined by measuring the diameter of the inhibition zones were measured in millimetre (mm) around using an antibiotic zone scale. The plates were incubated at 37°C in an incubator for 24 hrs to examine the zone of inhibition. All the experiments were carried out in triplicates and the results were expressed as mean ± standard deviations.

3. RESULTS AND DISCUSSION

3.1. Biosynthesis of silver nanoparticles

In this study, silver nanoparticles were biosynthesized from *Dypsis lutescens* leaves extract showed that the environmentally benign and renewable source of the plant can be used as an effective reducing as well as a stabilizing agent for the synthesis of AgNPs. This is the first assay using leaves of *D. lutescens* to synthesize of AgNPs. The overall schematic representation of the biosynthesize process for AgNPs is shown in Figure 1.

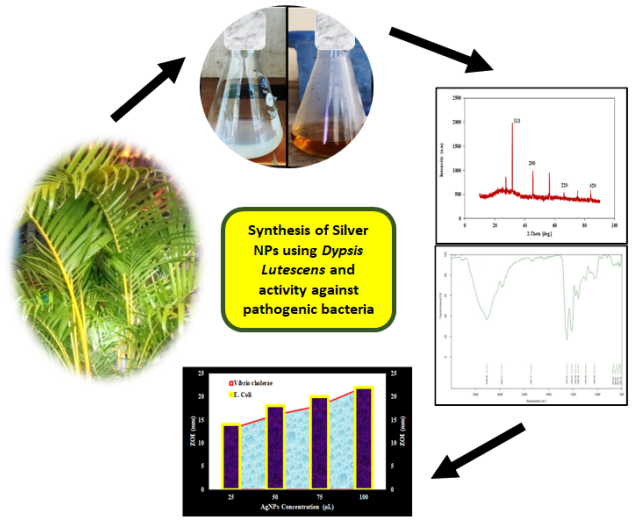

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Figure 1 The overall schematic representation of the biosynthesize process for AgNPs.

The leaf extracts were mixed in the aqueous solution of $AgNO_3$ at room temperature and then incubated overnight confirms the presence of silver nanoparticles. During the synthesis process, the solution of $AgNO_3$ and leaf extract shows the colour change from light yellow → orange → brown → dark brown (Figure 1). The visible colour change was observed by the direct naked eye and it indicates the reaction between the leaf extract and silver nitrate solution where reductant from plant extract directly reduced Ag^+ to Ag atoms (Ag^0). These colour changes preliminarily confirmed the generation of silver nanoparticles which has been due to the excitation of surface plasmon resonance, typical of the AgNPs (Lekeshmanaswamy and Anusiyadevi, 2020; Mathew et al., 2020).

The reductive actions of *D. lutescens* may be attributed due to the presence of various phytochemicals and bioactive compounds such as flavonoids, coumarins, sterols, terpenoids, quinones and alkaloids (El-Ghonemy et al., 2019). The same author reported nearly ten phenolic compounds accumulated with eight flavonoids (vicenin, prechafuroside, orientin, vitexin, violanthin, isoorientin, luteolin, apigenin with two phenolic acids) in leaf extract of *D. lutescens*. Therefore, the aqueous leaves extract of the selected species contains highly phenolic compounds, antioxidants and flavonoids. Hence, the specific species of the Arecaceae family has a certain medicinal property like anticancer, antioxidant and hypolipidemic activity (Chiduruppa et al., 2018). Moreover, the hydroxyl groups present in biomolecules are responsible for the bioreduction of Ag^+ ions as shown in the proposed mechanism (Figure 2). Flavonoids contain various functional groups, which have an enhanced ability reduce metal ions through the production of reactive hydrogen atom due to tautomeric transitions. During this transition, the enol-from is converted to keto-form, the process ensured by the reduction of metal ions into metal NPs (Singh et al., 2018). Zafar and Zafar, (2019) reported the bioreduction mechanism for the silver nanoparticles synthesized from *Phoenix dactylidera*, the proposed mechanism developed for this study. *D. lutescens* leaf extract plays a dual role as both reducing and stabilizing agents simultaneously without any involvement of chemicals. Proteins have a strong ability to create bonding on metal ions and may be encapsulated around the nanoparticles to avoid agglomeration which makes stabilization in an aqueous solution. During AgNPs formation, both reducing and capping mechanism plays a critical role (Ajitha et al., 2015). Initially, the NPs was confirmed visually through the colour changes from light yellow to dark brown which was due to the collective oscillation of free conduction electrons leads to surface plasmon resonance (SPR). The confirmed AgNPs were further admitted for characterizations and antimicrobial analyses.

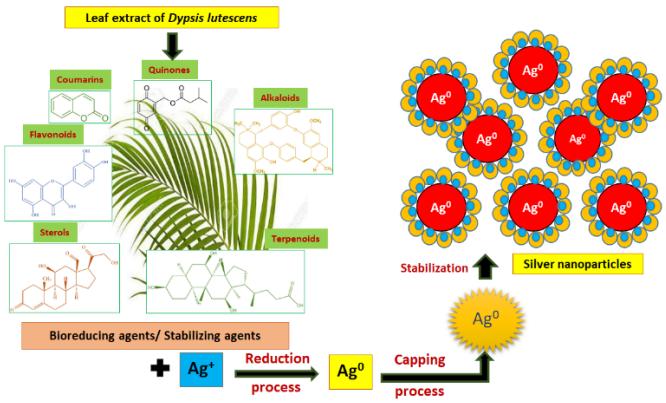


Figure 2. The possible mechanism of the formation of silver nanoparticles from *Dypsis lutescens* leaf extract.

3.2. UV-Visible absorption studies

The UV–visible spectroscopy is one of the essential analyses used to identify the primary appearance of metal nanoparticles in the plant aqueous medium. The phyto-reduction of silver ions in the aqueous solution of silver during the reaction with the biomolecules present in the *D. lutescens* leaf extracts was observed by UV–visible spectroscopy. The spectroscopic analysis of *D. lutescens* leaf extracts to identify absorption peaks for silver nanoparticles was performed at the wavelength range of 200 – 700 nm (Figure 3). The absorption peak corresponds to the surface plasmon resonance band observed at 421 nm and is clearly shown in Figure 3. It has a similar observation for silver nanoparticles

using other plant extracts (Mathew et al., 2020; Lekshmanswamy and Anusiyadevi, 2020). The metal nanoparticles have free electrons, which reflect the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with lightwave (Elangovan et al., 2015). Therefore, the strong absorption peak identified at wavelength 421 nm confirms the synthesized silver nanoparticles.

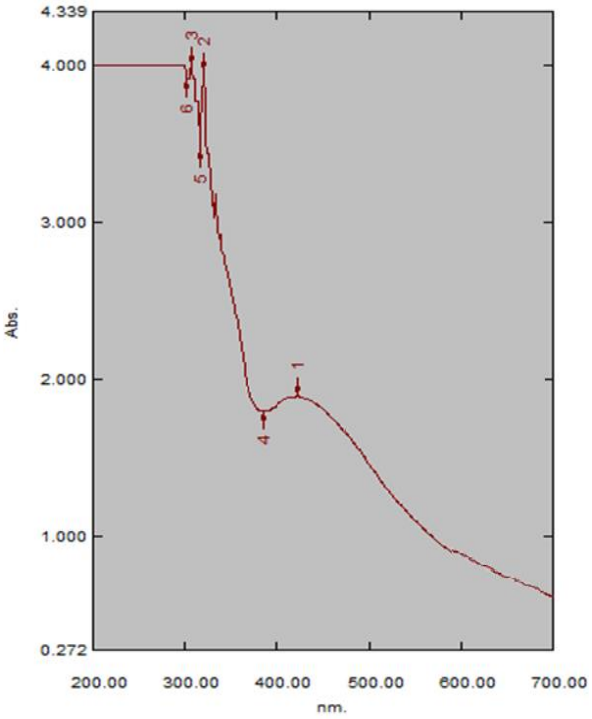


Figure 3. UV-Visible spectrum of *Dypsis lutescens* leaf extract with the plasmon resonance of silver nanoparticles at 421 nm.

3.3. Fourier transform infrared analysis

FTIR measurements were carried out to identify the major functional groups in the plant extract and their possible involvement of the synthesized silver nanoparticles (Babu and Prabu, 2011). FTIR spectrum (Figure 4) shows the presence of different functional groups at various positions which indicates the complex nature of the bioactive compounds and which have the dual efficiency of reducing agents and stabilizers. The bands appearing at 3268, 2962, 1625 cm^{-1} in Figure 4 were assigned to stretching vibrations of strong O-H bond of phenol group, C-H stretching of aromatic groups, and C=O for amide group, respectively. The bands observed at 528, 551, 598 and 670 cm^{-1} correspond to C-I stretching and C–Br stretching of halo compounds. The intense peak at 1392 cm^{-1} corresponds to C–N stretch vibrations. The bands at 1064 cm^{-1} are due to the ether linkages and suggest the presence of flavanones adsorbed on the surface of the nanoparticle. The weaker band at 1239 cm^{-1} corresponding to the C-O-C stretch was observed and is responsible for the capping ligands of the NPs. The main role of the capping ligands is to stabilize the NPs to prevent further growth and agglomeration. The band observed at 2361 cm^{-1} corresponds to C≡C stretching. The immediate reduction and capping of silver ions into silver nanoparticles might be due to the presence of phenolic compounds, flavonoids and antioxidants which are all limited by these stretching vibrations (Lekshmanswamy and Anusiyadevi, 2020; Kalishwaralal et al., 2010).

The functional groups (hydroxyls, carbonyls and aldehyde) present in flavonoids, coumarins, sterols, terpenoids, quinones and alkaloids play a role in synthesizing metallic nanoparticles (Sheny et al., 2011). The detailed synthesizing mechanism was shown in Figure 2. Hence, terpenoids can help in the reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids (Ovais et al., 2018). During the process, the covering agents present in the leaf extract can be actively covered the nanoparticles and preventing the aggregation of the nanoparticles to leads to stabilization (Shah et al., 2015). Therefore, the FTIR pattern confirmed the presence of all functional groups which corresponds to the bioreduction of Ag^{+} to Ag^{0} ions.

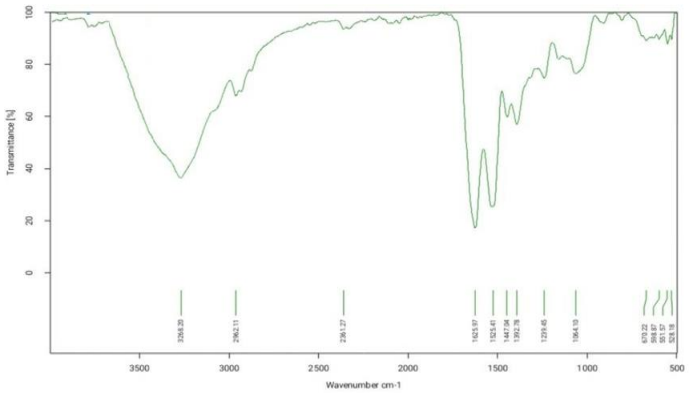


Figure 4. FTIR spectra of biosynthesized silver nanoparticles.

3.4. Crystallographic analysis of AgNPs

The crystal phase and structure of the green synthesized AgNPs were confirmed by XRD analysis. The crystalline structure of the NPs was examined by the XRD pattern has been shown in Figure 5. The highest 2θ diffraction peaks observed at 31.7°, 45.5°, 66.4°, 75.35° and 84.2° which corresponding to (111), (200), (220), (311) and (420) planes of Ag, respectively, which relates to the Face - Centered Cubic (FCC) structure of AgNPs and confirms the successful synthesis of nanoparticles (Esmaile et al., 2020). In addition, some extra peaks were also observed at 28.2°, 57.5° and 76.1° in the XRD pattern which could be due to the presence of phytochemical compounds available in the leaf extract. The presence of sharp peaks in the pattern displays a high level of crystallinity for the synthesized AgNPs. The wide peaks in XRD patterns of solids are related to the effect of the size of crystallite and showed the effects of crystalline nuclei during experimental conditions (Patil and Kim, 2017). The (111) orientation is more intense and considered as a predominant orientation as same reported in other literature (Ajitha et al., 2015; Gopinath et al., 2013; Krishnaraj et al., 2012). Therefore, the XRD pattern results proved that the synthesized NPs have a crystallite shape because of the crystallization of bioorganic phase observed on the surface of AgNPs.

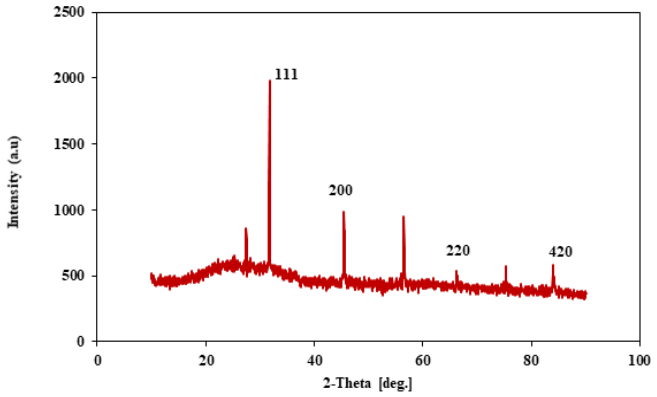


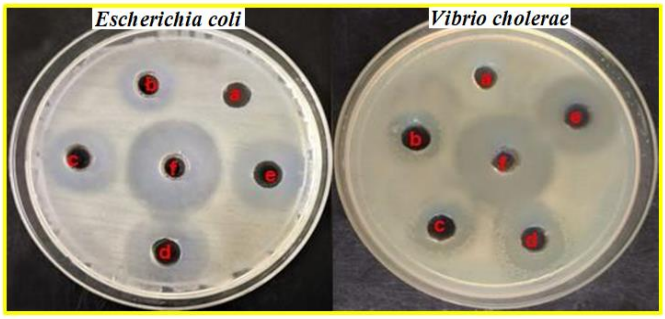
Figure 5. XRD analysis of biosynthesized silver nano particles.

The average size of the synthesized crystallites using the FWHM of peaks was calculated by the Debye-Scherrer equation. The wider peaks exhibit the smaller size of the crystalline NPs and are found to be 31 nm. The average size of synthesized AgNPs was confirmed and found to be 24 nm and 23.5 nm by Ajitha et al. (2015) and Esmaile et al. (2020), respectively using leaf extracts. The obtained size difference may be due to the family of the leaf extract and the operating conditions.

3.5. Antibacterial studies of AgNPs

The biosynthesized silver nanoparticles have been studied for their potential antibacterial activity against *Escherichia coli* and *Vibrio cholerae* using an agar well diffusion assay and shown in Figure 6. Different concentrations of aqueous silver nanoparticles of 0, 25, 50, 75 and 100 µL are placed into the agar well with

the positive control of azithromycin tested with the concentration of 30 µL/well. The diameter of the inhibition zone gradually increased with increasing the concentration of the AgNPs for both pathogenic bacteria and was visualized in Figure 6. Hence, the bactericidal studies have been analyzed in a dose-dependent manner (Zafar and Zafar, 2019). During comparison with the positive control, the diameter of the wells nearly closed to the azithromycin well for the respected concentration of inhibition zone. The obtained results of *Invitro* inhibitory activity of silver nanoparticles against pathogenic bacteria and all the mean values of both samples are tabulated in Table 2. The diameter of the inhibition zone increased from 14 to 22 mm and 13 to 22 mm for the gram-negative bacteria of *E. coli* and *V. cholerae*, respectively during increasing AgNPs concentration from 25 to 100 µL/well. A maximum inhibition zone of 22 mm was observed at 100 µL/well for both pathogenic strains of *E. coli* and *V. cholerae*. The observation values nearly match with the positive control values of 30 and 29 mm obtained at 30 µL/well for *E. coli* and *V. cholerae*, *respectively*. The results confirm that the inhibition zone is increased with an increase in the concentration of nanoparticles against the bacterial strains.



a) 0 µL; b) 25 µL; c) 50 µL; d) 75 µL; e) 100µL and f) Azithromycin (30 µL)

Figure 6. Agar well diffusion assay of bactericidal activity against pathogenic strains of synthesized AgNPs.

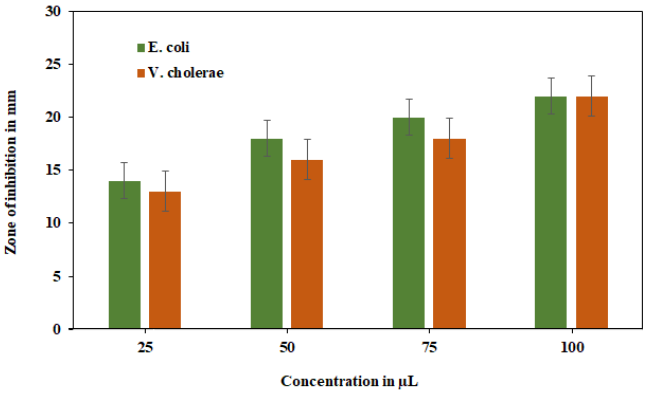


Figure 7. Effect of the zone of inhibition of silver nanoparticles against gram-negative bacteria.

The effect of the zone of inhibition of silver nanoparticles against gram-negative bacteria is shown in Figure 7. The zone of inhibition of *E. coli* always higher than the *V. cholerae* for each concentration of the AgNPs. Therefore, the present study proved that the synthesized AgNPs had the highest antibacterial activity against *E. coli*. A very good spectrum of antibacterial activity was found in the sample against both bacteria. Antibacterial activity is most likely derived from electrostatic attention between the negative charged microorganism cell membrane and positively charged NPs (Esmaielzadeh et al., 2015; Mushir et al., 2016).

The zones of inhibition formed were mainly due to the destabilization of the outer membrane, the collapse of the plasma membrane, and depletion of intracellular ATP by the silver nanoparticles (Morones et al., 2005). It increases the permeability of cell membranes, produces reactive oxygen species, and interrupts the replication of deoxyribonucleic acid by releasing silver ions. The low concentration of AgNPs showed a moderate inhibition zone against both pathogenic agents. The high concentration of AgNPs demonstrated a clear inhibition zone. Therefore, the diameter of the inhibition zone increases with increasing the concentrations of AgNPs. This study showed significant biocidal activity against E. coli compared to *V. cholerae* in green synthesized AgNPs due to the thin layer of peptidoglycan of *E. coli* (Bharathi et al., 2018).

Table 2. *In-vitro* inhibitory activity of silver nanoparticles against pathogenic bacteria

Bacterial Strains	Diameter of Zone of Inhibition (mm) of aqueous concentration of AgNPs			
	25 µL	50 µL	75 µL	100 µL
Escherichia coli	14±0.1	18±0.3	20±0.1	22±0.1
Vibrio cholerae	13±0.2	16±0.2	18±0.2	22±0.1

The category of alkaloids glycosides, flavanols, sterols and triterpenoids and these phytocompounds are the responsive group for the synthesis of silver nanoparticles (Dahibhate et al., 2020). The results from this study reveal that gram-negative bacteria are resistant to the action of tested extracts and the same has been published by Lekeshmanaswamy and Anusiyadevi. (2020) for *Perfularia daemia* leaf extract. Moreover, gram-negative bacteria are generally more resistant than gram-positive ones. This is because Gram-negative bacteria strains have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impermeable to antimicrobial agents (Singhal et al., 2011; Elumalai et al., 2010). Hence, antibacterial study reveals that silver nanoparticles possess great potential antibacterial activity against *Escherichia coli* and *Vibrio cholerae* may be the release of the reactive species oxygen which damage the membrane proteins (Azizi et al., 2017). Therefore, *Dypsis lutescens* derived silver nanoparticles have a maximum antibacterial activity against gram-negative pathogenic bacteria.

CONCLUSION

In this study, green synthesis of silver nanoparticles has been proposed by using *Dypsis lutescens* leaf extracts. The absorption peak of the synthesized nanoparticles was characterized by UV-visible spectroscopy. The crystalline nature of silver nanoparticles was studied using X-ray diffraction and the average size of nanoparticles was found to be 31 nm. The FTIR analyses revealed that the presence of silver nanoparticles with active biomolecules and phyto-molecules is responsible for the synthesis, capping and stabilization of the AgNPs. The significant antibacterial activity was tested for synthesized AgNPs which showed that biosynthesized AgNPs from the *D. lutescens* leaf extract has much resistance against gram-negative bacteria of *Escherichia coli and Vibrio cholerae*. The diameter range of the inhibition zone was revealed that the potential of antimicrobial activity of pathogens. Therefore, the present study concludes that biologically derived AgNPs using *Dypsis lutescens* is eco-friendly, rapid, non-toxic and cost-effective approaches.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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