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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM THE LEAF EXTRACT OF *DENDROPHTHOE FALCATA* (L.F.) ETTINGSH: PHYTOCHEMICAL ANALYSIS AND BIOACTIVITY STUDIES

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ABSTRACT: An evergreen semiparasitic plant belonging to the Loranthaceae family, *Dendrophthoe falcata* (L.f.) Ettingsh is common throughout India and feeds on a variety of host species. The potential of silver nanoparticles (AgNPs) produced by *D. falcata*-mediated processes is still unknown, despite the known bioactivity of *D. falcata* extracts. The present investigation examines the phytochemical profile of leaf extracts from *D. falcata*, their function in the synthesis of AgNPs, and the AgNPs' bioactivity. The existence of tannins, terpenoids, alkaloids, and flavonoids was verified by phytochemical analysis. AgNPs were synthesized and characterized using UV-Vis spectrophotometry, FTIR, SEM, TEM, and XRD, confirming their structural integrity and stability. The DPPH radical scavenging assay revealed AgNPs' robust antioxidant properties by showing that their capacity increased proportionately with incubation time and that their purple coloring significantly decreased. Also, AgNPs proved robust antibacterial activity, comparable to positive controls against *E. coli*, a gram-negative bacterium. They showed a notable inhibition zone against gram-positive bacteria at a 300 µl concentration. Further, AgNPs inhibited *S. aureus*, albeit only at higher concentrations. These findings indicate that *D. falcata*-mediated AgNPs are effective, eco-friendly biofunctional agents with potential applications in pharmaceutical, biotechnological, and biomedical fields.

INTRODUCTION: Medicinal herbs have been used for years to heal illnesses and infections. These plants are abundant in India, which is referred to as the "medical garden of the world." According to a WHO review, nearly 70–80% of today's population relies on alternative medicine, mainly from herbal sources [1, 2].

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Dendrophthoe falcata is a huge, grey-barked parasitic plant that grows in bushes. The flowers are orange-red and crimson and the leaves are positioned in opposition to one another with oblong berries. The plant is also called *Loranthus falcatus* Linn. F. *D. falcata* Ettingsh is Indigenous to Indo-China, Thailand, Sri Lanka, and Australia [3, 4]. The parasitic shrub has been shown to have about 3000

host plants, with roughly 7 different host species occurring in India [4]. *D. falcata* is reported to contain several bioactive compounds, such as tannins, ß-sitosterol, flavonoids, rutin, kaempferol, quercetin, ß-amyrin, oleanolic acid, and stigmasterol. Moreover, several enzymes, such as hexokinase, glucose phosphatase, and L-threonine dehydratase have been isolated from *D. falcata* leaves [3, 5]. The plant extract is useful in treating asthma, pulmonary tuberculosis, ulcers, menstrual disorders, swelling, wounds, inflammation and renal and vesical calculi. The decoction from the plant is used as an antifertility and anticancer agent. A paste prepared from *D. falcata* leaves is used in the treatment of skin disorders and boils, in pus extraction, and in setting dislocated bones [3, 6, 7].

Green technology can help overcome several health and environmental problems associated with synthetic compounds and drugs. In recent years, nanomaterial research has gained momentum towards developing eco-friendly materials for the agriculture, medicine, health, and pharmaceutical industries. Nanoparticles have been extensively used in drug delivery, drug development, therapy, and diagnostics [8]. In this context, silver nanoparticles, or AgNPs, have been extensively used. Silver has antifungal, antibacterial, and anti-inflammatory properties [8, 9]. The synthesis of AgNPs can be achieved using ion sputtering, sol-gel, and chemical reduction; however, such methods may have several negative environmental effects because of the harmful chemicals used; hence, eco-friendly alternatives are needed [10]. In this regard, plant extracts have been exploited as reducing agents or stabilizers in synthesizing platinum nanoparticles [11], zinc oxide nanoparticles [12], copper nanoparticles [13], and AgNPs [14].

D. falcata is widely used in rural areas as a component of ethnomedicine for treating several infections and diseases [15]. The plant extracts have been investigated for their antioxidant, thrombolytic, and antimicrobial activities. However, only limited reports are available on the bioactivity of nanoparticles synthesized using *D. falcata* extracts. Therefore, in this study, we aimed to characterize the extracts of *D. falcata*, explore their potential for the synthesis of AgNPs, and analyze the antibacterial and antioxidant potential of the synthesized AgNPs.

MATERIALS AND METHODS:

Sample Collection: Healthy and undamaged *D. falcata* leaves were collected from Thattapparai village near Gudiyattam, in Vellore district, Tamil Nadu. The leaf samples were washed and thoroughly shade-dried. The leaf material was then powered and used for further analysis.

Preparation of Leaf Extract for Qualitative Analysis: The powdered leaf sample (about 50 g) was soaked in methanol (150 mL). After covering the beaker with aluminum foil, the sample was shaken intermittently for 72 h. Next, the extract was filtered through grade 1 filter paper, then vacuum distillation was used to extract the solvent. Until it was examined further, the concentrated leaf extract was kept at 4°C.

Initial Qualitative Assessment:

Alkaloid Analysis:

Mayer's Test: A few drops of Mayer's reagent were put into the test tube's sidewalls and 1 ml of plant extract. The development of a white, creamy precipitate indicated the presence of alkaloids.

Wagner's Test: The existence of alkaloids is indicated by adding drops of Wagner's reagent to a plant extract, showing red-brown precipitation [16].

Phenols Analysis: $FeCl_3$ test: Two drops of 5% $FeCl_3$ solution were added into 5 ml of leaf extract. The formation of a dark green color confirmed the existence of phenols.

Gelatin Test: 2 mL of gelatin solution (1:10, gelatin: NaCl) was added to 5 mL of extract. The formation of white deposition indicated the existence of phenols.

Lead Acetate Test: Around 3 mL of lead acetate solution (10%) was added to the extract to form a colossal white deposition indicating the existence of phenols [16].

Terpenoids Analysis:

Salkowski Test: 1 mL of chloroform and 2 drops of H_2SO_4 were added to 2 mL of extract. The formation of a reddish-brown color confirms the existence of terpenoids.

Flavonoids Analysis:

Lead Acetate Test: 1 mL of lead acetate solution (10%) was added to 2 mL of extract, which resulted

in the formation of a yellow deposition, confirming the availability of flavonoids.

Tannins Analysis:

$FeCl_3$ Test: A few drops of a $FeCl_3$ solution (5%) were poured into 2 mL of extract. The appearance of a blue color exhibited the availability of tannins.

Phytosterols Analysis:

Libermann-Burchard's Test: About 2 mL of acetic anhydride was added to leaf extract (50 mg). Next, two drops of H_2SO_4 were added, which resulted in color changes that revealed the availability of phytosterols.

Phyto-Mediated Formulation of AgNPs: AgNPs were prepared using the crude leaf extract of *D. falcata* (L.f.) Ettingsh employs the reduction method. The fine leaf powder was mixed with distilled water (1:10) and allowed to boil at 60ºC for 5 min. Next, the extract was collected using grade 1 filter papers. The leaf extract was stored separately at 4ºC for further process. About 100 mL of 1 mM $AgNO_3$ solution was heated at 100ºC, and plant extract (1 mL) was mixed with $AgNO_3$ solution and allowed to boil for 20 min. After 20 min, the solution turned blackish-yellow, which indicated the formation of AgNPs [17]. The presence of AgNPs was confirmed by UV-visible spectrophotometry and Fourier transform infrared spectroscopy (FTIR). The prepared AgNPs were then centrifuged at 3500 rpm for 30 min. The collected pellets were centrifuged again at 14000 rpm for 10 min. The pellets obtained were then washed with ethanol to remove the plant extracts and contaminants. Subsequently, the ethanol-washed pellets were washed three times with distilled water and warmed in a hot air oven at 60ºC for 1 h and 30 min. The dried powder was collected and characterized by XRD, SEM, and TEM, to confirm their nano properties [18].

DPPH (1,1-diphenyl-2-picrylhydrazyl) Assay: The anti-oxidant property of the synthesized AgNPs was analyzed using a DPPH assay. A DPPH solution was prepared by dissolving 7.98 mg of DPPH in 99.5% ethanol and incubating it in the dark for 2 h. Different concentrations of AgNPs were used for the analysis. AgNPs (50 µL to 400 µL) were prepared from 1 mL plant extract. Approximately 5 mL of plant extract was used to analyze anti-oxidant activity.

Each concentration of AgNPs was mixed with 1 mL of DPPH, and the solution was mixed with 2 mL of Tris HCl. The solution was stored at room temperature for 24 h and 48 h, and absorbance at 517 nm was analyzed using a colorimeter. Ethanol (1200 µL) combined with 800 µL of Tris HCl was used as a blank. About 1 mL of DPPH with Tris HCl (800 µL) mixed with 200 µL of ethanol was used as a control [19].

The inhibition ratio was estimated using the equation,

$$\text{Inhibition ratio (\%)} = C - A / C$$

Where, A = DPPH exposed to different concentrations of AgNPs, C = Control

Examination of Antibacterial Activity: The antibacterial assay was conducted with the agar-well diffusion method. Gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria were inoculated and incubated for 24 h, after which they were uniformly streaked on plates. Wells were made on the media for the AgNP sample, plant extract, and positive and negative controls. The cultures were incubated for 24 h at 37 °C. Subsequently, the zone of inhibition was measured [20].

RESULTS:

Qualitative Analysis: The phytochemicals were detected based on our preliminary qualitative analyses of the plant extract **Table 1.**

TABLE 1: PHYTOCHEMICAL ANALYSIS OF THE CRUDE EXTRACT OF *DENDROPHTHOE FALCATE*

Phytochemical test	Indicator	Result
Alkaloids	A red precipitate was observed	+
Flavonoids	The yellow color was formed	+
Terpenoids	The reddish-brown precipitate was formed	+
Phenol	The blue or green color was observed	+
Tannins	The bluish-black color was formed	+
Steroids	Two layers formed an upper layer of red and a lower layer of yellow with green fluorescence	+

Characterization of AgNPs: The AgNPs prepared using the plant extract-mediated process were illustrated using UV spectrophotometry, FTIR, SEM, TEM, and XRD. The initial characterization was done using UV-visible spectrophotometry in the absorbance range of 300 to 500 nm. The absorbance maximum (λmax) value of the AgNPs synthesized through the plant extract-mediated process was found to be 420 nm, indicating the presence of nanoparticles **Fig. 1.** Further confirmation of the presence of AgNPs was done using FTIR analysis. The peaks were acquired at 3297 cm^{-1}, 2362.21 cm^{-1}, 2334.56 cm^{-1}, 1639.15 cm^{-1}, and 526.44 cm^{-1} **Fig. 2.** An FTIR study showed that nanoparticles were capped with plant compounds. The SEM analysis indicated that the moderate size of the AgNPs was 50 nm, and the particles were spherical and clumped together. Increasing the concentration of the plant extract might have some effects on maintaining the stability of the prepared AgNPs **Fig. 3.**

From the EDAX spectrum, it is evident that the characteristic peak of silver composition **Fig. 4.** The nanoparticles have an 84% silver weight on their own, as shown in **Table 2.** The crystallinity of the AgNPs was analyzed using XRD. The diffraction patterns of the peak correspond with the diffraction peaks of JCPDS card no. 04-0783. The diffraction pattern confirmed that the prepared particles have a face-centered cubic structure **Fig. 5.** The crystallite size was calculated using Scherrer's equation, and the mean of all the crystallite sizes **Table 3** indicated a value of 102.44 nm.

Scherrer's Equation:

$$D = k\lambda / (\beta \cos\theta)$$

The TEM investigation revealed that the AgNPs were spherical **Fig. 6.** Some agglomerates were found, and this might be due to organic constituents in the plant extract.

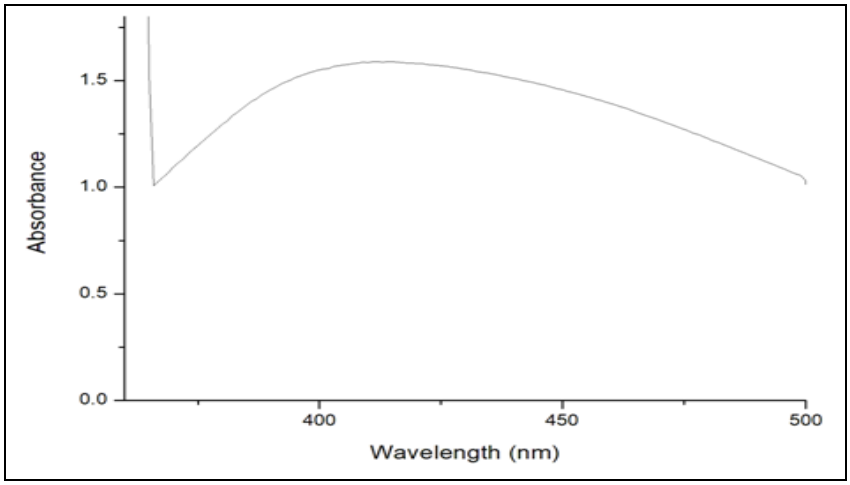

FIG. 1: UV-VISIBLE SPECTRUM OF *D. FALCATA* SHOWING THE PRESENCE OF AG NPS

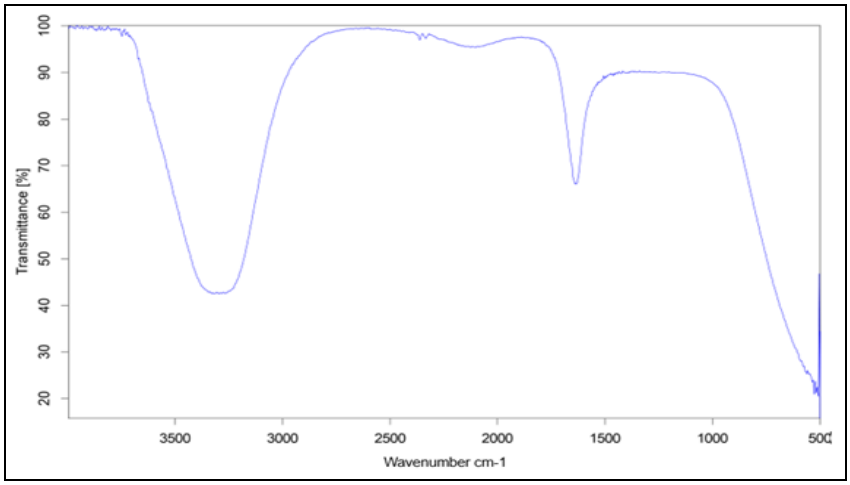

FIG. 2: FTIR ANALYSIS OF SILVER NANOPARTICLES SYNTHESIZED FROM LEAF EXTRACT OF *D. FALCATA*.

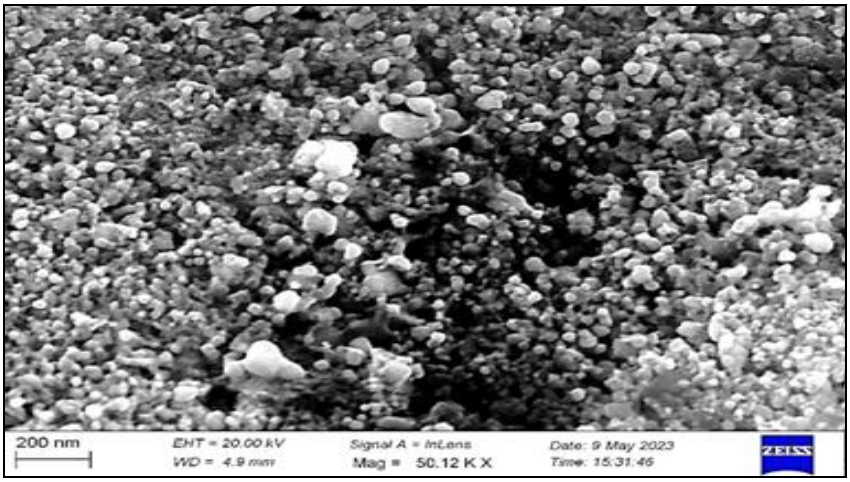

FIG. 3: SEM IMAGE OF THE SYNTHESIZED AG NPS FROM *D. FALCATA*

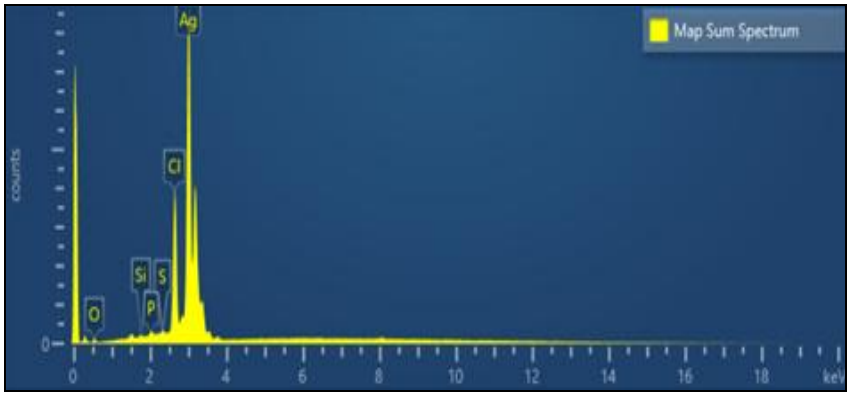

FIG. 4: EDAX SPECTRUM OF THE SILVER NANOPARTICLES OF *D. FALCATA*

TABLE 2: THE LIST OF ELEMENTS PRESENT IN AG NPS (SEM EDAX)

Element	Weight %	Atomic %
O	3.42	15.99
Si	0.19	0.50
P	0.39	0.94
S	0.21	0.49
Cl	10.96	23.15
Ag	84.84	58.92
Total	100	100

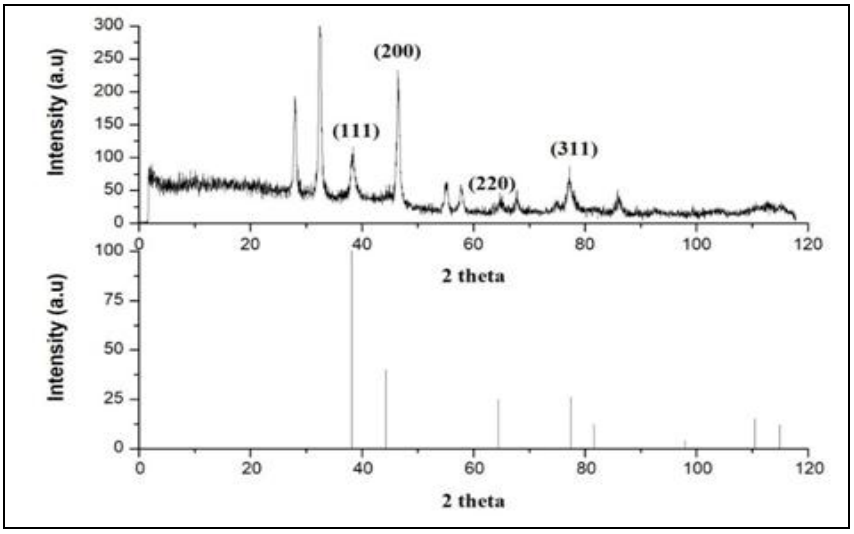

FIG. 5: XRD ANALYSIS OF THE AG NPS OF *D. FALCATA*

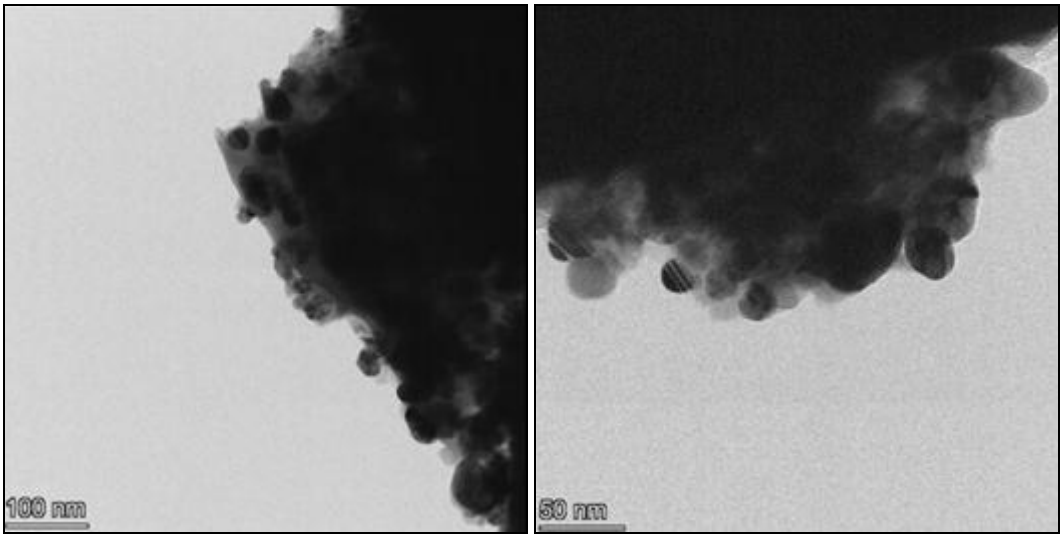

FIG. 6: TEM IMAGE OF THE AG NPS OF *D. FALCATA*

Antioxidant and Antibacterial Activity: The antioxidant activity of the AgNPs synthesized using *D. falcata* (L.f.) Ettingsh was studied using DPPH as free radicals. The antioxidant activity of AgNPs is directly proportional to the incubation period. However, the scavenging activity of the AgNPs is further confirmed by a decrease in the purple color of the DPPH, indicating the strong antioxidant properties of the AgNPs. The antimicrobial assay demonstrated that AgNPs showed excellent antibacterial activity against the gram-negative bacterium, *E. coli*, which was almost equivalent to the activity of the positive control. In contrast, the zone of inhibition for the gram-positive bacteria was found at 300 µL concentration **Table 3.** Therefore, this suggests that the plant extract has no antibacterial activity, and the inhibition zones formed were only due to the AgNPs. Thus, the prepared AgNPs are a potent antibacterial agent against *E. coli.* They are also capable of inhibiting *S. aureus* but only at higher concentrations.

TABLE 3: ANTIBACTERIAL ACTIVITY OF THE SYNTHESIZED AG NPS AGAINST *E. COLI* AND *S. AUREUS*

Precursors	Microorganism	Concentration (µL)	Diameter (mm)	Radius (mm)
Plant extract	*E. coli*	100	27	13.5
		200	36	18
		300	35	17.5
	S.aureus	100	43	21.5
		200	44	22
		300	50	25
Control positive		50	46	25

DISCUSSION: In this study, the extracts of *D. falcata* were analyzed for their bioactive compound content. The extracts were then used in the synthesis of AgNPs, which, in turn, were characterized and studied for their antioxidant and antibacterial activities. Several investigations have proved the existence of bioactive compounds in *D. falcata*. Sinoriya *et al*., 2011 declared that the plant extract contains several phytocompounds, such as quercetin, rutin, gallic acid, and beta-sitosterol [21]. In another study, aqueous soluble fractions of *D. falcata* were found to have potent antioxidant activity, as measured by their DPPH scavenging activity. Lupeol and beta-sitosterol were identified from the extracts of *D. falcate* [5]. The antioxidant potential of *D. falcata* extracts in terms of DPPH scavenging activity was also studied by Pattanayak *et al*., 2011 [22]. Notably, Satishkumar *et al*., 2014 [23] succeeded in synthesizing AgNPs using *D. falcata* extracts as a reducing agent and suggested that these AgNPs demonstrate cytotoxic activity against human breast carcinoma cell lines. Various chemical, physical, and biological techniques have been used in the past to produce different types of metal nanoparticles. AgNPs are a preferred choice based on their vast scope of applications. The formulation of AgNPs using various chemicals, such as citrate, hydrazine, sodium borohydrate,

ethylene glycol, and DMF as reducing agents may have hazardous effects. Further, these approaches are comparatively expensive. Therefore, phyto-mediated, eco-friendly, benign, and cost-effective methods of nanoparticle synthesis are the need of the hour. Currently, the green root approach, which employs a plant-based material acting as both a reducing and capping agent, is gaining traction [14]. The green synthesis approach to nanoparticles is aimed at reducing pollution and toxicity to the environment. The use of microbe- and plant-mediated synthesis of nanoparticles is easy and safe [24]. Moreover, the phytosynthesis of nanoparticles has several applications in the fields of medicine, agriculture, and the environment. *D. falcata* (L.f.) Ettingsh extracts possess phytochemicals of high therapeutic value, such as alkaloids, triterpenoids, flavonoids, tannins, and saponins. In agreement with several studies, which suggest that phyto or biologically synthesized nanoparticles possess biological activity [14, 25–27]. Our investigation suggests that AgNPs synthesized using *D. falcata* as a reducing agent possess antibacterial and antioxidant potential and can be effectively utilized in pharmaceutical, biotechnological, and biomedical applications.

CONCLUSION: In our study, the production and stabilization of the AgNPs were aided by the *D. falcata* leaf extract which was abundant in phytochemicals. The characterization analysis confirmed the structural integrity and consistent size of AgNPs. The nanoparticles show strong antioxidant properties which increased with longer incubation time. In addition, AgNPs demonstrated strong antibacterial activity against *S. aureus* and *E. coli*, indicating their efficacy as antimicrobial agents. Our findings confirm the potential of plant-based nanoparticle synthesis for the production of functional and sustainable materials with a wide range of uses, and they support the use of *D. falcata*-mediated AgNPs as environmentally benign, bioactive agents.

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CONFLICTS OF INTEREST: The authors declare that they have no competing interests.

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