

# Tailored Synthesis of Silver Nanoparticles using Polyphenolic and Flavonoid-rich Leaf Extract of *Dryopteris cristata* L. and Insights into its Antioxidant Potency

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**Abstract**—Biosynthesized nanoparticles are increasingly recognized for their potential due to the biologically active secondary metabolites in plants that facilitate green synthesis and offer unique biological applications. This study presents a simple, eco-friendly and cost-effective method for the synthesis of silver nanoparticles using the aqueous leaf extract of *Dryopteris cristata* L., which serves as both a reducing and capping agent. Characterization through UV-visible spectroscopy (UV-Vis.), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDX), and transmission electron microscopy (TEM) analyses confirmed the properties of the *Dryopteris cristata*-silver nanoparticles (Dc-AgNPs). Phytochemical analysis identified the compounds responsible for nanoparticle reduction and stabilization. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays, revealing significant antioxidant potential. These Dc-AgNPs exhibit potential for use as antioxidant, antimicrobial and antiproliferative agents, making them valuable candidate for diverse therapeutic applications.

**Keywords:** Phytochemicals; Green synthesis; Nanoparticles; Silver; *Dryopteris cristata* L.; Antioxidant.

## INTRODUCTION

In the field of nanotechnology, the synthesis of silver nanoparticles (AgNPs) has experienced a significant escalation, drawing considerable interest from both nanotechnologists and biologists. Silver, with an annual production of approximately 500 tons, stands as one of the most extensively commercialized nanomaterials, and future projections suggest further growth (Bruna *et al.*, 2021). The methods for synthesizing silver nanoparticles are diverse, spanning both physical and chemical approaches. These include sol-gel processing, chemical reduction, laser-mediated synthesis, chemical vapor deposition, hydrothermal synthesis, reverse micelle

methods, microwave and ultraviolet irradiation, and photochemical reduction (Rafique *et al.*, 2017; Wang *et al.*, 2005; Khaydarov *et al.*, 2009). Biological synthesis, utilizing plant and microbial sources, has gained particular prominence due to its environmentally friendly nature, cost-effectiveness, and reduced reliance on toxic chemicals (Tarannum *et al.*, 2019). Among these methods, plant-derived extracts have shown superior efficiency in reducing silver ions and stabilizing silver nanoparticles compared to microbial sources (Tarannum *et al.*, 2019). Extensive research has investigated the synthesis of silver nanoparticles using various plant materials, including

leaves, flowers, seeds, bark, roots, and fruits. Notable examples include *Ocimum sanctum* (Ramteke *et al.*, 2012), *Azadirachta indica* (Tripathy *et al.*, 2010), *Murraya koenigii* (Sajeshkumar *et al.*, 2015), *Carica papaya* (Banala *et al.*, 2015), *Moringa oleifera* (Prasad *et al.*, 2011), *Coffea arabica* (Dhand *et al.*, 2016), *Tanacetum vulgare* (Dubey *et al.*, 2010), *Punica granatum* (Shanmugavadivu *et al.*, 2014), *Vitis vinifera* (Roy *et al.*, 2014), *Berberis vulgaris* (Behravan *et al.*, 2019), *Citrus reticulata* (Shanmugavadivu *et al.*, 2017), and *Catharanthus roseus* (Mukunthan *et al.*, 2011). These plant extracts serve as reductases, converting  $Ag^+$  ions into elemental silver ( $Ag^0$ ) nanoparticles. Beyond their recognized antibacterial properties, silver nanoparticles are also pivotal in environmental pollutant remediation (Hazarika *et al.*, 2024; Behravan *et al.*, 2019). Despite advancements in nanobiotechnology, the specific roles of various bioactive compounds in plant extracts in the synthesis of nanoparticles remain inadequately understood. Variability in nanoparticle yield among different plant species, even when using identical protocols, underscores the need for a detailed exploration of the underlying bioactive components. Establishing standardized procedures based on these findings is crucial for ensuring consistent large-scale production, addressing a significant challenge in the current domain of biosynthesized nanoparticles (Dhand *et al.*, 2016; Shanmugavadivu *et al.*, 2017).

*Dryopteris cristata* L., known as the crested wood fern, is a member of the Dryopteridaceae family and is indigenous to wetlands across the northern hemisphere (Zuo *et al.*, 2022). Its name reflects the distinctive crested appearance of its fronds, which emerge from the central axis, contributing to its notable visual appeal. In Arunachal Pradesh, India, this species is referred to as "Pukutphet" within the *Tai-Khamti* community. Beyond its aesthetic qualities, *Dryopteris cristata* is recognized for its potential pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, and antitumor activities (Alam *et al.*, 2021; Erhirhie *et al.*, 2019). While qualitative phytochemical analyses and quantitative assessments have been extensively documented for other *Dryopteris* species, such as *Dryopteris ramosa* (Alam *et al.*, 2021), *Dryopteris juxtapostia* (Rani *et al.*, 2022), *Dryopteris cochleata* (Kathirvel *et al.*, 2016), and *Dryopteris filix-mas* (Femi-Adepoju *et al.*, 2021), there is a notable absence of studies focused on the phytochemical profile of *Dryopteris cristata*. This gap presents a novel research opportunity. The current study aims to develop a rapid, simple, and eco-friendly method for the synthesis of silver nanoparticles (AgNPs) using the leaf extract of *Dryopteris cristata*, which is abundant in polyphenols and flavonoids. This research will identify the role of these phytochemicals in the nanoparticle synthesis process and characterize

the silver nanoparticles' morphological properties using different analytic techniques. Additionally, the antioxidant activity of the synthesized silver nanoparticles will be evaluated to assess their potential applications in the biomedical field.

## MATERIALS AND METHODS

### MATERIALS AND REAGENTS

The fresh leaves of *Dryopteris cristata* L. were collected from the botanical garden of the University campus. Analytical-grade reagents used in the study, including  $\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), L-ascorbic acid, Folin-Ciocalteu reagent, catechin, sodium bicarbonate, aluminum chloride, methanol, silver nitrate and deionized water, were purchased from HiMedia Laboratories and Sigma-Aldrich, India.

### PREPARATION OF THE PLANT EXTRACT

Briefly, 10 g of air-dried *Dryopteris cristata* leaves were washed thoroughly with distilled water and finely ground using a mortar and pestle. The resulting leaf powder was then immersed in 100 mL of deionized water and boiled for approximately 15 min at 60°C. After boiling, the mixture was filtered through Whatman filter paper (No. 1) and centrifuged at 5000 rpm for 10 min. The supernatant was collected and stored at 4°C for future applications.

### PHYTOCHEMICAL SCREENING

Phytochemical analysis was conducted to identify the presence of various phytoconstituents in the freshly prepared *Dryopteris cristata* leaf extract, including phenols, tannins, terpenoids, steroids, flavonoids, saponins, and alkaloids. This analysis was carried out using established methodologies as described by Sofowora (1993).

### DETERMINATION OF PHENOLIC CONTENT

The phenolic content of the *Dryopteris cristata* leaf extract was quantified using the Folin-Ciocalteu (FC) assay, with modifications based on Singleton *et al.* (1999). In this method, 150  $\mu$ L of the leaf extract was combined with 1.25 mL of a tenfold diluted FC reagent. After 5 min incubation, 1.25 mL of 6% (w/v) sodium bicarbonate was added to neutralize the reaction. The mixture was then incubated in the dark for 90 min. Absorbance was measured at 760 nm, and phenolic content was determined using a gallic acid equivalent standard curve, with results expressed as mg/mL of gallic acid equivalent (GAE).

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## DETERMINATION OF FLAVONOID CONTENT

The flavonoid content of the *Dryopteris cristata* leaf extract was quantified using a colorimetric assay, adapted from Woisky and Salatino (1998) with modifications. Specifically, 1 and 1.5  $\mu\text{L}/\text{mL}$  of the leaf extract were combined with 2 mL of ethanol containing 0.1 mL of 10% aluminum chloride and 2.5 mL of distilled water. After 20 min of incubation at room temperature, the absorbance of the reaction mixture was recorded at 415 nm. The total flavonoid content was determined using a catechin equivalent standard, and results were expressed as catechin equivalents (CAE).

## BIOSYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) were synthesized from *Dryopteris cristata* leaf extract using the biological method

(Mohanta *et al.*, 2017). In this procedure, 10% (v/v) of leaf extract was added dropwise to 45 mL of 2 mM  $\text{AgNO}_3$  solution, maintaining a 5:1 ( $\text{AgNO}_3$ : extract) ratio and a pH of approximately 7.2. The mixture was stirred vigorously at room temperature until a notable color change from light brown to reddish brown occurred, indicating the reduction of  $\text{Ag}^+ \rightarrow \text{Ag}^0$ . The resulting colloidal solution was allowed to stand undisturbed for 24 h at room temperature to facilitate nucleation. Following this period, the solution was centrifuged at 5000 rpm for 20 min. The precipitate was then washed with an ethanol-water mixture (3:2) and dried at 60°C for 24 h to yield the desired Dc-AgNPs. Figure 1 depicts the synthetic pathway for Dc-AgNPs, where phytochemicals in the leaf extract play a crucial role in capping and stabilizing the nanoparticles.

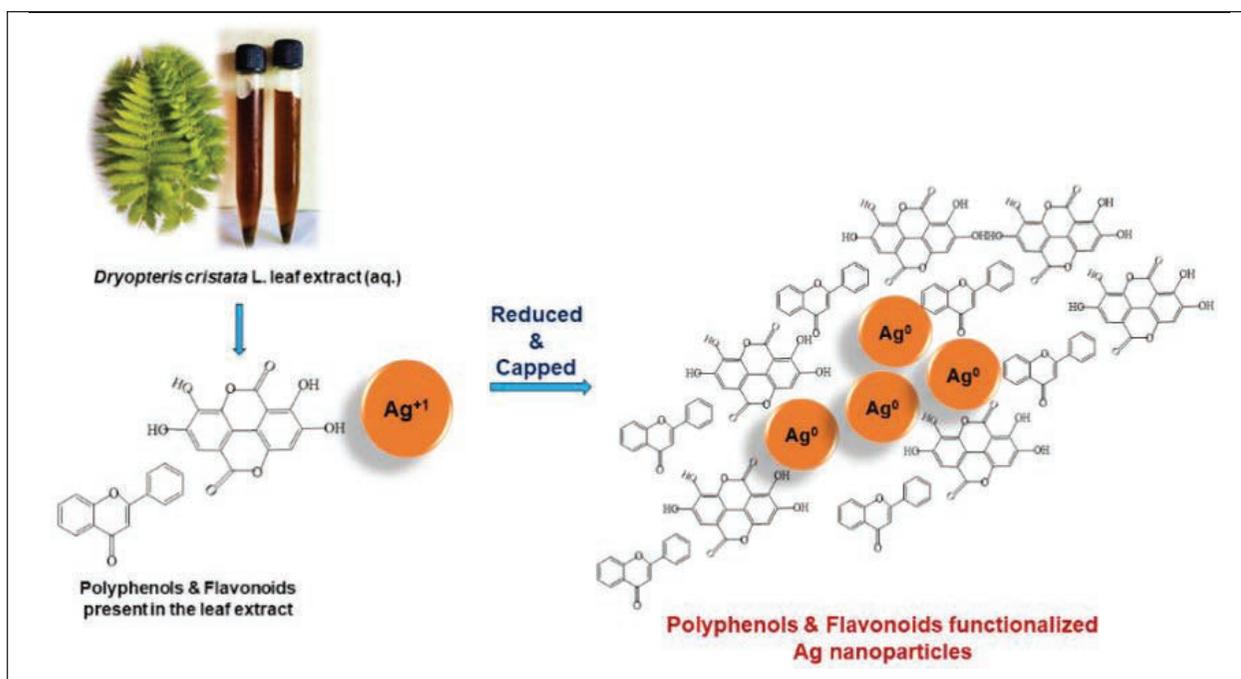


Fig. 1: Plausible Mechanism for the Formation of Dc-AgNPs by the Utilization of *Dryopteris cristata* L. Leaf Extract.

## ANTIOXIDANT ACTIVITY

### DPPH Free Radical Scavenging Assay

The antioxidant activity of the leaf extract was evaluated using the DPPH radical scavenging assay, following the protocol outlined by Sekar *et al.* (2020). Various concentrations of Dc-AgNPs, leaf extract, and ascorbic acid (30, 50, 70, 90 and 110  $\mu\text{g}/\text{mL}$ ) were mixed with 100  $\mu\text{L}$  of

0.1 mM DPPH solution in 80% ethanol. The mixtures were incubated in darkness at room temperature for 30 min. The absorbance of the samples was measured at 517 nm using a LABTRONICS LT-291 spectrophotometer, with 80% ethanol as the blank. The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{DPPH radical scavenging ability (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

The IC<sub>50</sub> values for the leaf extract and Dc-AgNPs were determined from the plotted graph using the equation  $Y = mX + C$  and the linear regression coefficient.

### ABTS Free Radical Scavenging Assay

The ABTS radical scavenging activity of various concentrations of the leaf extract and Dc-AgNPs was assessed using a standardized method (Sekar *et al.*, 2020). A stock solution of ABTS was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate, followed by incubation in darkness at room temperature for 16 h. The working solution was then prepared by diluting the stock solution with methanol to achieve an absorbance of approximately  $0.80 \pm 0.15$  at 734 nm. For the assay, 20  $\mu$ L of each concentration (30, 50, 70, 90 and 110  $\mu$ g/mL) of the leaf extract and Dc-AgNPs were mixed with 100  $\mu$ L of the ABTS working solution. The mixture was incubated for 30 min at room temperature. Absorbance was measured at 734 nm using a LABTRONICS LT-291 spectrophotometer,

with ascorbic acid serving as the standard. The percentage of ABTS radical scavenging activity was calculated using the formula:

$$ABTS \text{ radical scavenging ability (\%)} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

## RESULTS AND DISCUSSION

### PHYTOCHEMICAL SCREENING

Table 1 illustrates a detailed phytochemical analysis of *Dryopteris cristata* leaf extract. The presence of various phytochemicals was confirmed through distinctive reactions, such as the development of specific colorations and precipitates. The analysis identified a diverse range of phytoconstituents in the leaf extract, including phenols, tannins, flavonoids, glycosides, steroids, terpenoids, and alkaloids. These findings highlight the complex phytochemical profile of the leaf extract.

**Table 1: Phytochemical Constituents Present in *Dryopteris cristata* Leaf Extract (aq.).**

Sl. No.	Phytochemical	Test performed	Observation	Inference
1	Saponins	Foam test	The emergence of a dense and persistent layer of frothy.	(+)
2	Quinones	Borntreger's test	There was no formation of red coloration upon the addition of KOH solution.	(-)
3	Terpenoids and Steroids	Salkowski test	Reddish-brown coloration is formed in the organic layer.	(+)
4	Phenols and Tannins	FeCl <sub>3</sub> test	Blue-black colored solution is formed.	(+)
5	Flavonoids	Alkaline reagent test	An intense yellow-colored solution is formed, which turns colorless upon the addition of few drops of dilute HCl.	(+)
6	Alkaloids	Wagner's test	Reddish-brown colored precipitate is formed.	(+)

(+) means presence and (-) means absence of the respective phytochemicals.

### CHARACTERIZATION OF DC-AGNPS

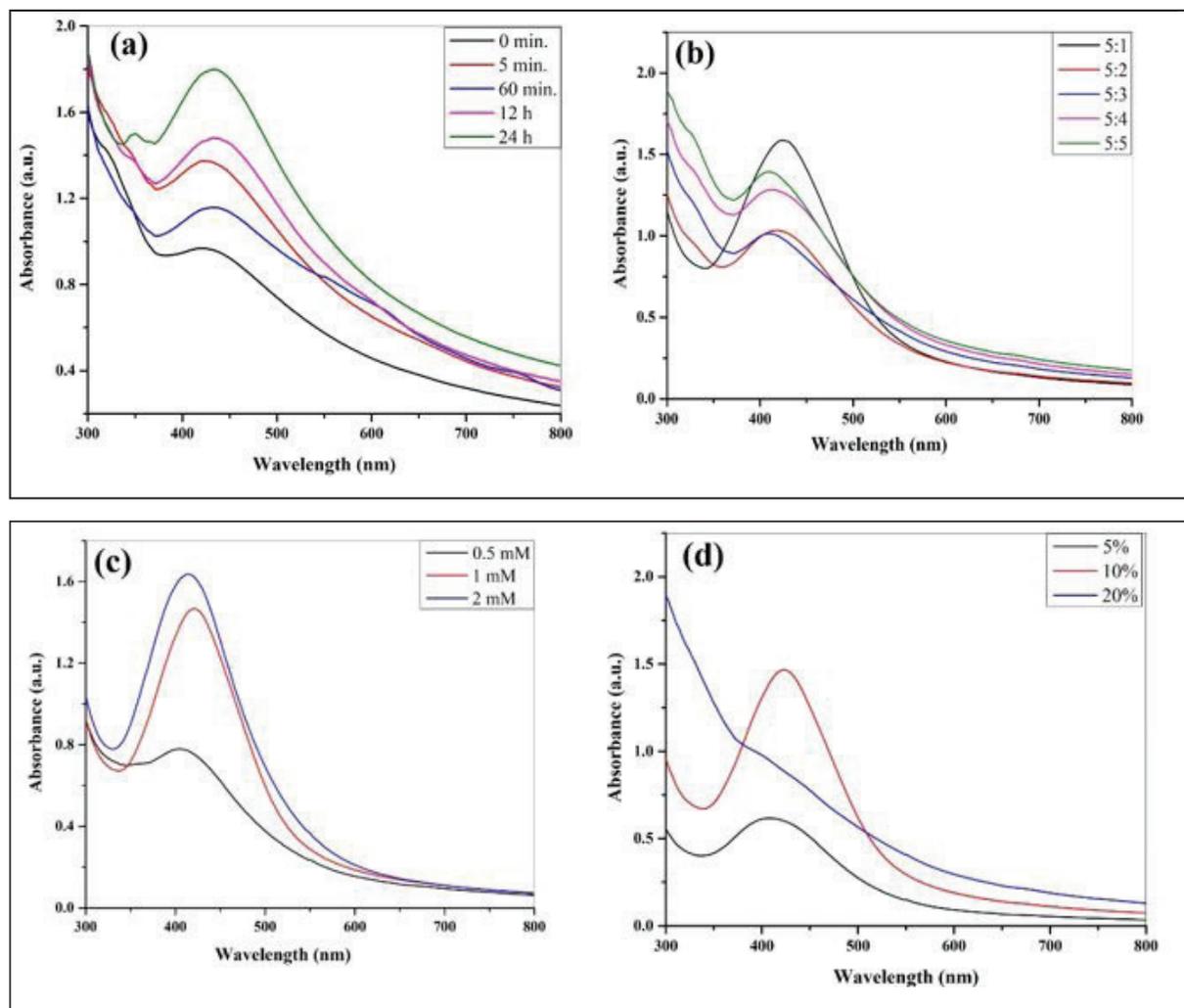
The synthesis of Dc-AgNPs was verified through UV-Vis. spectroscopy, with spectra recorded at specific intervals to monitor the progress of nanoparticle formation. Samples were withdrawn at 0 min, 5 min, 60 min, 12 h, and 24 h to track the kinetics of Dc-AgNPs synthesis. The UV-Vis. spectra exhibited a distinct bell-shaped peak, confirming nanoparticle formation, as depicted in Fig. 2(a). This peak is indicative of surface plasmon resonance (SPR), where the free electrons in the nanoparticles oscillate in response to incident light, resulting in an absorption band in the 390-420 nm range due to Mie scattering phenomena (Ouedraogo *et al.*, 2022; Nayak *et al.*, 2016). A broad absorption band within the 400-460 nm range was observed, characteristic of Mie scattering. Optimization of synthesis parameters was performed, including the concentration of the leaf

extract, the ratio of precursor solution to leaf extract, and the concentration of the precursor solution. Fig. 2(b) illustrates the effect of varying volumes of *Dryopteris cristata* leaf extract mixed with a 2 mM precursor solution at a 1:5 ratio. Increased leaf extract volume led to a broader SPR peak and a shift in the absorption band, resulting in the selection of a 5:1 ratio of precursor solution to leaf extract as optimal. The effect of precursor concentration on Dc-AgNPs synthesis was examined, as shown in Fig. 2(c). Different concentrations of AgNO<sub>3</sub> (0.5 mM, 1 mM, and 2 mM) were evaluated while maintaining constant other parameters. Higher concentrations of AgNO<sub>3</sub> enhanced the intensity of the absorbance peak, indicating that increased precursor concentration facilitates the formation of smaller and more numerous nanoparticles. Thus, a 2 mM AgNO<sub>3</sub>

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solution was identified as optimal. Fig. 2(d) presents the influence of leaf extract concentration (v/v) on Dc-AgNPs synthesis. A 5% leaf extract concentration resulted in a broad SPR peak with lower intensity, while a 20% concentration produced no significant peak. Conversely, 10% leaf extract concentration yielded a prominent peak

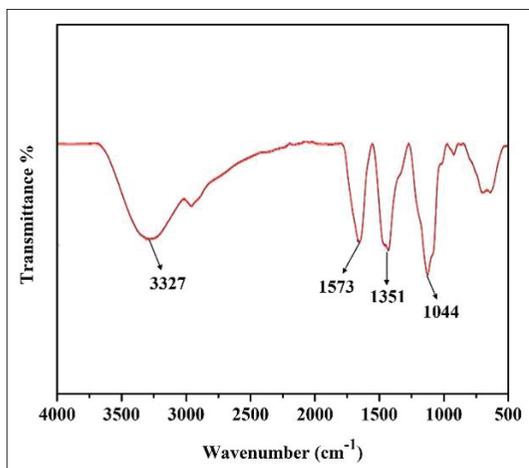
at approximately 425 nm, suggesting improved interaction between bioactive compounds and the precursor solution, leading to the formation of highly stable Dc-AgNPs. Consequently, 10% (v/v) leaf extract concentration was selected for further optimization.



**Fig. 2: UV-Vis. Analysis of Dc-AgNPs Colloidal Solution; (a) Absorbance Spectra at Different Time Intervals, (b) Effect of Leaf Extract Volumes, (c) Effect of Precursor Concentrations and (d) Effect of Leaf Extract Concentrations.**

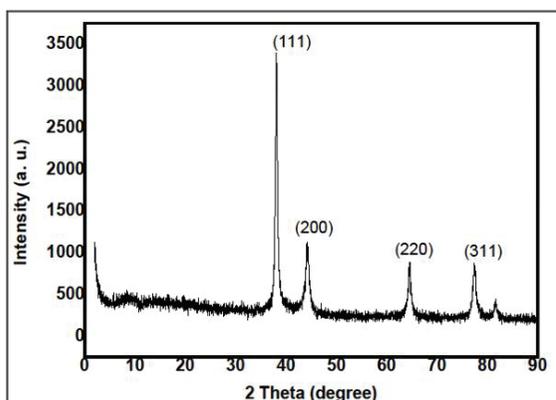
FT-IR spectroscopy was employed to characterize the secondary metabolites involved in the reduction and stabilization of Dc-AgNPs. The spectrum (Fig. 3) revealed prominent absorption bands/peaks at  $3327\text{ cm}^{-1}$ ,  $1573\text{ cm}^{-1}$ ,  $1351\text{ cm}^{-1}$  and  $1044\text{ cm}^{-1}$ , indicating the presence of phytoconstituents that function as capping agents. The broad band at  $3327\text{ cm}^{-1}$  corresponds to O-H stretching vibrations of phenolic compounds in the leaf extract. The

peak at  $1573\text{ cm}^{-1}$  is attributed to C-C stretching vibrations in alkenyl or aromatic rings. The peak at  $1351\text{ cm}^{-1}$  represents O-H bending vibrations, while the peak at  $1044\text{ cm}^{-1}$  is associated with C-O stretching in phenolic groups (Ramteke *et al.* 2012). These band/peaks are indicative of the phenolic groups in polyphenols and flavonoids present in the leaf extract, suggesting their involvement in the interaction with the biosynthesized nanoparticles.



**Fig. 3: FT-IR Spectrum of Dc-AgNPs.**

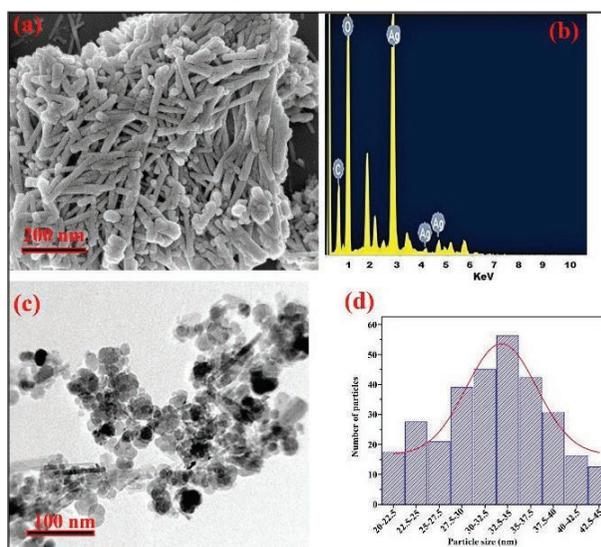
Fig. 4 illustrates the XRD analysis of the biosynthesized Dc-AgNPs, and four distinct diffraction peaks are observed at  $38.26^\circ$ ,  $44.45^\circ$ ,  $64.56^\circ$ , and  $77.48^\circ$ , which correspond to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes, respectively, as indicated by JCPDS card No. 04-0783 (Syed *et al.*, 2016). These peaks confirm the face-centered cubic (fcc) crystalline structure of the Dc-AgNPs. The observed XRD patterns are influenced by the nanoparticle size, which affects peak positions and intensities. The leaf extract's reducing agents contribute to the stabilization of Dc-AgNPs and support their crystalline formation, as extensively documented in biosynthesis studies (Meng, 2015). Using Scherrer's equation, the average crystallite size of the Dc-AgNPs was found to be 20.12 nm.



**Fig. 4: XRD Pattern of Dc-AgNPs.**

The morphology of Dc-AgNPs was characterized using SEM, which revealed that the nanoparticles exhibited polymorphic shapes, including irregularly granulated and ellipsoidal forms, and were highly aggregated (Fig. 5(a)). The ellipsoidal and aggregated morphology was further confirmed by the

TEM image, as depicted in Fig. 5(c). The average length of the nanoparticles ranged from 20 to 45 nm (Fig. 5(a)), consistent with previous studies on silver nanoparticles synthesized from *Prosopis juliflora* (Raja *et al.*, 2012), where the majority of nanoparticles had length between 30 and 37.5 nm, with only a small fraction exhibiting extreme values (Fig. 5(d)). Elemental analysis using EDX identified a prominent peak at 3 keV, confirming silver as the primary component (Fig. 5(b)). Additionally, the detection of carbon and oxygen peaks suggests the involvement of bioactive organic compounds from the leaf extract in the reduction and stabilization processes of Dc-AgNPs. The elemental composition of the nanoparticles was determined to be 78.35% silver, 18.91% carbon, and 2.74% oxygen.



**Fig. 5: Electron Micrograph Analysis of Dc-AgNPs; (a) SEM Image, (b) EDX Spectrum, (c) TEM Image and (d) Particle Size Distribution.**

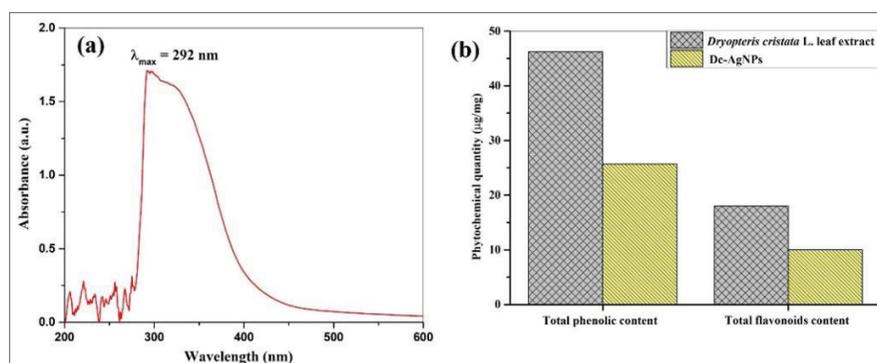
### ESTIMATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT IN THE LEAF EXTRACT AND DC-AGNPS

The absorbance peak at 292 nm in the *Dryopteris cristata* leaf extract indicates the presence of polyphenols and flavonoids (Fig. 6(a)). Quantitative analysis revealed that the total phenolic content in the leaf extract was  $46.21 \pm 1.25$  mg gallic acid equivalents (GAE) per 100 g, and the flavonoid content was  $18.02 \pm 0.13$  mg catechin equivalents (CAE) per mL. In contrast, the colloidal solution of Dc-AgNPs contained  $25.67 \pm 2.13$  mg GAE per 100 g and  $10.04 \pm 1.36$  mg CAE per mL (Fig. 6(b)). These results indicate a higher concentration of phenolic and flavonoid compounds in the leaf extract compared to the Dc-AgNPs. The data suggest that the polyphenolic and flavonoid compounds in the

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*Dryopteris cristata* leaf extract function as effective reducing agents, facilitating the conversion of Ag<sup>+</sup> ions to Dc-AgNPs. This observation is consistent with findings from other plant extracts, including leaves, stems, fruits, barks, and roots, which have demonstrated potential in nanoparticle synthesis via Ag<sup>+</sup> ion reduction. The leaf extract showed approximately twice the phenolic and flavonoid content compared to the resulting Dc-AgNPs. Given their known antioxidant properties, these bioactive compounds suggest that the *Dryopteris cristata* leaf extract has significant

antioxidant activity. Consequently, Dc-AgNPs synthesized from *Dryopteris cristata* leaves could function as potent antioxidants with potential therapeutic applications, utilizing the leaf extract as a sustainable and cost-effective bio-reducing agent. Similar findings have been reported for *Erythrina suberosa* (Roxb.) (Mohanta *et al.*, 2017) and *Tagetes erecta* L. (Nagaich *et al.*, 2016), underscoring the versatility of plant-derived bio-reducing agents in nanoparticle synthesis for commercial applications.

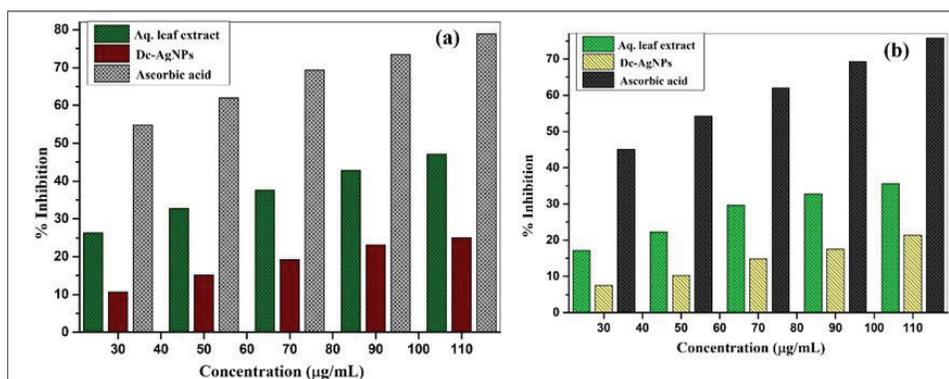


**Fig. 6: *Dryopteris Cristata* L. aq. Leaf Extract, (a) Absorption Spectrum; (b) Total Phenolic and Flavonoid Content of the Leaf Extract and Dc-AgNPs Colloidal Solution.**

### ANTIOXIDANT ACTIVITY USING DPPH AND ABTS ASSAY

The *in vitro* antioxidant activity of *Dryopteris cristata* leaf extract and Dc-AgNPs was evaluated using DPPH (Fig. 7(a)) and ABTS assays (Fig. 7(b)). The results demonstrated a dose-dependent increase in scavenging activity for both the leaf extract and Dc-AgNPs relative to ascorbic acid, the standard antioxidant. Notably, Dc-AgNPs exhibited superior radical scavenging efficacy, with IC<sub>50</sub> values of 15.27  $\mu\text{g/mL}$  for DPPH and 10.23  $\mu\text{g/mL}$  for ABTS, compared to the leaf extract's IC<sub>50</sub> values of 32.71  $\mu\text{g/mL}$  and 22.35  $\mu\text{g/mL}$ ,

respectively. These assays highlighted the Dc-AgNPs' ability to neutralize both neutral and cationic radicals, with the DPPH assay reflecting the nanoparticles' capacity to transfer electrons and neutralize DPPH radicals, while the ABTS assay indicated their ability to scavenge ABTS cationic radicals through both electron and hydrogen transfer mechanisms (Sekar *et al.*, 2020). These findings elucidate the multifaceted antioxidant mechanisms of Dc-AgNPs, demonstrating their effective role in neutralizing unstable radicals.



**Fig. 7: Antioxidant Activity Study of Different Concentrations of *Dryopteris Cristata* L. aq. Leaf Extract and Dc-AgNPs; (a) DPPH Scavenging Assay and (b) ABTS Assay.**

Table 2 illustrates the comparative analysis of various green synthesized AgNPs regarding their antioxidant properties, assessed through DPPH and ABTS scavenging assays. The IC<sub>50</sub> values obtained from these assays are indicative of the antioxidant efficacy, with a lower IC<sub>50</sub> value signifying stronger activity, as they represent a reduced concentration required to inhibit 50% of free radicals. The data shows that *Dryopteris cristata* leaf extract exhibits a lower IC<sub>50</sub> value in both assays compared to *Cucumis prophetarum* (Table 2, Entry 2) and *Alnus nitida* (Table 2, Entry 1) leaf extracts, indicating its superior antioxidant potential. Similarly, Dc-AgNPs present a markedly lower IC<sub>50</sub> value in both assays compared to other biosynthesized AgNPs (Table 2, Entries 1-5), demonstrating their enhanced antioxidant activity. This enhanced antioxidant capacity of Dc-AgNPs is attributed to the synergistic interaction between the

*Dryopteris cristata* leaf extract and AgNPs, which collectively increase the efficacy of free radical neutralization. The leaf extract independently shows strong antioxidant activity, and its incorporation into AgNPs further amplifies this effect. In comparison, Bhutto *et al.* (2018) reported that AgNPs synthesized with polyphenolic compounds exhibited higher IC<sub>50</sub> values, suggesting lower antioxidant activity relative to Dc-AgNPs. Similarly, Alves *et al.* (2018) observed that poly(3-aminophenyl) boronic acid-poly (vinyl alcohol) (PABA-PVA) mediated AgNPs also demonstrated higher IC<sub>50</sub> values compared to Dc-AgNPs. Therefore, Dc-AgNPs exhibit significant synergistic antioxidant activity owing to the combined effect of the *Dryopteris cristata* leaf extract, underscoring their potential for use in therapeutic applications as effective antioxidants.

**Table 2: Comparative Study of the Antioxidant Activity of Green Synthesized AgNPs.**

Sl. No.	Sample	Method of preparation	IC <sub>50</sub> value (µg/mL)	Ref.
1	<i>Alnus nitida</i> leaf extract (aq.)  AgNPs	Biological synthesis	37.35 (DPPH assay) 24.25 (ABTS assay)  19.25 (DPPH assay) 16.46 (ABTS assay)	Khuda <i>et al.</i> , 2023
2	<i>Cucumis prophetarum</i> leaf extract (aq.)  Cp-AgNPs	Biological synthesis	33.31 (DPPH assay) 50 (ABTS assay)  29.2 (DPPH assay) 34.5 (ABTS assay)	Hemlata <i>et al.</i> , 2020
3	<i>Reynoutria japonica</i> Houltt root extract (aq.)  AgNPs	Biological synthesis	27.80 (DPPH assay) 25.25 (ABTS assay)  19.25 (DPPH assay) 18.88 (ABTS assay)	Khuda <i>et al.</i> , 2022
4	AgNPs ( <i>Alcaligenes faecalis</i> GH3 cell extract aq.)	Biological synthesis	710 (DPPH assay) 220 (ABTS assay)	Badoei-dalfard <i>et al.</i> , 2019
5	CA-AgNPs	Biological synthesis	55.66 (DPPH assay) 25.77 (ABTS assay)	Mendam <i>et al.</i> , 2023
6	<i>Dryopteris cristata</i> leaf extract (aq.)	Biological synthesis	32.71 (DPPH assay) 22.35 (ABTS assay)	Present work
	Dc-AgNPs		15.27 (DPPH assay) 10.23 (ABTS assay)	

## CONCLUSION

In summary, the leaf extract of *Dryopteris cristata* was employed to synthesize Dc-AgNPs, with the extract's phytochemicals acting as both reducing and capping agents, thereby stabilizing the silver nanoparticles. FT-IR analysis further substantiated the involvement of these

phytochemicals in the nanoparticle synthesis. The leaf extract is rich in polyphenols and flavonoids, which are indicative of its inherent antioxidant properties. Antioxidant assays, including DPPH and ABTS scavenging tests, demonstrated that the synthesized Dc-AgNPs exhibited

superior radical scavenging activity compared to the leaf extract, with efficacy increasing with higher concentrations. These results highlight the potential of Dc-AgNPs as effective free-radical scavengers and underscore their promise in developing potent antioxidants for addressing diseases associated with oxidative stress.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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