

Article

Green Fabrication of Silver Nanoparticles Using Secondary Metabolites of *Leptogium austroamericanum*, *Parmotrema melanothrix*, and *Heterodermia diademata*: A Sustainable Bio-reduction Approach

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Abstract: Nanoparticles (NPs) are synthesized from natural sources, including cyanobacteria, algae, plants, fungi, and lichens, offer an eco-friendly, safe, and cost-effective alternative to conventional methods. This work highlights the phytochemical extraction of secondary metabolites, which encompass several bioactive compounds with anti-inflammatory, antitumor, cytotoxic, antibacterial, and antifungal properties. Compounds such as benzene, 1-ethyl-3-methyl-, nonadecane, 1-octadecanesulphonyl chloride, decane, 2,3,5,8-tetramethyl-, and chloroxine exhibit antitumor properties. Furthermore, AgNPs biosynthesized by *L. austroamericanum*, *P. melanothrix*, and *H. diademata* demonstrated unique and potent biological activities and held promise as sustainable alternatives to synthetic antimicrobial and therapeutic agents. This study underscores the potential of lichen-based nanotechnology for biomedical applications as a sustainable, environmentally friendly approach.

Keywords: *L. austroamericanum*; *P. melanothrix*; *H. diademata*; lichens; Phytochemical analysis; Secondary metabolites; Silver nanoparticles

1. Introduction

Lichens are complex symbiotic organisms composed of a fungal partner (mycobiont) and a photosynthetic partner (photobiont), which may be an alga or a cyanobacterium [1]. Over 20,000 species have been reported worldwide, thriving in terrestrial ecosystems and diverse ecological conditions, and contributing significantly to nutrient cycling, soil formation, and habitat provisioning. Lichens have traditionally been valued for their medicinal properties [2–5]. In India, the Western Ghats represent a major biodiversity hotspot for lichen flora, with 54 families and nearly 950 species across approximately 150 genera, including foliose, fruticose, crustose, and squamulose growth forms [6,7]. The most prominent families, such as Parmeliaceae, Graphidaceae,



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Physciaceae, Pyrenulaceae, Thelotremaaceae, Arthopyreniaceae, Astrotheliaceae, Porinaceae, Pannariaceae, Lobariaceae, and Teloschistaceae, are particularly abundant on bark and rock substrates [8,9]. Parmeliaceae species, including *Lobaria pulmonaria* and *Pseudevernia furfuracea*, have been extensively studied [10]. Members of the Lecanoraceae, such as *Protoparmeliopsis muralis*, *Lecanora muralis*, and *Xanthoria elegans*, have also been widely reported [11–13]. Several lichen families have been exploited for nanoparticle synthesis, including Graphidaceae (*Sticta* spp.), Peltigeraceae (*Acroscyphus* spp.), Collemataceae (*Leptogium puberulum*), and Usneaceae (*Usnea antarctica*) [13,14]. Recent studies have expanded the scope of green nanoparticle synthesis to include Parmeliaceae species such as *Parmeliopsis ambigua*, *Punctelia subrudecta*, *Evernia mesomorpha*, *Xanthoparmelia plitti*, *Parmotrema praesorediosum*, *Parmotrema tinctorum*, *Parmelia perlata*, *Parmelia sulcata*, *Usnea longissima*, *Ramalina dumeticola*, and *Ramalina sinensis* [15–18]. Similarly, species of Cetrariaceae, such as *Cetraria islandica* and *Cladonia rangiferina*, have been investigated for the synthesis of metal and metal-oxide nanoparticles [13,19,20]. Advancements in metabolomic tools, including high-resolution LC–MS/MS, UPLC–MS/MS, and advanced mass spectrometry platforms, have greatly facilitated the identification of lichen-derived secondary metabolites [21,22]. These metabolites exhibit a wide range of biological activities, including antioxidants, antimicrobial, cytotoxic, wound-healing, enzyme-inhibitory, anti-inflammatory, and analgesic effects Kumar et al. [23].

The green synthesis of nanoparticles harnesses the natural biochemical processes of lichens to produce metal-based nanostructures (1–100 nm) in an eco-friendly and cost-effective manner without toxic reagents [16,24]. Lichens have been used to synthesize a wide range of metal and metal oxide nanoparticles, including those of silver (Ag), gold (Au), copper (Cu), iron (Fe), zinc (Zn), nickel (Ni), cobalt (Co), titanium dioxide (TiO₂), and zinc oxide (ZnO), each exhibiting unique physicochemical properties not observed in their bulk materials [25–28]. Biosynthesized NPs are commonly characterized using analytical techniques such as ultraviolet–visible spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and X-ray diffraction [14,18]. Elemental mapping and morphology analysis have revealed well-defined NP structures, including uniformly distributed cubic particles [13,29]. Moreover, one-pot synthesis methods have enabled the fabrication of complex nanocomposites, such as ZnO/TiO₂/SiO₂ and Fe₃O₄/SiO₂, using extracts of *Lecanora muralis* [10,12]. Lichens are attractive platforms for green nanotechnology because they accumulate structurally diverse secondary metabolites by reducing chelating, antioxidant, and surface-stabilizing capabilities [30,31]. Compared with a purely taxonomic description, this chemical rationale better explains why lichen extracts can drive AgNP formation and simultaneously influence the particles' biological behavior [32,33]. Their metabolites also provide a mechanistic basis for the antibacterial and anticancer effects observed in lichen-mediated nanoparticles. Lichen-derived nanoparticles, particularly those composed of silver, copper, and iron oxide, have been synthesized from *Parmotrema praesorediosum*, *Xanthoria elegans*, *Cetraria islandica*, *Usnea antarctica*, *Leptogium puberulum*, and *Usnea longissimi* [34]. Although lichen-mediated AgNP synthesis has been reported for several species, including *Usnea*, *Parmotrema*, and *Xanthoria*, the present study is distinct because it integrates GC–MS-defined metabolite chemistry with AgNP formation, stability, and bioactivity across three lichen species [35–37]. This approach provides a species-resolved chemical rationale for nanoparticle reduction and capping, rather than only documenting nanoparticle production. These nanoparticles exhibited broad-spectrum antimicrobial activity and effectively inhibited gram-positive bacteria, including *Staphylococcus aureus*, *S. mutans*, *S. pyogenes*, *S. viridans*, *Corynebacterium diphtheriae*, and *C. xerosis*. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are gram-negative bacteria [13,16,17]. Metal oxide nanoparticles synthesized from *Protoparmeliopsis muralis* and *Lecanora muralis* exhibit potent antibacterial, anti-quorum-sensing, antioxidant, and antifungal activities. These NPs are effective against multidrug-resistant pathogens and fungal species, including *Candida albicans*, *Candida* spp., *Aspergillus flavus*, *A. niger*, and *A. terreus* [11,12].

Leptogium (Ach.) Gray (Collemataceae, Ascomycota) is a foliose lichen genus that has been extensively studied, with approximately 70 and 180 taxonomically identified species [6,38]. The genus was first discovered in Brazil by Eschweiler (1833). *Leptogium* species, such as *L. bullatum* (Ach.) Nyl., are widely distributed in humid temperate and tropical regions, and *L. rivulare* (Ach.) Mont. and *L. tremelloides* (L.f.) are also reported from these regions [39]. Heterodermia species (Physciaceae; Lecanorales; Ascomycota) constitute some of the most common foliose lichens in tropical and subtropical regions. The genus comprises approximately 210 species within 59 families and is characterized by the absence of a lower cortex and the presence of norstictic and salazinic acids as the predominant medullary compounds [6,40,41]. *Parmotrema* (Hale) (Parmeliaceae, Lecanorales) is another diverse genus of foliose lichens commonly found on tree bark and rocks in tropical and subtropical environments [42–45]. While numerous studies have reported plant and fungal-mediated synthesis of silver nanoparticles (AgNPs), reports employing lichen-derived secondary metabolites remain comparatively few studies. In this

present study, three key findings were highlighted: (a) GC-MS-based identification of lichen-derived secondary metabolites revealed significant antimicrobial and anticancer potential. (b) *L. austroamericanum*, *P. melanothrix*, and *H. diademata* lichen extracts effectively mediate the green synthesis of stable, crystalline, and monodispersed silver nanoparticles (AgNPs); and (c) the biosynthesized AgNPs physicochemical characteristics played a critical role in determining their biological activity and stability. Therefore, this work advances lichen-mediated nanotechnology by establishing a mechanistic relationship between lichen metabolites, nanoparticle formation, and potential biomedical applications. Therefore, the present study investigates the phytochemical composition and nanoparticle synthesis potential of *L. austroamericanum*, *P. melanothrix*, and *H. diademata*. Overall, this study paves the way for future applications in antimicrobial therapy, cancer treatment, and nanotechnology-based drug delivery systems. Unlike conventional plant-fungal-mediated syntheses, lichen-derived AgNPs are formed in the presence of a chemically distinct metabolite repertoire that includes halogenated aromatics, phenolics, esters, and lichen-specific secondary metabolites. These compounds can simultaneously reduce Ag⁺ ions, cap the nanoparticle surface, and contribute intrinsic bioactivity, thereby integrating nanoparticle formation and functional performance within a single biologically derived system. The present study is therefore novel in linking GC-MS-identified lichen metabolites with AgNP_s formation, physicochemical stability, and antibacterial/anticancer potential across three lichen species.

2. Results

2.1. Morphological Characterization of Lichens

The morphological examination of the three lichen species revealed distinct structural features adapted to their respective habitats. *L. austroamericanum* showed a soft, gelatinous, foliose thallus that appeared bluish gray to dark brown when dry and turned olive-green when wet, containing nitrogen-fixing components (Figure 1A). *P. melanothrix* (Figure 1B) exhibited a large, loosely attached foliose thallus with broad, gray to greenish-gray lobes, black ciliate margins, and a black lower surface with dense rhizines aiding attachment. In contrast, *H. diademata* possessed a whitish-gray foliose thallus with narrow lobes, white margins, and a black underside, often bearing soredia or isidia for vegetative propagation (Figure 1C). These morphological variations reflect their adaptation to humid, well-lit, and aerated environments, contributing to their ecological diversity and functional significance. The materials and methods are detailed in the Supporting Information file.

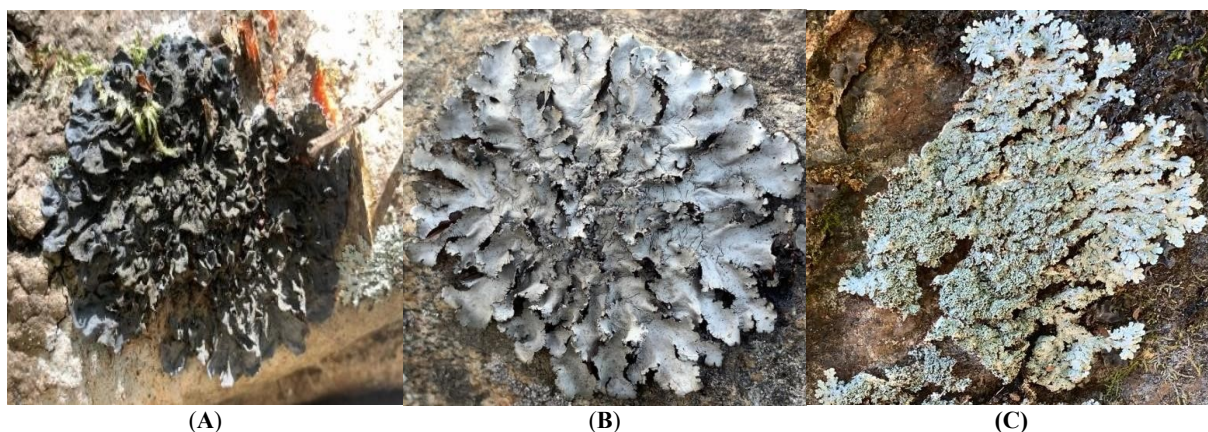


Figure 1. Lichen samples from different phenotype species (A) *Leptogium austroamericanum* (B), *Parmotrema melanothrix*, and (C) *Heterodermia diademata*.

2.2. Phytochemical and Biological Activity of Lichen Extracts

Metabolic compounds were extracted using acetone from various lichen species, including *L. austroamericanum*, *P. melanothrix*, and *H. diademata*, and evaluated for anti-tumor, antimicrobial, and anti-inflammatory activities (Figure 2A–C). Based on GC-MS profiling, varying numbers of compounds were identified in the extracts from *L. austroamericanum* (41), *P. melanothrix* (35), and *H. diademata* (33), respectively (Figure 2), including diverse functional groups such as alcohols, aldehydes, aromatic hydrocarbons, carboxylic acids, esters, hydrocarbons, chlorine-containing compounds, bromine-containing compounds, iodine-containing compounds, and low-reliability compounds with distinct biological roles (Table 1). Alcohols played an essential role; for example, 1-octanol, 2,7-anti-microbial, ethyl-, and α -acorenol acted as penetration enhancers with mild antimicrobial and anti-inflammatory properties, whereas tridecanol derivatives functioned as surfactants.

Aldehydes such as 7-hexadecenal (Z) served as signaling molecules and displayed moderate antimicrobial effects. Aromatic hydrocarbons such as Mesitylene and Naphthalene exhibited cytotoxic properties, whereas halogenated compounds (e.g., 2-bromotetradecane, Octadecane, 1-chloro-) showed antibacterial potential due to halogen substitution. Benzoic acid, a major carboxylic acid, is a well-known antimicrobial preservative (Table 1). Several metabolites identified through GC-MS analysis likely contributed directly to AgNP formation. Phenolic and aromatic compounds such as chloroxine and benzoic acid derivatives can donate electrons for Ag⁺ reduction, while ester-containing compounds such as Butyl everninate may adsorb onto the nanoparticle surface and act as stabilizing agents. These metabolites collectively regulate nucleation, growth, and long-term colloidal stability. GC-MS identification of chloroxine, butyl everninate, benzoic acid derivatives, and related aromatic/oxygenated metabolites suggests a dual role in AgNP biosynthesis. Phenolic and carbonyl-containing molecules can transfer electrons to Ag⁺, initiating nucleation, whereas esterified and hydrophobic metabolites may adsorb onto the nanoparticle surface and suppress uncontrolled aggregation. In addition, chloroxine may contribute intrinsic antibacterial and anticancer activity through membrane perturbation and signaling interference, while butyl everninate likely supports colloidal stabilization and augments the bioactivity profile of the final nanomaterial.

Other notable compounds include chloroxine, recognized for its antibacterial and antifungal activity, and butyl everninate, a lichen-derived ester with strong antioxidant and antibiotic properties. Fumaric acid derivatives (esters) exhibit anti-inflammatory effects, whereas phthalate esters, though widely used industrially, have endocrine-disrupting potential. Ethers and hydrocarbons serve as emollients and hydrophobic agents, with some, such as longifolene, offering anti-inflammatory benefits. Iodine-containing compounds, such as 1-iodo-2-methylundecane, may act as antiseptics, and steroidal compounds like estra-1,3,5(10)-trien-17 β -ol (estradiol) exhibit strong hormonal activity. Among the identified compounds, five demonstrated notable anticancer potential: butyl everninate, benzoic acid, chloroxine, estradiol, and longifolene. These compounds are known to induce apoptosis, inhibit cancer cell proliferation, and interfere with tumor-related enzymatic pathways (Table 1). Overall, the lichen extracts contained a broad range of bioactive compounds such as benzoic acid, butyl everninate, chloroxine, α -acorenol, and longifolene, which showed strong antibacterial and antifungal effects. Mesitylene, benzene derivatives, octadecane (2-methyl-), and heptadecane (2,6,10,15-tetramethyl-) displayed antioxidant and anti-inflammatory activity, while 1-octadecanesulphonyl chloride, 2-bromotetradecane, and chlorinated alkanes contributed to cytotoxic responses. These metabolites reflect the potential of lichens for pharmacological and ecological applications, providing a biochemical basis for the comparative biological activity observed among the three samples. These results support the biochemical potential of lichen-derived AgNPs, which may be correlated with their observed biological activities.

Table 1. GC-MS elution and analysis of bioactive compounds from Lichen species.

Functional Group	Name of the Compounds	Molecular Formula	MW	RT	S1	S2	S3	Biological Activity
					Peak Area (%)			
Alcohols	1-Octanol, 2,7-dimethyl-	C ₁₀ H ₂₂ O	158	3.79	3.19	4.19	4.86	Microbial metabolic pathways, antimicrobial
	Tridecanol, 2-ethyl-2-methyl-	C ₁₆ H ₃₄ O	242	31.89	1.36	1.63		antimicrobial and antifungal activities and pharmacological
	α -acorenol	C ₁₅ H ₂₆ O	222	20.12	0.97	0.58		Antimicrobial, antifungal, with alpha-glucosidase
Aldehydes	7-Hexadecenal, (Z)-	C ₁₆ H ₃₀ O	238	39.27	1.26		4.78	Insect sex pheromone
Aromatic Hydrocarbons	Benzene, 1,2,3-trimethyl-	C ₉ H ₁₂	120	6.44	2.24	3.57	4.71	Neurotoxin in a plant metabolite and antioxidants.
	Benzene, 1-ethyl-3-methyl-	C ₉ H ₁₂	120	5.02	5.63	8.74	8.67	Antifungal, anti-inflammatory, and antitumor properties
	Mesitylene	C ₉ H ₁₂	120	5.71	11.16	17.64	21.71	Solvent, low bioactivity, toxic at high exposure
	Naphthalene	C ₁₀ H ₈	128	11.59	1.81	1.32	1.6	Insecticidal, fumigant, toxic/carcinogenic
Bromine-containing	2-Bromotetradecane	C ₁₄ H ₂₉ Br	276	21.18	2.86			Biocidal/reactive
Carboxylic acids	Benzoic acid	C ₇ H ₆ O ₂	122	19.28	31.72			Antibacterial, antifungal, food,
Chlorine-containing	Octadecane, 1-chloro-	C ₁₈ H ₃₇ Cl	288	27.9	3.12	1.37		Chlorinated alkane, mild biocidal/toxic effects
Diverse functional groups	1-Octadecanesulphonyl chloride	C ₁₈ H ₃₇ ClO ₂ S	352	41.63	1.1	6.41	7.64	Cytotoxic, reactive reagent, biocidal action
	2-Piperidinone, N-[4-bromo-n-butyl]-	C ₉ H ₁₆ BrNO	233	24.8	0.68			Pharmacological or enzyme inhibitory
	Butyl everminate	C ₁₃ H ₁₈ O ₄	238	32.84	8.76	33.96	30.05	Antimicrobial activity
	Chloroxine	C ₉ H ₅ Cl ₂ NO	213	26.8		2.83	1.68	Antibacterial, antifungal
	Hexasiloxane, tetradecamethyl-	C ₁₄ H ₄₂ O ₅ Si ₆	458	29.48	0.72			Biologically inert, cosmetic-grade silicone
Esters	Fumaric acid, decyl non-5-yn-3-yl ester	C ₂₃ H ₃₈ O ₄	378	36.32		1.05	1.06	Antimicrobial, antioxidant
	Phthalic acid, butyl tetradecyl ester	C ₂₆ H ₄₂ O ₄	418	40.48	1.48		1.52	Endocrine disruptor, toxic, environmental hazard
	Isopropyl tetradecyl ether	C ₁₇ H ₃₆ O	256	23.28	1.61			Chemically inert, bioactivity
	1-Hexene, 4-methyl-	C ₇ H ₁₄	98	3.19	4.44	8.48	8.05	Reactive, toxic at high exposure
Hydrocarbons	2-Methyltetracosane	C ₂₅ H ₅₂	352	22.59	1.15			Insect pheromone role
	Decane	C ₁₀ H ₂₂	142	7.29			0.91	Solvent, low biological activity
	Decane, 2,3,5,8-tetramethyl	C ₁₄ H ₃₀	198	15.28	0.82	1.58	1.22	Metabolite in cancer cell in terpenoids
	Dodecane, 2,6,10-trimethyl-	C ₁₅ H ₃₂	212	21.98	0.65		1.01	Pheromonal function
	Octadecane, 2-methyl-	C ₁₉ H ₄₀	268	23.89	1.33	1.74	0.82	antioxidant, anti-inflammatory & antibacterial
	Longifolene	C ₁₅ H ₂₄	204	20.23	2.36			Antimicrobial, anti-inflammatory
	Heptadecane, 2,6,10,15-tetramethyl-	C ₂₁ H ₄₄	296	39.13	1.75	1.45		Antioxidant and antimicrobial
	Nonadecane	C ₁₉ H ₄₀	268	35.08	1.02			Antimicrobial and anticancer
	1-Iodo-2-methylundecane	C ₁₂ H ₂₅ I	296	17.13	0.78	1.02	0.73	Analgesic and anti-diarrheal
Low reliability	4,7-Methanoindene, 3a,4,5,6,7,7a-hexahydro-, endo-	C ₁₀ H ₁₄	134	7.67	0.61	1.66		Halogenated alkane, toxic/reactive
	Estra-1,3,5(10)-trien-17 β -ol	C ₁₈ H ₂₄ O	256	41.96	0.47			Hormonal, estrogenic, endocrine-active

GC-MS analysis showing their functional groups, molecular formula, compound class, molecular weight (MW), retention time (RT), percentage (%) of area, and retention index (RI) of different lichen species (**S1**), *L. austroamericanum*, (**S2**), *P. melanothrix*, and (**S3**), *H. diademata*.

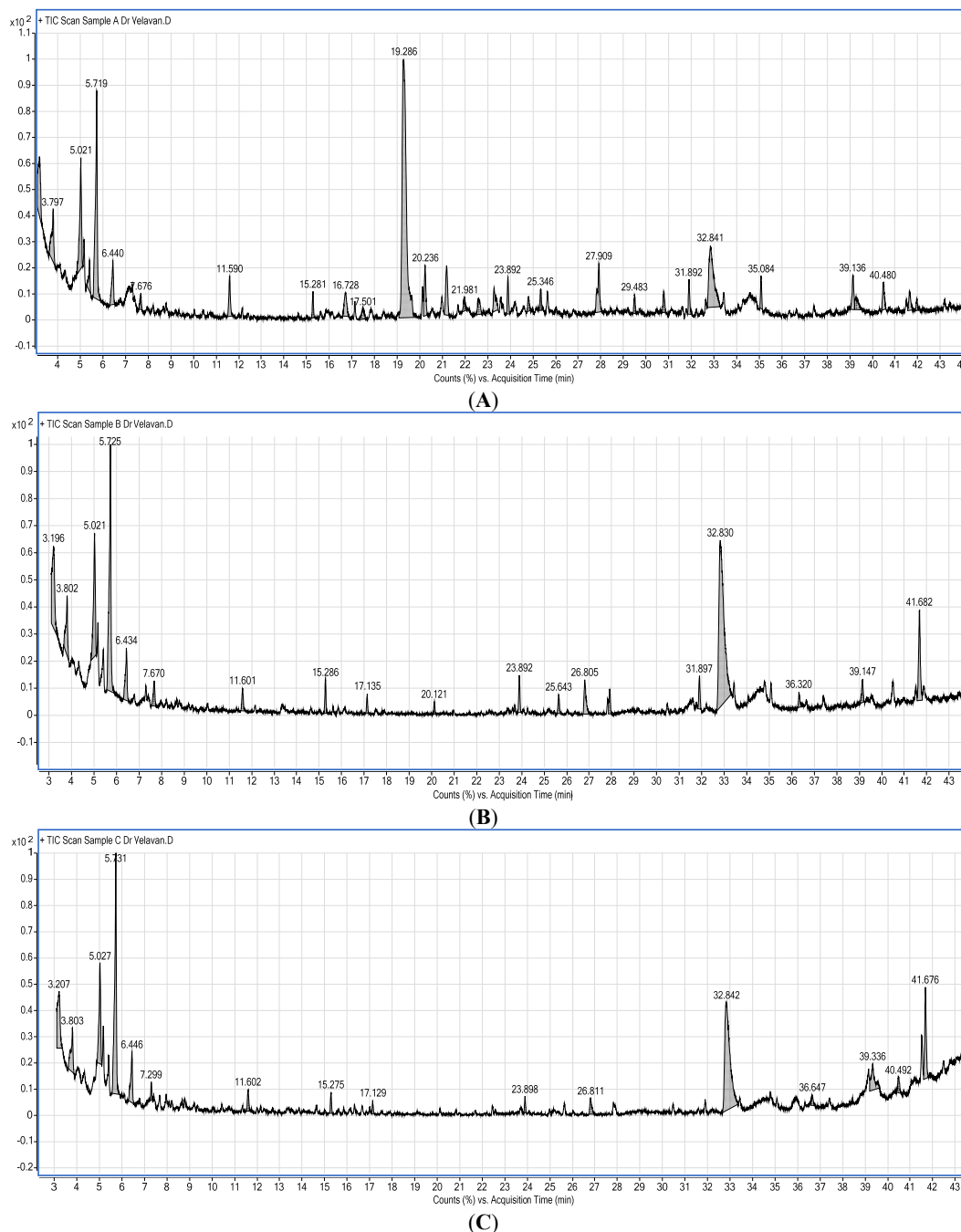


Figure 2. GC-MS analysis for methanol extraction from lichens and chromatogram showing peak (A) *L. austroamericanum*, (B) *P. melanothrix*, (C) *H. diademata*.

2.3. UV-Spectrophotometer

The formation of silver nanoparticles (AgNPs) is typically indicated by a color change in the solution, which turns dark brown or purple owing to the interaction between the nanoparticles and the organic molecules present in the acetone extract. In this study, silver nitrate (AgNO_3) was mixed with an aqueous-acetone extract of *L. austroamericanum*, *P. melanothrix*, and *H. diademata* and incubated at room temperature. After 72 h, the solution developed a purple color, with no further noticeable changes, confirming the successful synthesis of AgNPs. UV-Vis spectral analysis of the colloidal solution, conducted between 200 and 500 nm at room temperature, revealed peaks at 350 and 400 nm. The prominent peak at approximately 390–420 nm corresponds to the excitation of the surface plasmon resonance of AgNPs from *L. austroamericanum* (Figure 3A), *P. melanothrix* (Figure 3B), and *H. diademata* (Figure 3C). The control solution, without silver nitrate, showed no evidence of absorption in the range of 300 to 900 nm. The presence of a broad resonance indicates the aggregation of silver nanoparticles in the solution.

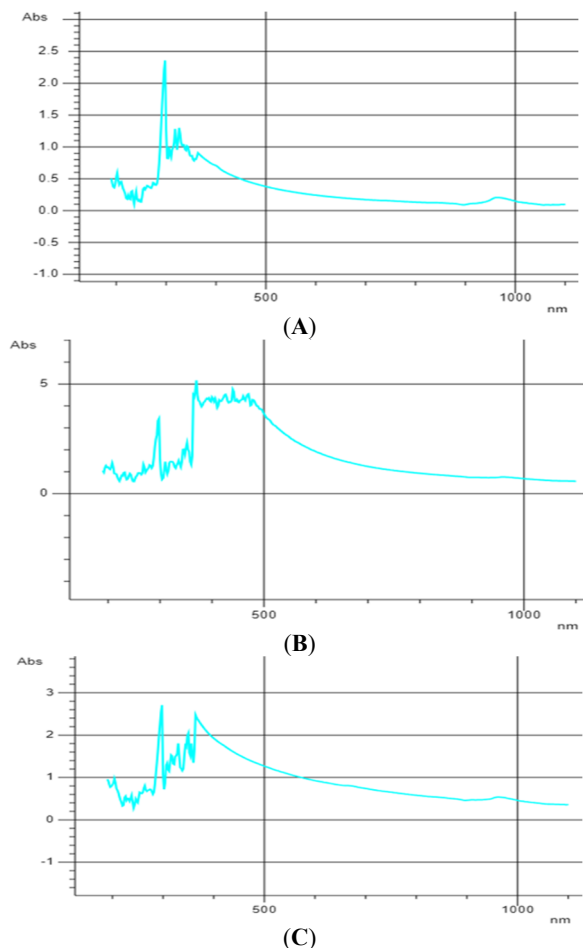


Figure 3. UV absorption spectrum of silver nanoparticle synthesized by methanolic extracts of (1 mM), (A) *L. austroamericanum*, (B) *P. melanothrix* and (C) *H. diademata*.

2.4. Fourier-Transform Infrared Spectroscopy (FTIR)

Infrared (IR) spectroscopy is an essential tool for identifying molecular functional groups by recording the absorption of infrared light at specific frequencies, producing distinct vibrational signatures (Figure 4A–C). For instance, stretching vibrations of hydroxyl (O–H) groups in alcohols and carboxylic acids are typically observed between 3200 and 3600 cm^{-1} , as seen in ethanol. Hydrocarbons such as alkanes exhibit C–H stretching between 2850 and 2960 cm^{-1} , while thiol groups (S–H) generally absorb in the 2550–2600 cm^{-1} range, as seen in ethanethiol. Carbonyl-containing compounds display strong C=O stretching bands; esters (e.g., methyl acetate) show absorptions near 1735–1750 cm^{-1} , whereas aldehydes and ketones absorb around 1700–1750 cm^{-1} . Acid anhydrides exhibit two distinctive bands due to symmetric and asymmetric carbonyl stretching, occurring in the 1800–1850 cm^{-1} and 1740–1790 cm^{-1} ranges, respectively. Alkenes demonstrate C=C stretching near 1620–1680 cm^{-1} , while nitro compounds (e.g., nitrobenzene) reveal asymmetric N–O stretching absorptions in the 1500–1570 cm^{-1} range and symmetric N–O stretching between 1300 and 1370 cm^{-1} . The bending vibrations of methyl (–CH₃) groups appear at approximately 1375–1450 cm^{-1} , as illustrated for isopropanol (Table 2). Alcohols and ethers, such as glucose and diethyl ether, exhibit C–O stretching within the 1000–1300 cm^{-1} range. Aromatic structures, including benzene and styrene, show C–H bending absorptions in the 675–900 cm^{-1} region. Frequencies below 1500 cm^{-1} comprise the fingerprint region, which contains numerous unique absorption bands that help identify individual molecules. For instance, C–Br stretching vibrations are typically observed between 500 and 600 cm^{-1} , as in bromobenzene. These spectral ranges may vary slightly depending on the molecular environment, serving as a general reference for interpreting IR spectra (Figure 4A–C). The observed shifts in O–H, C=O, and C–O stretching frequencies after nanoparticle synthesis suggest active participation of hydroxyl and carbonyl groups in silver ion reduction and nanoparticle capping. These findings support the hypothesis that lichen metabolites function simultaneously as reducing and stabilizing agents.

The FTIR data were systematically organized to categorize the absorption peaks based on their chemical relevance. The “Compound Class” column groups the observed peaks according to their molecular type, such as alcohols, hydrocarbons, or aromatic and carbonyl compounds. Each vibrational motion, including stretching or

bending, in the “Group” column describes the molecular activity responsible for the absorption. The “Absorption (cm^{-1})” column records the exact wavenumber at which absorption occurs, indicating the corresponding energy transition. The “Appearance” column provides qualitative details such as broad, sharp, or strong, reflecting factors like hydrogen bonding or structural uniformity. Further clarification is offered in the “Comments” column, which explains the relevance of each band to the molecular structure (Table 2). The FTIR peak (cm^{-1}) values reaffirm the measured data, while the “Possible Functional Group/Vibration” column matches these peaks to known spectral assignments such as O–H stretching or C–H bending based on literature and reference databases. These columns provide a comprehensive framework for interpreting the FTIR spectrum, allowing for a detailed and justified assignment of functional groups to their corresponding absorption peaks.

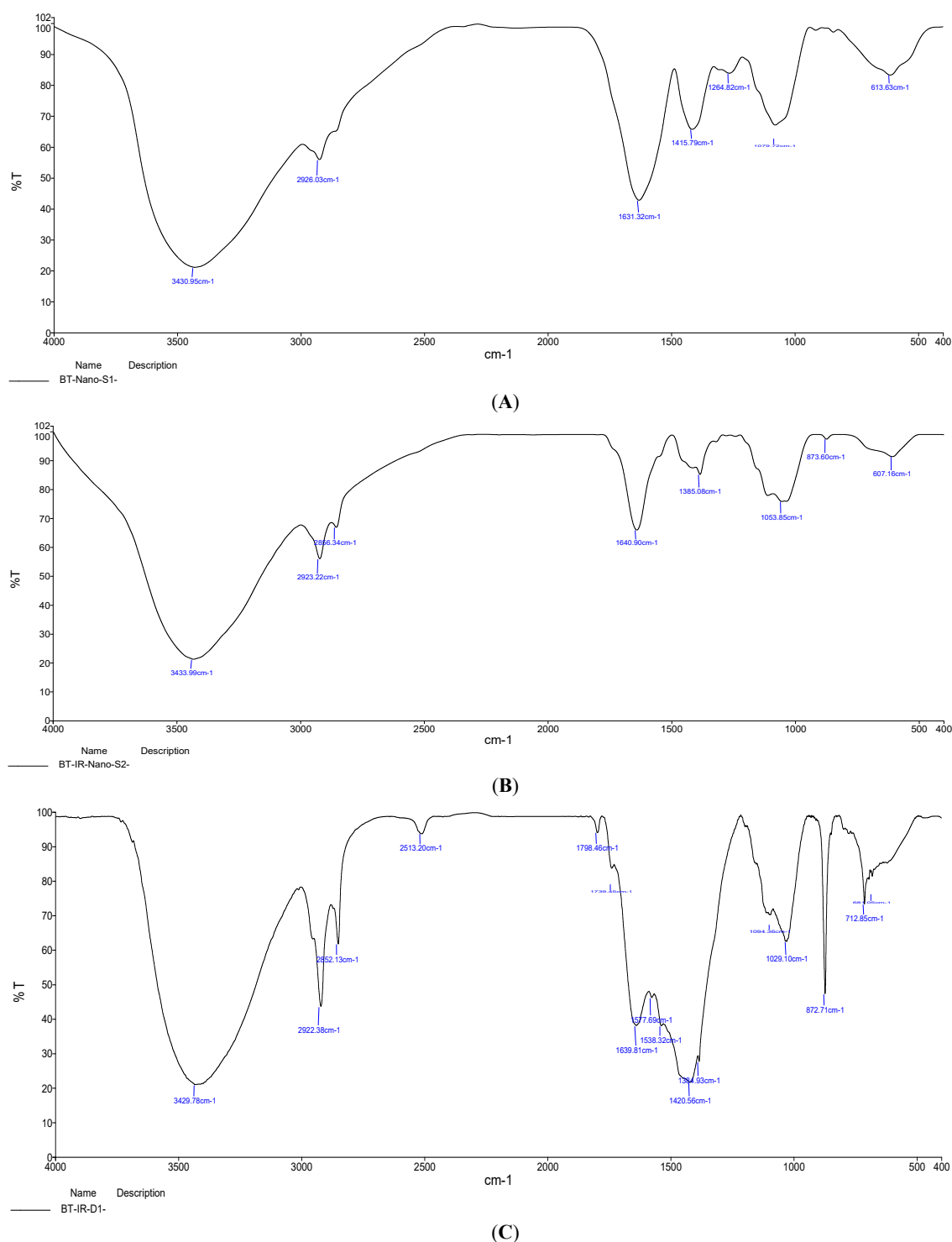


Figure 4. FTIR spectral images for silver nanoparticles synthesized by methanolic extracts of (A) *L. austroamericanum*, (B) *P. melanothrix* and (C) *H. diademata*.

Table 2. List of various peak values and functional group interpretations of biosynthesized silver nanoparticles (AgNPs), aqueous leaf extract of *L.austroamericanum*, *P. melanothrix*, and *H. diademata* by FT-IR analysis.

FTIR Peak (cm ⁻¹)	Molecular Formula	Compound Class	Example Chemical	Appearance	Functional Group/Bond Assignment	Probable Compound Class/Indication
3439.76	C ₂ H ₆ O	Alcohols/Phenols	Ethanol	Broad, strong	O–H stretching vibration	Alcohols, phenols, or adsorbed water; indicates hydrogen bonding
3433.56	C ₆ H ₆ O	Phenols	Phenol	Broad, strong	O–H stretching vibration	Alcohols or phenolic compounds; hydrogen bonding from biomolecules or adsorbed moisture
3430.56	C ₂ H ₆ O	Alcohols/Water	Ethanol	Broad, strong	O–H stretching vibration	Alcohols or phenolic –OH groups; possible adsorbed water on nanoparticle surface
2926.48	C ₆ H ₁₄	Aliphatic Hydrocarbons	Hexane	Sharp, strong	C–H asymmetric stretching	Aliphatic –CH ₂ or –CH ₃ groups (alkanes)
2925.63	C ₆ H ₁₄	Aliphatic Hydrocarbons	n-Hexane	Sharp, strong	C–H asymmetric stretching	Aliphatic –CH ₂ and –CH ₃ groups (alkanes, lipids)
2922.32	C ₆ H ₁₄	Aliphatic Hydrocarbons	Hexane	Sharp, strong	C–H asymmetric stretching	Aliphatic –CH ₂ and –CH ₃ groups (alkanes, fatty acids, or lipids)
2856.10	C ₆ H ₁₄	Aliphatic Hydrocarbons	n-Hexane	Sharp, moderate	C–H symmetric stretching	Aliphatic hydrocarbons; long-chain compounds
2852.64	C ₆ H ₁₄	Aliphatic Hydrocarbons	n-Hexane	Sharp, moderate	C–H symmetric stretching	Aliphatic hydrocarbons; long-chain alkane presence
1746.06	C ₄ H ₆ O ₃	Anhydrides/Esters	Acetic anhydride	Strong, sharp	C=O stretching	Esters, aldehydes, or carboxylic acids
1639.80	C ₃ H ₅ NO	Amides/Alkenes	Acrylamide	Moderate	Amide I (C=O stretching or C=C stretching)	Proteins, amides, or conjugated double bonds
1636.84	C ₂ H ₅ NO	Amides	Acetamide	Moderate	C=O stretching or amide I (C=O–N)	Proteins, enzymes, or amide linkages—biological capping/stabilization
1631.75	C ₂ H ₅ NO	Amides	Acetamide	Moderate	C=O stretching or amide I (C=O–N)	Proteins, amides, or conjugated carbonyl groups involved in nanoparticle capping
1537.64	C ₆ H ₅ NO ₂	Nitro Compounds	Nitrobenzene	Strong	N–O asymmetric stretching	Nitro compounds or secondary amides
1384.62	C ₃ H ₈ O	Alcohols	Isopropanol	Medium	C–H bending (methyl group)	Alkanes or alcohol derivatives
1384.16	C ₃ H ₈ O	Alcohols	Isopropanol	Medium	C–H bending (methyl group)	Methyl symmetric deformation in alkanes or alcohols
1384.13	C ₃ H ₈ O	Alcohols	Isopropanol	Medium	C–H bending (methyl group)	Alkane or alcohol bending vibrations
1318.18	C ₂ H ₇ N	Amines/Amides	Ethylamine	Medium	C–N stretching vibration	Amines or amides; possibly biomolecules
1242.60	C ₄ H ₁₀ O	Ethers/Esters	Diethyl ether	Medium	C–O stretching	Ethers, esters, or carboxylic acids
1098.46	C ₄ H ₈ O ₂	Esters/Ethers	Ethyl acetate	Strong	C–O stretching	Alcohols, esters, or polysaccharides; biomolecular origin
1085.98	C ₆ H ₁₂ O ₆	Carbohydrates	Glucose	Moderate	C–O stretching (fingerprint region)	Alcohols, esters, or carbohydrate linkages
1078.64	C ₄ H ₈ O ₂	Esters/Ethers	Ethyl acetate	Strong	C–O stretching	Alcohols, ethers, esters, or polysaccharides—typically from plant biomolecules
1016.82	C ₆ H ₁₂ O ₆	Carbohydrates	Glucose	Moderate	C–O–C stretching	Ether or carbohydrate structures (possible polysaccharide capping agents)
977.65	C ₈ H ₈	Alkenes	Styrene	Sharp	=C–H bending (alkene)	Unsaturated hydrocarbons
967.06	C ₈ H ₈	Aromatic Alkene	Styrene	Sharp	C=C bending	Possible alkene or aromatic substitution band
862.72	C ₆ H ₆	Aromatic Compounds	Benzene	Sharp	Aromatic C–H bending	Substituted aromatic ring
842.14	C ₆ H ₆	Aromatic Compounds	Benzene	Sharp	Aromatic C–H out-of-plane bending	Substituted benzene derivatives; aromatic compounds
792.94	C ₆ H ₆	Aromatic Compounds	Benzene	Sharp	Aromatic C–H out-of-plane bending	Mono- or di-substituted benzene ring
614.85	C ₆ H ₅ Br	Aromatic Halides	Bromobenzene	Weak	C–Br or metal–oxygen vibration	Possible C–Br stretch or Ag–O/Ag–N bonding indicating nanoparticle formation
612.48	C ₆ H ₅ Br	Aromatic Halides	Bromobenzene	Weak	Metal–oxygen (M–O) or C–Br stretching	Metal–ligand vibration confirming AgNP formation
549.36	Ag ₂ O	Metal Oxide	Silver oxide	Weak	Metal–oxygen (M–O) vibration	Confirms silver–oxygen or silver–nitrogen bonding indicating nanoparticle formation

2.5. Particle Size Analysis

Particle size analysis (PSA) is an important technique used to measure the size distribution of silver nanoparticles synthesized from lichens, including *L. austroamericanum*, *P. melanothrix*, and *H. diademata*. PSA plays a crucial role in characterizing silver nanoparticles for applications in proteins, polymers, cosmetics, fertilizers, soils, abrasives, and many more. Using this technique, the mean particle size and polydispersity index can be calculated. Figure 5A–C shows the particle size distribution of the three lichen species, with particle sizes of 140.6, 121.5, and 192.4 nm and polydispersity index (PDI) values of 0.227, 0.287, and 0.154, respectively. Based on the results obtained, the nanoscale particles were highly monodispersed. As discussed later, for silver nanoparticles (AgNPs), particle size also influences critical characteristics, including content uniformity, dissolution, and absorption rates. The AgNPs exhibited particle sizes ranging from approximately 121–192 nm. Smaller nanoparticles possess higher surface-area-to-volume ratios, facilitating enhanced interaction with bacterial membranes and cancer cells. The comparatively smaller particles synthesized from *P. melanothrix* may therefore demonstrate superior biological activity due to increased cellular penetration and reactive oxygen species generation.

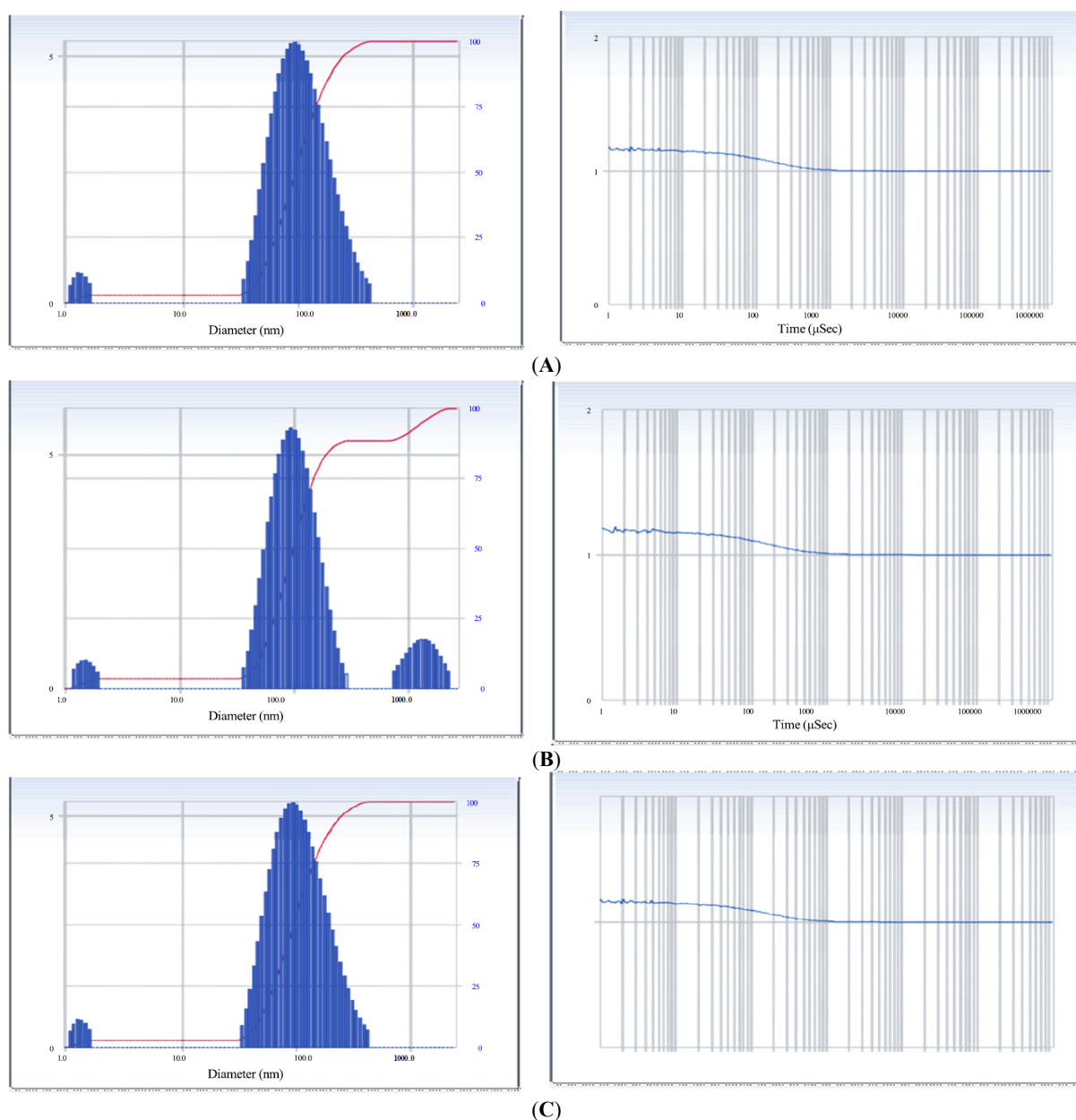


Figure 5. PSA of silver nanoparticles in particle size distribution histogram of biosynthesized AgNPs showing the size range and frequency, providing insights into their uniformity and average size (A) *L. austroamericanum*, (B) *P. melanothrix*, and (C) *H. diademata* mediated silver nanoparticles.

2.6. X-ray Diffraction (XRD) Analysis

The X-ray diffraction (XRD) patterns of tAgNPs synthesized using methanolic extracts of *L. austroamericanum* (Figure 6A), *P. melanothrix* (Figure 6B), and *H. diademata* (Figure 6C). The XRD analysis should be interpreted with caution. The low-angle peaks observed at $2\theta = 8.59^\circ$, 10.73° , 13.88° , 19.22° , and 22.97° are not characteristic of FCC metallic silver and therefore cannot be used to support Ag (0) formation. Metallic silver, when present, should be identified using the standard FCC reference pattern (JCPDS/ICDD No. 04-0783), with reflections indexed to the (111), (200), (220), (311), and (222) planes at approximately 38.1° , 44.3° , 64.4° , 77.4° , and 81.5° , respectively. Any additional low-angle reflections likely originate from lichen-derived organic residues or amorphous/capped components associated with the nanoparticle surface. Therefore, the XRD section has been revised to distinguish extract-derived features from metallic silver reflections and to avoid overinterpretation. The sharp diffraction peaks observed in XRD analysis indicate high crystallinity of the AgNPs. Increased crystallinity is associated with improved physicochemical stability and may contribute to enhanced antimicrobial efficacy through sustained silver ion release.

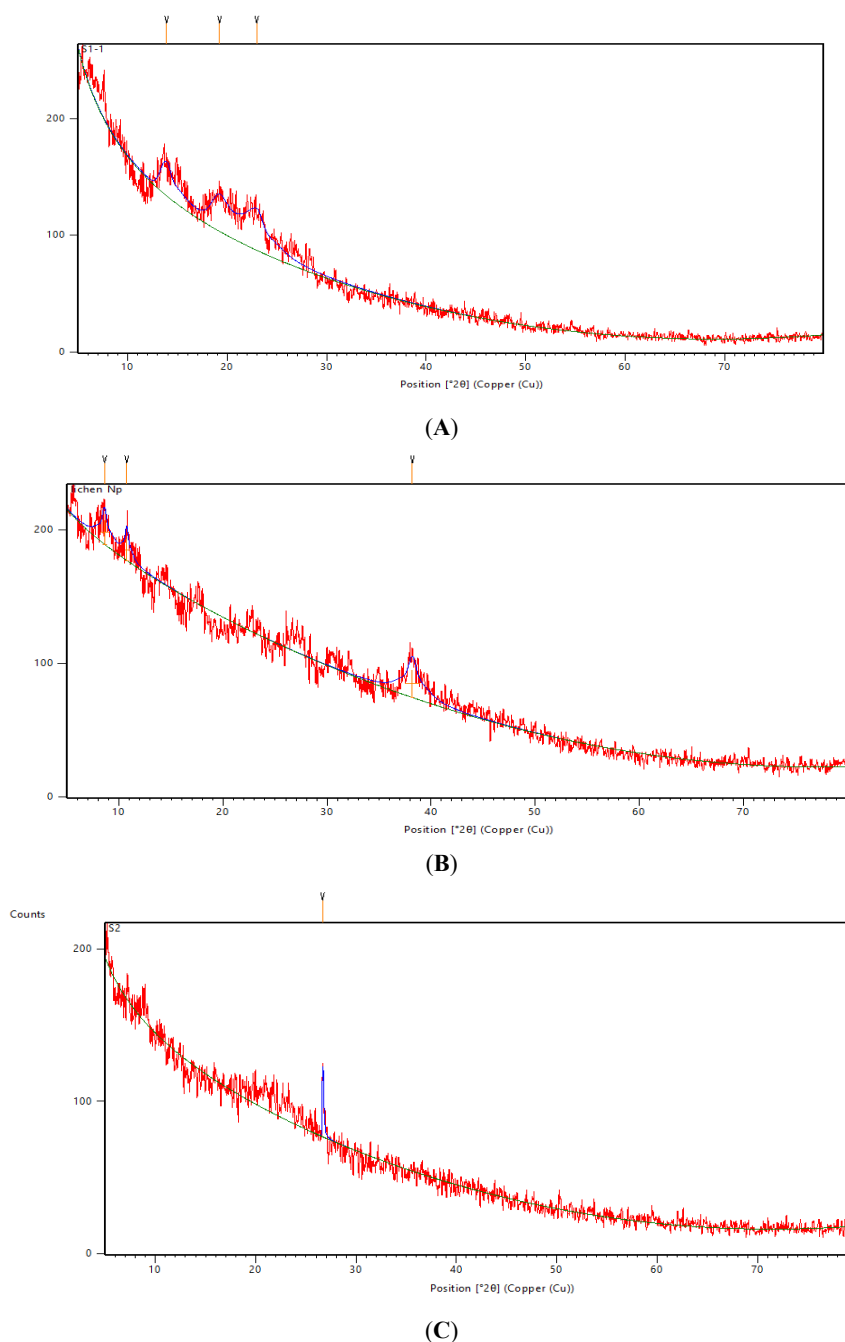


Figure 6. X-ray Diffraction (XRD) patterns of the silver nanoparticles (AgNPs), synthesized by methanolic extracts of (A) *L. austroamericanum*, (B) *P. melanothrix*, and (C) *H. diademata*.

2.7. Scanning Electron Microscopy (SEM) Analysis of Silver Nanoparticles

Phyto-synthesis of silver nanoparticles (AgNPs) using lichen acetone extracts, including *L. austroamericanum*, *P. melanothrix*, and *H. diademata*, was successfully confirmed through scanning electron microscopy (SEM). SEM analysis revealed the morphological characteristics of the nanoparticles, which appeared as a polydispersed mixture with sizes ranging from 150 to 200 nm for *L. austroamericanum* (Figure 7), *P. melanothrix* (Figure 8), and *H. diademata* (Figure 9). Most of the nanoparticles appeared predominantly spherical, although some exhibited slight variations (Figures 7–9). In some regions, slight aggregation occurred, likely due to the high surface energy of the AgNPs. The micrographs indicated that the particles were well developed with a relatively smooth and uniform surface topology (Figures 7–9). The observed polydispersity reflects differences in nucleation and growth processes during biosynthesis. Overall, the SEM findings provide valuable information on the size distribution and morphology of AgNPs, highlighting their suitability for potential applications in biomedical, antimicrobial, and nanotechnological fields.

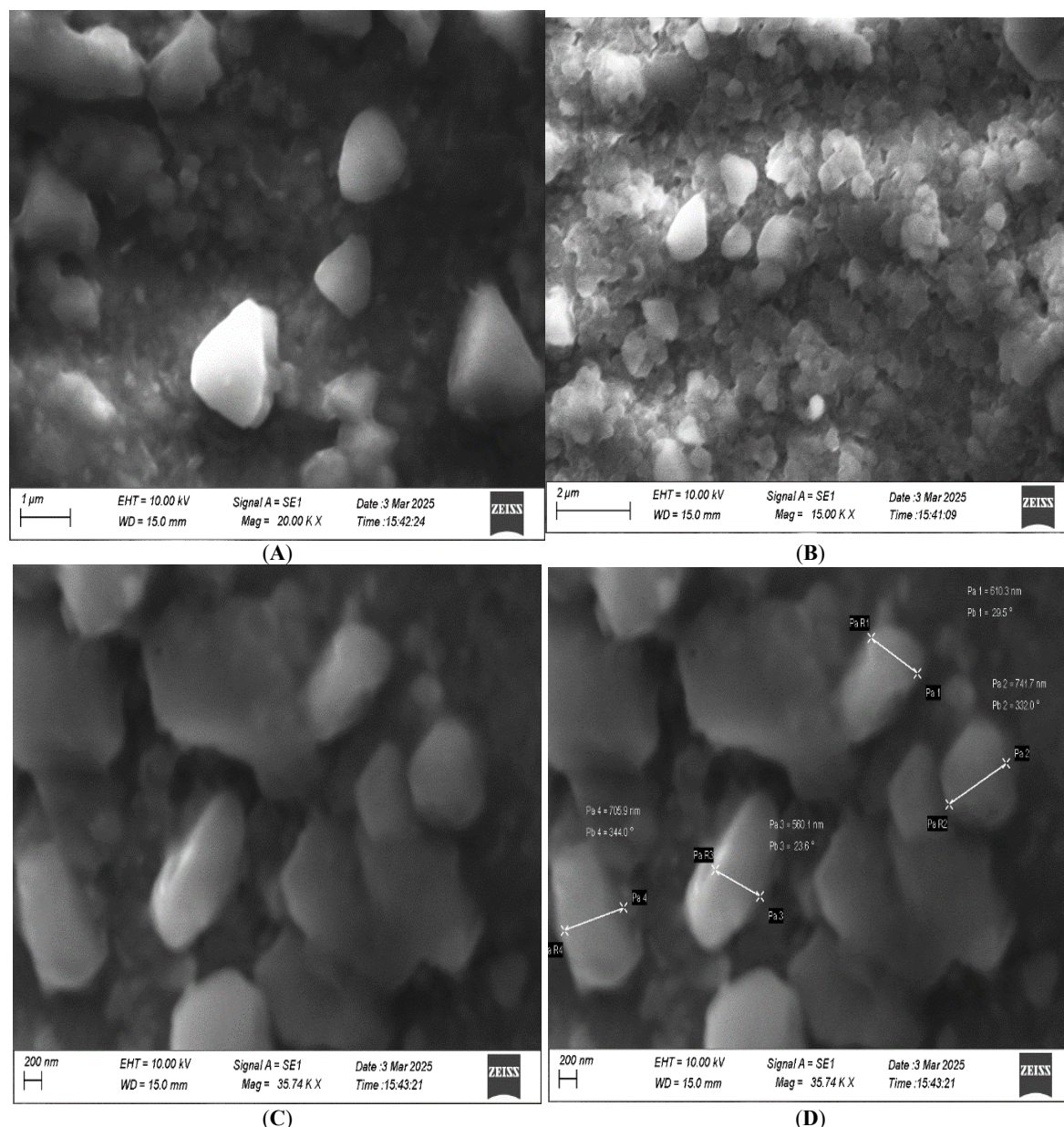


Figure 7. Scanning electron microscope image of silver nanoparticles synthesized by using methanolic extracts of *L. austroamericanum*, (A) 1 μm of AgNPs, (B) 2 μm of AgNPs, (C & D) 200 nm of AgNPs.

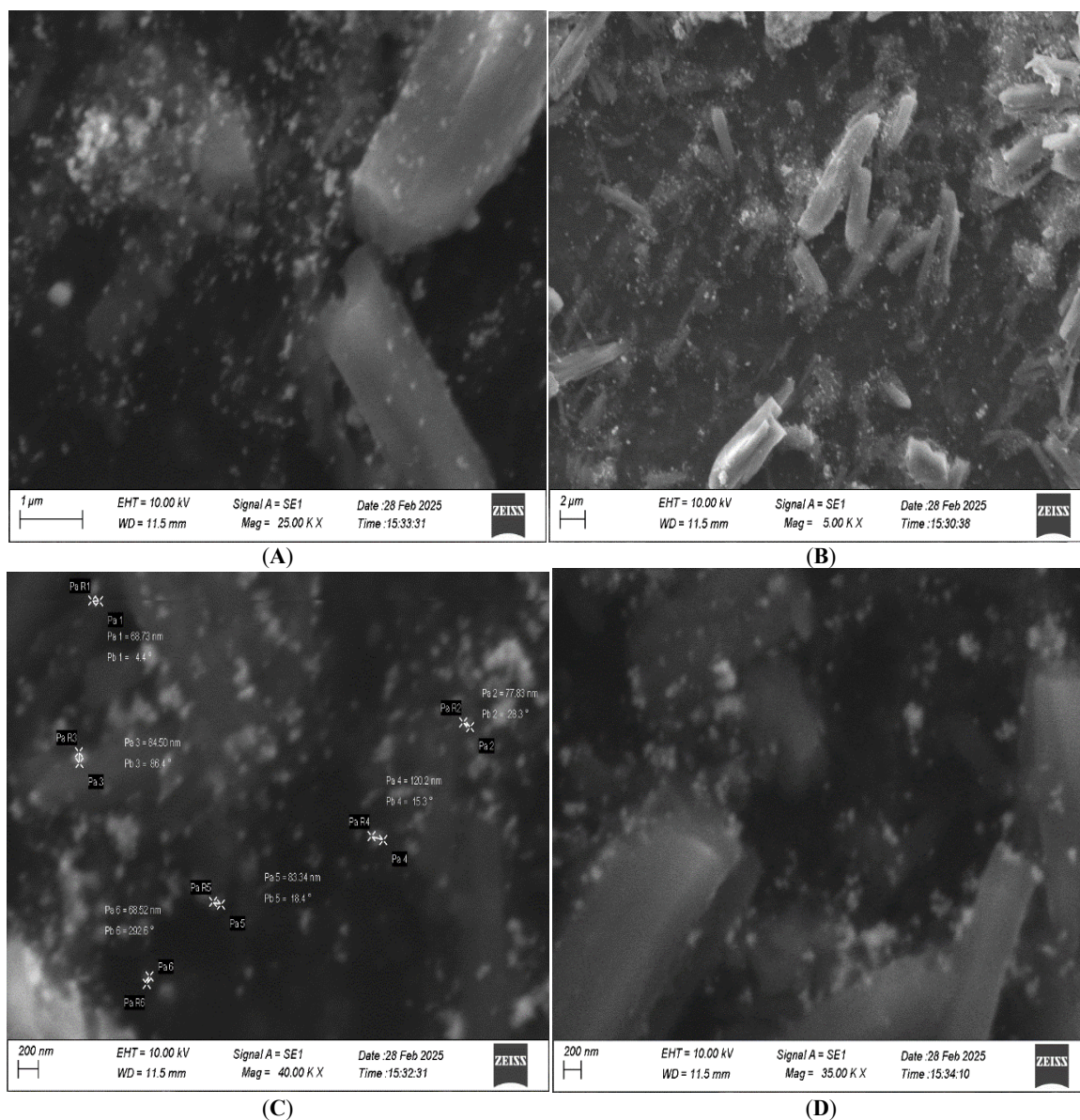
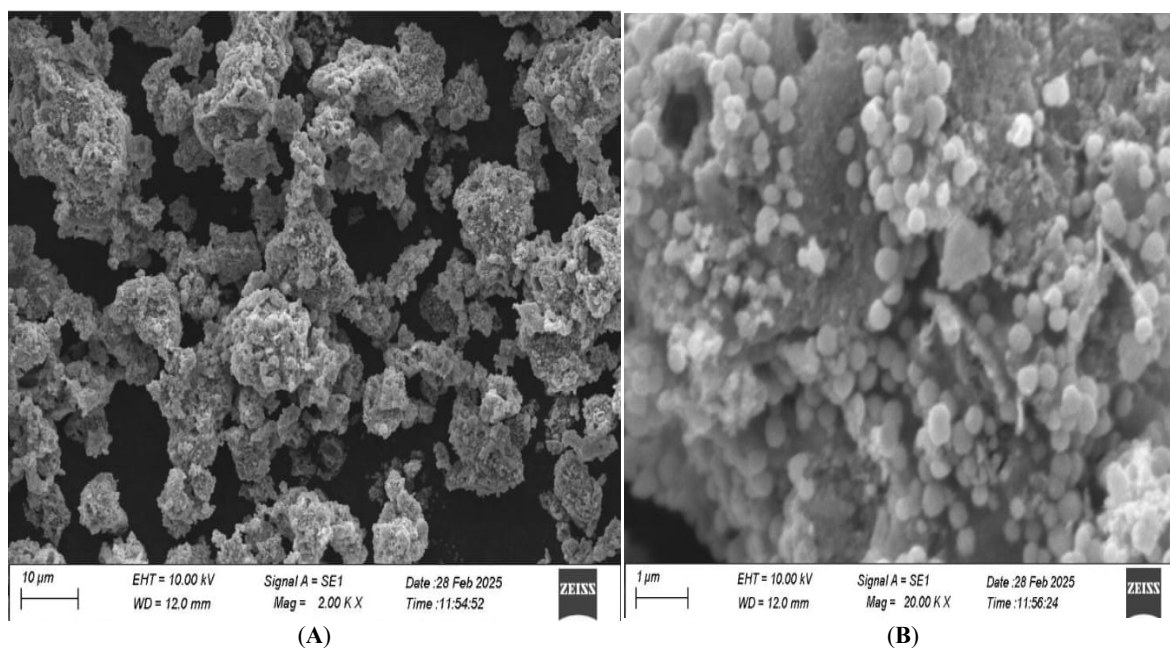


Figure 8. Scanning electron microscope image of silver nanoparticles synthesized by using methanolic extracts *P.melanothrix*, (A) 1 µm of AgNPs, (B) 2 µm of AgNPs, (C & D) 200 nm of AgNPs.



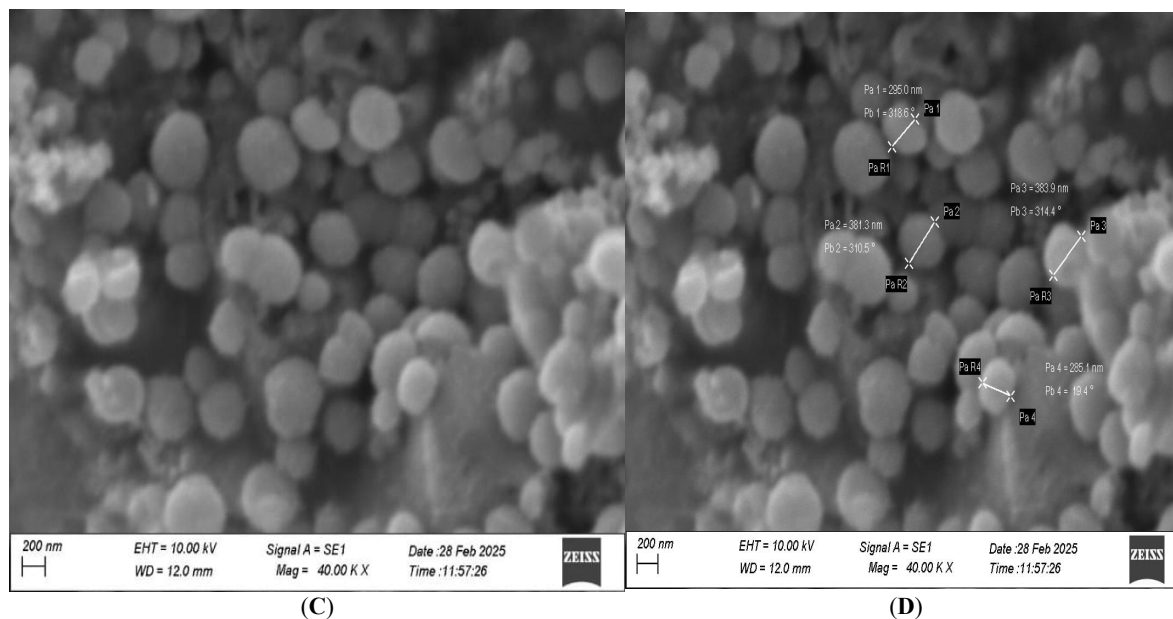


Figure 9. Scanning electron microscope image of silver nanoparticles synthesized by using methanolic extracts *H. diademata* (A) 1 μm of AgNPs, (B) 2 μm of AgNPs, (C & D) 200 nm of AgNPs.

3. Discussion

Nanostructure analyses of *L. austroamericanum*, *P. melanothrix*, and *H. diademata* revealed distinct adaptive traits and bioactive potential. Furthermore, GC–MS profiling confirmed the presence of diverse secondary metabolites responsible for antimicrobial, antioxidant, and anticancer activity. The successful green synthesis of crystalline silver nanoparticles (AgNPs) using lichen extracts was validated through comprehensive characterization techniques, including UV–Vis, FTIR, XRD, particle size analysis, and SEM. Recent studies from our research group have demonstrated that lichens produce a wide range of bioactive compounds with significant pharmacological properties, including antimicrobial, anti-inflammatory, and anticancer effects. Additionally, previous reports have identified five major growth forms of lichen species, such as *P. aurata*, *S. fuliginosa*, *L. leucomelos*, *P. reticulatum*, and *H. leucomelos*, which were investigated for their secondary metabolites as documented by Aiswarya et al. [45].

Leptogium austroamericanum, *L. juressianum*, *L. krogiae*, *L. marginellum*, and *L. resupinans*, along with 25 taxa previously reported from East Africa, have been molecularly identified using the mtSSU and 5.8S markers [46–49]. Secondary metabolites such as usnic acid, atranorin, and gyrophoric acid have demonstrated significant cytotoxic potential against various cancer cell lines by inducing apoptosis, suppressing tumor proliferation, and modulating key molecular pathways involved in cancer development [50–52]. Moreover, fatty acid amides such as (13Z)-13-docosenamide and 9-octadecenamide (Z) have recently gained attention as potential anticancer molecules due to their role in regulating cell membrane fluidity and intracellular signaling pathways [36,43,44]. Comparable findings by Sadowski et al. [53] indicated that biosynthesized nanoparticles often exhibit size variation, with an average diameter of around 200 nm, and form polydispersed systems, which are consistent with the present observation.

The FTIR spectra of the synthesized nanoparticles displayed characteristics corresponding to hydroxyl, carbonyl, and amide C=C functional groups, confirming the presence of diverse biomolecules such as polyphenols, alkaloids, flavonoids, fatty acids, amino acids, and proteins/enzymes [54]. Fluorinated and organophosphorus compounds detected in the present study have been reported to interfere with critical metabolic pathways essential for cancer cell function and viability [23]. The presence of polyphenolic constituents likely imparts a variable surface charge to the AgNPs, influencing their electrostatic stability. Additionally, benzoic acid derivatives and lactones, commonly found in lichen extracts, are known for their pronounced cytotoxic and chemotherapeutic activities, as these compounds can inhibit tumor cell growth and induce apoptotic processes [55]. A high zeta potential value is generally indicative of nanoparticle stability, as it minimizes aggregation through electrostatic repulsion among particles [56]. Phytochemical components such as tannins, alkaloids, and saponins, present in *Eucalyptus camaldulensis* [57], along with polyphenols, flavonoids, tannins, and glycosides found in *Terminalia arjuna* [58], may also contribute to the reduction and stabilization of nanoparticles. Furthermore, the observed shift in the C=O stretching band in the silver nanoparticle spectra suggests the active participation of carbonyl groups

in the reduction of silver ions, while the downshifts of O–H and N–H stretching vibrations indicate the binding or capping of these functional groups onto the nanoparticle surface [59].

The unique physicochemical and biological properties of silver nanoparticles, coupled with their nanoscale dimension, make them promising candidates for developing next-generation therapeutics and addressing global health challenges such as infectious and parasitic diseases [11,60]. Silver has historically been recognized for its strong antimicrobial potential and its long-standing use in medicine [61]. In recent years, AgNPs have gained particular attention for their multifunctional role in infection control, drug delivery, and targeted colon cancer therapies, including applications in cancer and gene therapy [60,62]. Lichen-mediated AgNPs have exhibited remarkable antibacterial efficacy, with mechanochemical synthesis further enhancing their selectivity and biological performance [16]. For instance, AgNPs synthesized from *P. tinctorum* showed strong inhibitory effects against multiple pathogenic bacteria, whereas neutral or negatively charged nanoparticles displayed comparatively lower antibacterial activity [60–62]. However, the biological activity and potential cytotoxicity of AgNPs are closely influenced by parameters such as surface charge, solubility, shape, and size, necessitating detailed toxicological evaluation before large-scale pharmaceutical application [63,64].

Recent researchers have also extended lichen-based green synthesis to other metal and metal oxide nanoparticles, including ZnO/TiO₂/SiO₂ and Fe₃O₄/SiO₂ systems [12,13]. Lichen species such as *Lecanora muralis*, *Ramalina sinensis*, *Ramalina dumeticola*, and *Parmotrema praesorediosum* have been successfully used for nanoparticle biosynthesis and characterized through UV–visible spectroscopy, FT-IR, and field emission scanning electron microscopy (FESEM). Energy-dispersive X-ray spectrometry (EDX), scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), and elemental mapping confirmed the formation of nanostructures with cubic morphology and average particle sizes between 13 and 19 nm [16,18]. The increased adoption of lichen-derived biosynthesis is primarily attributed to its eco-friendly nature, which eliminates the need for hazardous reducing agents and enhances nanoparticle safety for biomedical and pharmaceutical applications [13,65]. The biological activities of AgNPs are known to depend on multiple physicochemical factors, including particle shape, size, surface morphology, pH, ionic strength, and capping molecules [66]. In the present study, particle size analysis revealed that the synthesized AgNPs ranged between 277 and 918 nm, consistent with previous reports [67,68]. Particle dispersity, assessed through the polydispersity index (PDI), provides insight into nanoparticle uniformity. According to the International Organization for Standardization (ISO), samples with PDI values below 0.05 are considered monodisperse. In contrast, values above 0.7 indicate a broad size distribution typical of a polydisperse system [69]. Compared with recent lichen-based AgNP studies, the present nanoparticles fall within the reported active range for biosynthesized systems and are consistent with the general trend that smaller, more stable, and more strongly capped particles exhibit stronger antibacterial effects [36]. Plant extracts are typically rich in polyphenols, proteins, and sugars that can rapidly reduce Ag⁺ whereas lichen extracts contain specialized metabolites such as depsides, depsidones, halogenated aromatics, and related secondary metabolites that may impart stronger capping and bioactivity. Recent reviews of plant-mediated AgNPs highlight their scalability and broad biomedical use, but lichen-based systems may provide a more chemically unique surface corona and a different bioactivity profile. Previous lichen-derived AgNPs have shown potent inhibition against *E. coli*, *P. aeruginosa*, and *S. aureus*, and mechanistic reviews attribute this to membrane disruption, ROS generation, and intracellular damage [32,33]. Our data, therefore, extend the current literature by linking the observed antibacterial response to the chemistry of specific lichen metabolites identified by GC–MS.

In addition, silver solid-state mechanochemical synthesis has been effectively employed to produce various lichen-based nanostructures, including lichen-reduced graphene oxide (LrGO) composites, bimetallic nanoparticle nanocomposites (LrGO AgAu), and magnesium nanoparticles. Lichens such as *X. elegans*, *C. islandica*, *U. antarctica*, *C. rangiferina*, *L. puberulum*, *Acroscyphus sp.*, and *Sticta sp.* have been utilized for this purpose. Analytical techniques, including UV spectroscopy, SEM, XRD, FT-IR, and selected area diffraction (SAD), confirmed the successful formation of silver nanoparticles with an average size of approximately 23 nm [29,70,71]. A plausible anticancer mechanism involves a combined effect of AgNP-mediated ROS generation, mitochondrial dysfunction, and DNA damage, together with metabolite-specific signaling inhibition. Chloroxine may contribute to suppression of the PI3K/AKT/mTOR pathway and induction of apoptosis, whereas other aromatic and halogenated lichen metabolites may enhance membrane permeability, oxidative stress, and cell-cycle arrest. The lichen-derived capping layer may further improve cellular interaction and facilitate uptake, resulting in synergistic cytotoxicity against cancer cells [31,34,35]. Collectively, these findings reinforce that lichen-mediated synthesis represents a sustainable and biologically driven approach to nanoparticle production. The resulting nanoparticles (NPs) show a wide spectrum of activity, including antimicrobial, anti-diabetic, antioxidant, and anticancer properties, making them strong candidates for therapeutic applications. Future studies focusing on structure-

activity relationships, mechanistic pathways, and cytotoxicity will be crucial to validate the biomedical potential of lichen-driven nanoparticles.

4. Conclusions

This study highlights that lichens serve a dual function, acting both as a natural reservoir of bioactive secondary metabolites and as an efficient biological system for the eco-friendly synthesis of silver nanoparticles (AgNPs). The GC–MS profiles of *L. austroamericanum*, *P. melanothrix*, and *H. diademata* revealed diverse bioactive compounds with notable antimicrobial activity against *E. coli*, *K. pneumoniae*, and *S. aureus*. These metabolites exhibited significant pharmaceutical properties. Furthermore, the lichen extracts from the tested species successfully facilitated the green synthesis of stable and crystalline silver nanoparticles (AgNPs), as confirmed by UV-Vis, FTIR, XRD, PSA, and SEM analyses. The integration of antimicrobial, anticancer, and nanotechnological properties observed in *L. austroamericanum*, *P. melanothrix*, and *H. diademata* highlights the promising role of lichen-derived compounds and nanoparticles in biomedical and pharmaceutical applications. Future research should focus on validating in vivo efficacy, elucidating synergistic mechanisms, and optimizing production methods for scalable use in clinical and industrial settings.

Supplementary Materials

The additional data and information can be downloaded at: <https://media.scilit.com/articles/others/2607151013295820/AABB-26040238-SM.pdf>. Materials and Methods.

Author Contributions

V.V.: Conceptualization, methodology, and original draft preparation; V.A.H.; S.A.T.; S.S.; K.S.M. and S.P.: performed the experiments; V.A.H.: Statistical analysis and editorial assistance; R.N.: lichen sample analysis; V.V.; M.M.: visualization and data curation. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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