



PHYTOCHEMICAL PROFILING AND BIOLOGICAL EVALUATION OF METHANOLIC AND N-HEXANE EXTRACTS OF *COTONEASTER MICROPHYLLUS* WALL. EX LINDL.

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Abstract *Cotoneaster microphyllus* Wall. ex-Lindl. is a medicinal plant with limited knowledge available on its phytochemical profile and biological activities. Present study, methanolic and n-hexane extracts of *C. microphyllus*, from its leaves, shoots, and roots were analyzed for their total phenolic and flavonoid contents and evaluated for antioxidant and other biological activities. Overall, the methanolic extracts showed higher levels of phytochemicals and stronger in vitro antioxidant activity than the n-hexane extracts. The methanolic extract of the roots exhibited the highest TPC ($28.5 \pm 1.7 \mu\text{g GAE/mg}$), whereas the leaves methanolic extract gave the highest TFC ($16.4 \pm 1.47 \mu\text{g QE/mg}$) and showed the strongest DPPH scavenging activity ($89.6 \pm 5.3\%$). The n-hexane extracts showed greater α -amylase inhibition, with the leaves extract producing the highest inhibition ($52.5 \pm 2.1\%$). Antibacterial activity was most pronounced against *Bacillus subtilis*, whereas antifungal activity was mainly observed against *Aspergillus fumigatus*. No significant protein kinase inhibition was detected. In the brine shrimp assay, the shoots n-hexane extract showed the lowest LC_{50} among the plant extracts ($36.7 \pm 0.9 \mu\text{g/mL}$), indicating the greatest lethality. Overall, the results demonstrate distinct biological profiles among extracts according to solvent and plant part and support further chemical characterization and biological evaluation of the most active fractions.

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Introduction

The medicinal plants are used as an important source of natural products, either directly employed as therapeutic agents or serve as precursors for drug derivatives. Many clinically important medicines are derived from plant secondary metabolites, and natural product research is going to offer valuable chemical scaffolds for discovery of new drugs (Newman, and Cragg, 2020; Harvey et al., 2015). Advances in analytical techniques and metabolomics have made it easier to study the chemical composition of medicinal plants and investigate their biological activities. Bioactivity-guided approaches have also helped researchers identify extracts and compounds with potential biological effects (Skalicka-Woźniak, 2021). Nevertheless, a large number of medicinal plants remain underexplored, especially those with established traditional uses (Lautié et al., 2020). Furthermore, rising

antimicrobial resistance and the growing burden of chronic inflammation, metabolic diseases, and oxidative stress-related conditions have intensified the need for safer therapeutic options with chemically diverse profiles (Sathishkumar, 2025; Mashele, 2025). Together, these factors support further research on medicinal plants and their potential therapeutic uses. Plants biological activities are largely due to their secondary metabolite profiles. Their expression and distribution vary among species, plant organs, developmental stages, and environmental conditions (Alami et al., 2024; Reshi et al., 2023). Researchers mainly focus on flavonoids and phenolic compounds because of their antioxidant potential and their roles in biological pathways associated with oxidative stress and inflammation (Shahidi, and Ambigaipalan, 2015; Sun, and Shahrajabian, 2023). Among some other specialized secondary metabolites, tannins, alkaloids, terpenoids, and saponins have also shown prominent

antimicrobial, anti-inflammatory, cytotoxic, and enzyme-modulating actions (A. M. Abdel-Mageed et al., 2024; Hilal et al., 2024). However, the biological action of crude plant extracts often comes from the combined effect of several compounds working together, not just one active ingredient. This shows why thorough phytochemical analysis is important for correctly interpreting pharmacological results (Chatterjee et al., 2026).

Because medicinal plant extracts contain numerous biologically active substances, integrating phytochemical characterization with biological evaluation has become a key approach in pharmacognostic studies. Chemical profiling provides information on secondary metabolite composition, whereas biological assessment determines whether these compounds are associated with measurable biological effects (Mihaylova, and Popova, 2026). Biological activity without proper chemical characterization provides an inadequate understanding of the compounds associated with these effects and reduces the reproducibility of studies (Babalola et al., 2025). Moreover, the extraction solvent, polarity, and plant tissue selection strongly influence metabolite recovery and biological responses (Azmir et al., 2013; Do et al., 2014). Therefore, integrated phytochemical and biological investigations provide a reliable approach for linking chemical composition with biological action and identifying promising medicinal plant extracts. Within medicinally important plant families, Rosaceae represents a potential source of bioactive natural products, with its members producing a variety of secondary metabolites (Cristea et al., 2025; Fayaz et al., 2024), particularly phenolics, flavonoids, tannins, and terpenoid compounds (Jakimiuk, and Tomczyk, 2024; Fayaz et al., 2024) which are reported to exhibit antioxidant, antimicrobial and anti-inflammatory effects (Cristea et al., 2025; Mo et al., 2025). However, this family exhibits significant diversity in its phytochemical composition, with studies indicating that the content of flavonols, tannins, and catechins varies considerably across different genera, even between different plant parts of the same species (Khranova et al., 2020).

Among the less-studied Rosaceae genera, *Cotoneaster* has gained attention because of its diverse metabolite profile and promising pharmacological relevance. Studies across the genus have reported flavonoids, phenolic acids, proanthocyanidins, triterpenoids, and several other potential bioactive compounds, although their distribution differs significantly among species and even plant parts (Kicel, 2020). This chemical variability provides a strong basis for investigating individual *Cotoneaster* species to better understand their phytochemical compositions and medicinal potentials. Studies on selected *Cotoneaster* species have confirmed that their biological activities are

closely associated with their phenolic-rich phytochemical profile. Investigations of species such as *C. bullatus*, *C. zabelii*, and other related taxa have identified flavonol derivatives, phenolic acids, flavan-3-ols, and proanthocyanidins as key constituents contributing to their antioxidant and other biological effects (Kicel et al., 2016; Kicel, 2020). Comparative studies have revealed considerable interspecific differences in metabolite composition, indicating that chemical profiles and biological activities may differ among species within the genus. In addition to antioxidant properties, several species of *Cotoneaster* have shown enzyme inhibitory, antimicrobial, and other biological activities. These available studies highlight the importance of the genus as a possible source of plant-derived compounds. (Kicel et al., 2018; Kicel et al., 2022). However, researchers have focused on its few species, leaving many taxa insufficiently characterized. Therefore, a comprehensive investigation of underexplored *Cotoneaster* species is important for increasing our understanding of the genus and discovering new sources of plant-derived bioactive compounds.

Cotoneaster microphyllus Wall. ex Lindl. an evergreen shrub belongs to Rosaceae family which is one of the less studied species of genus. It occurs in the Himalayan region, including northern Pakistan, northwestern India, Nepal, northern Myanmar, and southwestern China, where it grows in temperate and subalpine habitats (Royal Botanic Gardens, Kew, 2026; *Cotoneaster microphyllus* in Flora of China @ efloras.org). Environmental conditions, including altitude and temperature, can influence the biosynthesis, accumulation, and distribution of specialized metabolites in plants, making Himalayan species of particular interest for phytochemical and pharmacological investigations (Pant et al., 2021). Despite its broad geographical distribution and reported traditional uses, *C. microphyllus* has received relatively little scientific attention. Compared with other *Cotoneaster* species, relatively few experimental studies have examined the phytochemical nature and pharmacological effects of *C. microphyllus*. The first reported phytochemical and biological investigation of *C. microphyllus* examined its root and aerial-part extracts and reported antioxidant, antimicrobial, anthelmintic, and anticholinesterase activities (Ali et al., 2021). More recently, Dadhwal et al. (2026) investigated *C. microphyllus* for its nutritional value, phytochemicals, and antioxidant activity, providing further information on its metabolites and their biological actions. However, neither study systematically compared the phytochemical composition or biological actions of different plant parts extracted in different solvents with different polarities. Consequently, the effects of the plant tissues and polarity of the extraction solvent on the phytochemical and biological activities of *C.*

microphyllus remain poorly understood. While earlier studies have confirmed the occurrence of biologically active constituents and affirmed the medicinal potential of *C. microphyllus* (Ali et al., 2021; Dadhwal et al., 2026). However, very little data still exists on the comparative phytochemical and biological properties of its different vegetative parts extracted using different solvents. Secondary metabolite accumulation can vary among plant tissues, while solvent polarity influences the kinds and amounts of extracted compounds. So, leaves, shoots, and roots may differ in chemical composition and biological actions depending on the extraction solvent used. (Azmir et al., 2013; Do et al., 2014). Therefore, comparing extracts from different plant parts using different solvents may provide a clearer picture of the differences in phytochemistry and biological activities of *C. microphyllus*. In this study, methanolic and *n*-hexane extracts of leaves, shoots, and roots of *C. microphyllus* were examined to assess their phytochemical content and biological activities. The findings provide additional information on the chemical properties and biological potential of this relatively understudied Himalayan plant.

Materials and Methods

Collection and authentication of plant material

Leaves, shoots, and roots of *C. microphyllus* Wall. ex Lindle. were collected from the hilly area of the former Federally Administered Tribal Areas (FATA), district Khyber, Pakistan. The botanical authentication of the plant was confirmed by the Herbarium Section, Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan. Collected plant materials were cleaned with tap and distilled water sequentially. The plant material was dried in the shade at room temperature.

Extract preparation

Dried plant samples were separately crushed to powder form and extracted by maceration using analytical-grade solvents of different polarity. The powdered material was sequentially macerated in methanol (MeOH) and *n*-hexane (*n*-Hex) for 3 days with each solvent. After each extraction step, the material was filtered through fine muslin cloth, and Whatman No. 1 filter paper. Each Filtrate underwent solvent removal under vacuum through a rotary evaporator (R-200, Büchi, Switzerland) and was further subjected to vacuum drying (VacuCell, Einrichtungen GmbH). The resulting six crude extracts were designated as L-MeOH (leaves in methanol), R-MeOH (roots in methanol), S-MeOH (shoots in methanol), L-*n*-Hex (leaves in hexane), R-*n*-Hex (roots in hexane), and S-*n*-Hex (shoots in hexane) from the leaves, shoots, and roots and kept at 4 °C for further analysis.

Extract recovery

The extraction yield of crude extracts was determined using this equation:

Extract recovery (%)

$$= \frac{\text{Weight of crude extracts}}{\text{Weight of powdered plant material}} \times 100$$

Preparation of extract solutions

Stock solutions from the crude extracts were prepared in DMSO at concentrations of 4 and 20 mg/mL. The 4 mg/mL solutions were used for phytochemical, antioxidant, and α -amylase assays, whereas the 20 mg/mL solutions were used for antibacterial, antifungal, protein kinase inhibitory, and brine shrimp lethality assays.

Total phenolic content

Total phenolic content (TPC) of *C. microphyllus* was determined following the procedure described by Fatima et al., 2015, with few adjustments. For every sample, 20 μ L of the extract solution was transferred from the stock to a separate microplate well. Gallic acid and DMSO served as the standard and blank, respectively. Folin–Ciocalteu reagent (90 μ L), diluted ten-fold, was subsequently added to the wells containing samples. After a 5 min reaction time, the mixtures were incubated for 1 hour. A microplate reader (ELx800, BioTek, USA) was used to measure absorbance at 650 nm. A calibration curve was prepared using gallic acid at concentrations ranging from 5 to 25 μ g/mL. All measurements were performed in triplicate; TPC values were reported as micrograms of gallic acid equivalents per milligram of extract (μ g GAE/mg extract).

Total flavonoid content

Total flavonoid content (TFC) of *C. microphyllus* extracts was determined by adapting the aluminium chloride colorimetric protocol previously defined (Fatima et al., 2009), with little changes. For the flavonoid assay, the extract samples, quercetin standard, and blank were dispensed into separate microplate wells at 20 μ L per well. Aluminium chloride (10 μ L, 10%) and potassium acetate (10 μ L, 1 M) were then added to each well. After adding distilled water, the reaction mixture was incubated for 30 min. Absorbance readings were taken at 415 nm by a microplate reader (ELx800, BioTek, USA).. Calibration was performed using quercetin concentrations: 2.5, 5, 10, 15, 20, and 40 μ g/mL. This assay was conducted in triplicate; TFC values were expressed as microgram quercetin equivalents per milligram of extract (μ g QE/mg extract).

DPPH activity

DPPH radical scavenging capacity of *C. microphyllus* was assessed (Fatima et al., 2015), with only slight changes. The DPPH working solution was obtained by taking 9.6 mg of DPPH dissolved in 100 mL analytical-grade methanol, sonicating it for 30 min before use. Each well of a microplate received 10 μ L of the respective extract, standard, or blank, along with 190 μ L of DPPH solution. The resulting reaction mixtures were then incubated for 1 h in the dark at 37 °C, and absorbance readings were taken at 515 nm by a microplate reader (ELx800,

BioTek, USA). Ascorbic acid and DMSO were taken as Positive and negative controls, respectively. The assay was conducted in triplicate.

Percent DPPH activity was calculated as:

$$\%RSA = \left(1 - \frac{A_s}{A_c} \right) \times 100$$

Where A_s and A_c represents absorbance of the sample reaction mixture and negative control, respectively.

Total antioxidant capacity

Total antioxidant capacity of *C. microphyllus* extracts was assessed using a modified phosphomolybdenum reduction assay in accordance with the procedure (Fatima et al., 2015). The reagent was ready by dissolving 1.67 g sodium dihydrogen phosphate and 0.247 g ammonium molybdate in distilled water containing 1.63 mL of concentrated sulfuric acid, followed by reaching to a final volume of 50 mL. Each extract (100 μ L) was mixed with 900 μ L of the prepared reagent and maintained at 95 °C for 90 min. Upon reaching room temperature, 200 μ L aliquots of reaction mixtures were taken into separate wells of a 96-well microplate. A microplate reader (ELx800, BioTek, USA) was used for measuring absorbance at 630 nm. Ascorbic acid was taken as the standard, and the results were given as microgram ascorbic acid equivalents per milligram of extract (μ g AAE/mg extract).

Total reducing power

Total reducing power of *C. microphyllus* extracts was assessed using the potassium ferricyanide method (Jafri et al., 2017), with some adjustments. For the reducing power assay, 100 μ L of each extract was mixed with 200 μ L of phosphate buffer (0.2 M, pH 6.6) and 250 μ L of 1% potassium ferricyanide. This reaction mixture was maintained at 50 °C for 20 min, and then 200 μ L of 10% trichloroacetic acid. Centrifugation was performed at 3000 rpm for 10 min. 150 μ L portions of supernatant from each sample were placed in a microplate well and mixed with 50 μ L of 0.1% ferric chloride solution. Absorbance measurements were recorded at 630 nm using a microplate reader (ELx800, BioTek, USA). Ascorbic acid was taken as positive and DMSO as a negative control. Values were expressed as microgram ascorbic acid equivalents per milligram of extract (μ g AAE/mg extract).

Antibacterial activity

C. microphyllus crude extracts were screened for their potential antibacterial action against the tested strains by the disc diffusion protocol following (Bibi, 2011), with some modifications. Extracts were prepared at a concentration of 20 mg/mL in DMSO; 5 μ L from each sample was placed on 5-mm filter-paper discs. After inoculation with the respective bacterial strains, the discs were placed onto nutrient agar plates. Roxithromycin and Cefixime (4 mg/mL each) were taken as standard positive controls, whereas discs treated with DMSO served as the negative control. After incubation for 24 h at 37 °C,

measurements of inhibition zone diameters were recorded in millimeters with a Vernier caliper. Extracts producing inhibition zones ≥ 9 mm were classified as active, and each assay was conducted in triplicate. The bacterial strains comprised two Gram-positive strains, *Staphylococcus aureus* ATCC 6538, and *Bacillus subtilis* ATCC 6633 and three Gram-negative strains, *Klebsiella pneumoniae* ATCC 4619, *Escherichia coli* ATCC 15224, and *Pseudomonas aeruginosa* ATCC 9721.

Antifungal activity

Antifungal activity of *C. microphyllus* was screened using the disc diffusion procedure following (Bibi, 2011), with slight modifications. For antifungal screening, 6-mm Whatman No. 1 filter paper discs were impregnated with 5 μ L of each sample prepared in DMSO (20 mg/mL). Following inoculation of the Sabouraud dextrose agar plates with 100 μ L of the respective fungal suspension, the filter-paper discs were placed on the agar surface. Terbinafine (4 mg/mL; 2.5 μ L per disc) served as the reference antifungal agent, while clotrimazole and DMSO were used as the positive and negative controls, respectively. Following incubation for 24 h at 37 °C, the diameters of inhibition zones were recorded in millimeters using a Vernier caliper. Inhibition zones ≥ 9 mm were classified as significant, and the assay was conducted in triplicate.

The fungal panel comprised *Aspergillus flavus* FCBP 0064, *Aspergillus niger* FCBP 0198, and *Fusarium solani* FCBP 0291.

Protein kinase inhibition assay

The protein kinase inhibitory activity of *C. microphyllus* crude extracts was measured using the disc diffusion method with *Streptomyces* 85E (Fatima et al., 2015), with some minor changes. Discs (6 mm) of Whatman filter paper containing 5 μ L extract solution (20 mg/mL in DMSO) were placed on ISP4 mineral medium inoculated with *Streptomyces* 85E. DMSO and Surfactin were included as the negative and positive controls, respectively, and incubated at 28 °C for 72 h. Protein kinase inhibitory action was identified by the presence of a clear zone surrounding the extract- or control-treated disc.

α -Amylase inhibition assay

α -Amylase inhibition potential of *C. microphyllus* extracts was evaluated using the microplate-based starch-iodine protocol (Xiao et al., 2006), with some adjustments. In each well of a 96-well microplate, 15 μ L of phosphate buffer, 10 μ L of extract, 25 μ L of α -amylase enzyme, and 40 μ L of starch solution were added and incubated at 50°C for 30 min. Following incubation, each reaction mixture was treated with 20 μ L of 1 M HCl and 90 μ L of iodine solution. Acarbose and DMSO were used as the positive and negative controls, respectively. A blank mixture containing buffer, starch, and DMSO was also included. Absorbance readings were noted at 540 nm with a microplate reader (ELx800, BioTek, USA).

Percentage of enzyme inhibition was determined using the given equation:

$$\% \text{ Enzyme Inhibition} = \frac{A_s - A_n}{A_b} \times 100$$

Where A_s , A_n and A_b denote the absorbance of the negative control, sample, and blank, respectively.

Brine shrimp lethality assay

Cytotoxic activity of *C. microphyllus* crude extracts was evaluated via a brine shrimp lethality test (Fatima et al., 2009), with slight changes. *Artemia salina* eggs were placed in artificial seawater (38 g/L) containing 6 mg/L of dried yeast and kept under continuous aeration and light for 24–48 h to hatch. The hatched nauplii were transferred to a 96-well microplate using a Pasteur pipette. Each extract was tested at final concentrations of 25, 50, 100, and 200 µg/mL, with 10 nauplii maintained in 300 µL seawater in separate wells. DMSO and doxorubicin (4 mg/mL) were included as the negative and positive controls, respectively. After an incubation period of 24 h, the surviving nauplii were counted. Median lethal concentration (LC_{50}) values were generated using TableCurve 2D software. The assay was conducted in triplicate.

Statistical analysis

Data are reported as mean $\pm$ standard deviation (SD). LC_{50} was calculated using TableCurve 2D Ver. 4 software. Results from the cytotoxicity, antimicrobial, enzyme inhibition, antioxidant, and phytochemical assays were subjected to analysis of variance (ANOVA). Differences between means were evaluated using the least significant difference (LSD) test at $P \leq 0.05$. Regression analysis was performed using Microsoft Excel.

Results

Extraction yield (% Weight/Weight)

Extraction yield varied according to both plant part and solvent, as shown in Table 1. Methanolic extract of the leaves (L-MeOH) was recorded with the highest extraction yield, 8.18% (w/w), followed by the roots methanolic extract (R-MeOH) of 2.27%. Among the *n*-hexane extracts, the leaves *n*-hexane extract (L-n-Hex) gave the highest yield (1.44%), whereas the *n*-hexane extract of the shoots (S-n-Hex) showed the lowest yield. Overall, methanolic extracts yielded more crude material than the corresponding *n*-hexane extracts.

Table 1. Extraction yield (%) of methanolic and *n*-hexane crude extract from the leaves, roots and shoots of *Cotoneaster microphyllus* Wall

Extract/Fraction	Solvent	% Extraction Yield
L-MeOH	Methanol	8.18%
L-n-Hex	N-hexane	1.44%
S-MeOH	Methanol	1.57%
S-n-Hex	N-hexane	0.106%

R-MeOH	Methanol	2.27%
R-n-Hex	N-hexane	0.142%

L-MeOH: leaves methanolic extract; *L-n-Hex*: leaves *n*-hexane extract; *S-MeOH*: shoots methanolic extract; *S-n-Hex*: shoots *n*-hexane extract; *R-MeOH*: roots methanolic extract; *R-n-Hex*: roots *n*-hexane extract. Values are expressed as the percentage of dried extract recovered from the dry mass of the starting plant material.

Total phenolic content

Methanolic extract of the roots (R-MeOH) was found with the highest phenolic content (TPC), 28.5 ± 1.7 µg GAE/mg extract, whereas *n*-hexane shoot extract (S-n-Hex) showed lowest value, 15.4 ± 0.92 µg GAE/mg extract (Figure 1A). The followed the order R-MeOH > L-MeOH > S-MeOH > L-n-Hex > R-n-Hex > S-n-Hex. Methanolic extracts generally showed higher TPC than the corresponding *n*-hexane extracts.

Total flavonoid content

Methanolic leaf extract (L-MeOH) recorded the highest flavonoid content, 16.4 ± 1.47 µg QE/mg extract, while *n*-hexane root extract (R-n-Hex) showed the lowest value, 4.8 ± 0.70 µg QE/mg extract (Figure 1B). The overall order was L-MeOH > S-MeOH > L-n-Hex > S-n-Hex > R-MeOH > R-n-Hex. Methanolic extracts generally exhibited higher TFC than the *n*-hexane extracts.

DPPH radical-scavenging activity

The leaf extract prepared in methanol (L-MeOH) contained the highest value ($89.6 \pm 5.3\%$) for DPPH radical-scavenging activity, whereas the *n*-hexane extract of the shoots (S-n-Hex) showed the lowest value ($42.6 \pm 2.5\%$), as illustrated in Figure 2. The activity followed the order L-MeOH > S-MeOH > R-MeOH > L-n-Hex > R-n-Hex > S-n-Hex. Generally, methanolic extracts displayed greater radical-scavenging activity than the corresponding *n*-hexane extracts.

Total antioxidant capacity

The methanolic leaf extract (L-MeOH) recorded the highest total antioxidant capacity (TAC), 53.5 ± 3.21 µg AAE/mg extract, whereas the roots *n*-hexane extract (R-n-Hex) showed the lowest value, 17.6 ± 1.06 µg AAE/mg extract (Figure 4). Methanolic extracts generally showed higher TAC than the *n*-hexane extracts.

Total reducing power

The leaves methanolic extract (L-MeOH) showed the highest reducing power, 90.3 ± 4.2 µg AAE/mg extract, whereas *n*-hexane extract of the roots (R-n-Hex) showed the lowest activity, 35.2 ± 2.11 µg AAE/mg extract (Figure 5). The extracts followed the order L-MeOH > S-MeOH > L-n-Hex > R-MeOH > S-n-Hex > R-n-Hex.

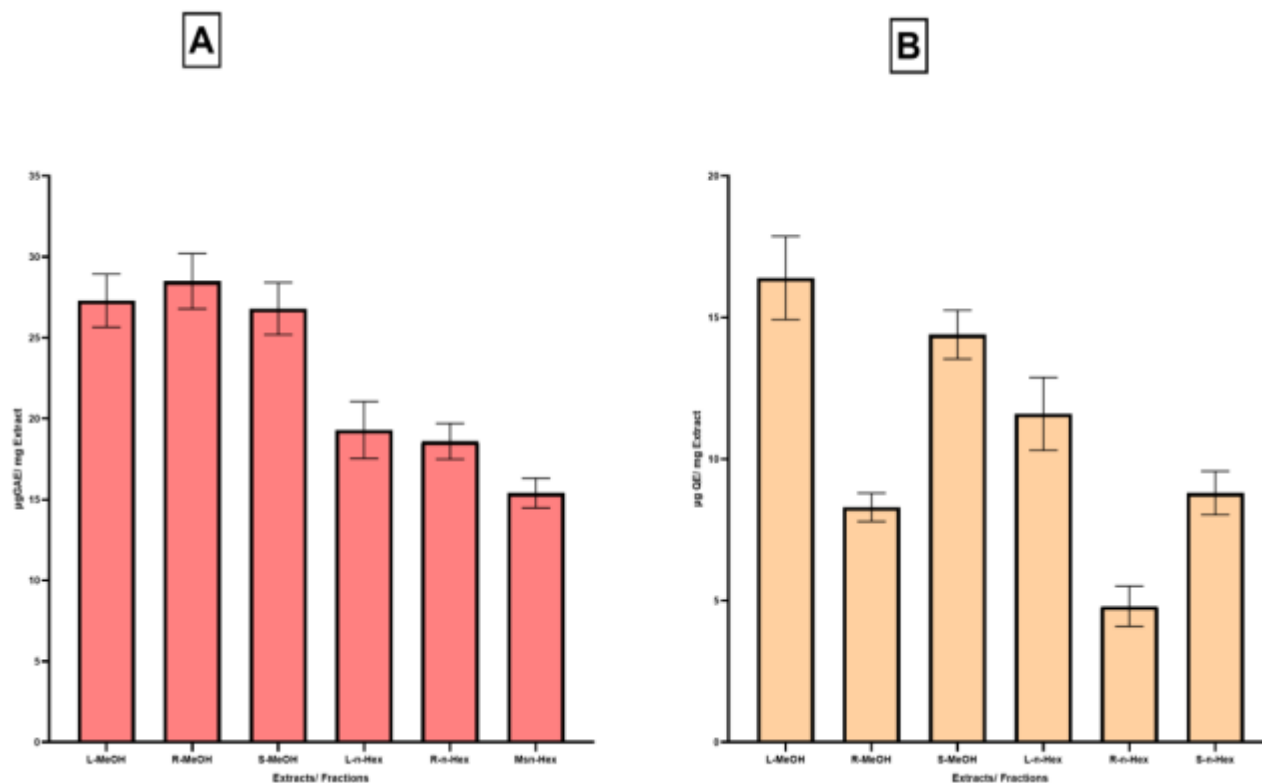


Figure 1. Total flavonoid and phenolic contents of crude extracts of *C. microphyllus* Wall. L-MeOH, leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH, shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. (A) Total phenolic content (µg GAE/mg extract) (B) total flavonoid content (µg QE/mg extract). Results are reported as mean $\pm$ SD

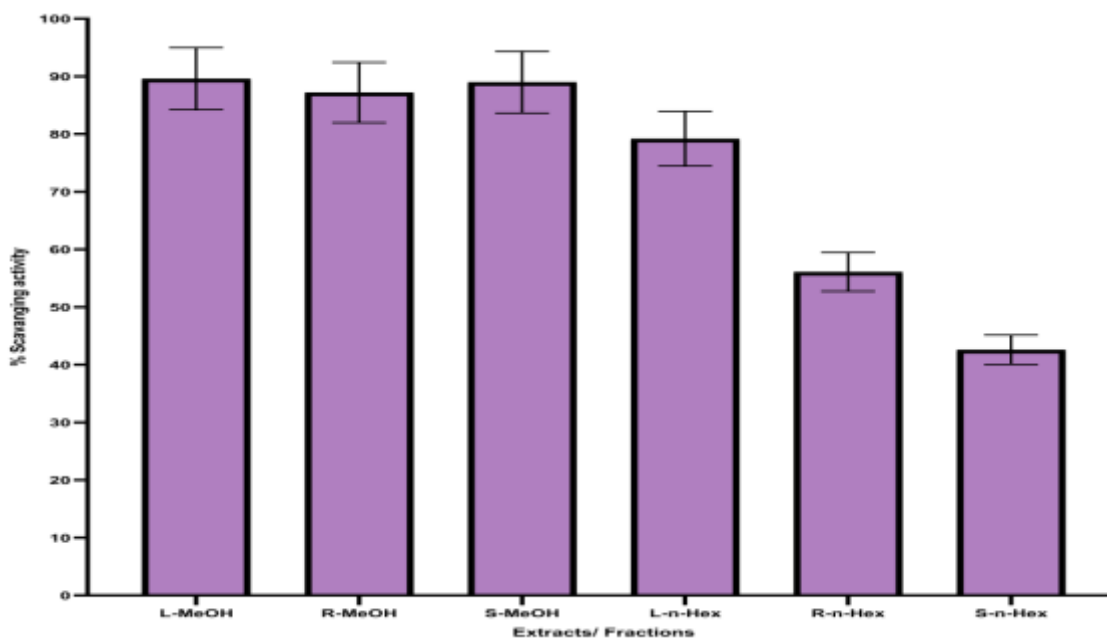


Figure 2. Radical-scavenging activity of crude *C. microphyllus* Wall. extracts determined by the DPPH assay. L-MeOH, leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH, shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. Values are expressed as percentage radical-scavenging activity and presented as mean $\pm$ SD

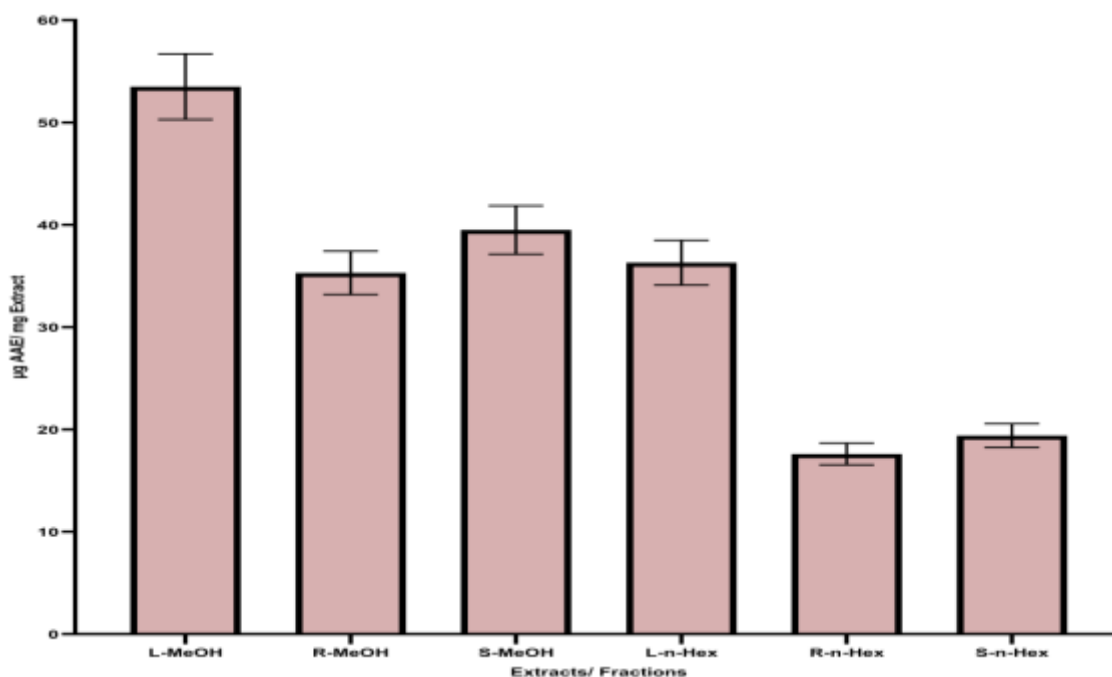


Figure 3. Total antioxidant capacity of crude extracts of *C. microphyllus* Wall. L-MeOH, leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH, shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. The results were reported as µg AAE/mg extract and presented as mean ± SD

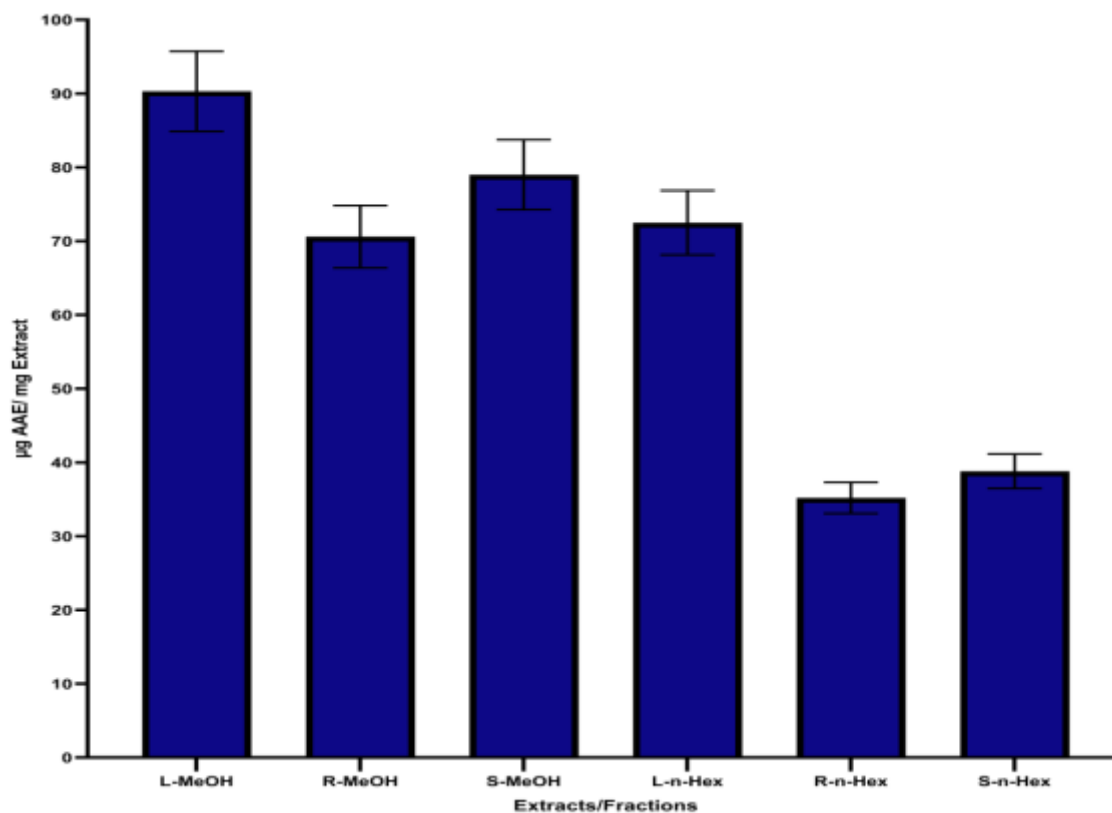


Figure 5. Total reducing power of *n*-hexane and methanolic crude extracts of *C. microphyllus* Wall. L-MeOH, leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH, shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. Results are reported as mean ± SD, with values given in µg AAE/mg extract

Antibacterial activity

The antibacterial action varied among the bacterial strains tested in this study (Table 2). The strongest activity was observed against *Bacillus subtilis*, with the leaves methanolic extract (L-MeOH) producing the largest inhibition zone 20.0 ± 1.2 mm, among the plant extracts. The other extracts also showed greater

activity against *B. subtilis* than against the remaining bacterial strains. Overall, the extracts showed limited activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Table 2. Antibacterial effects of methanolic and *n*-hexane extracts of *C. microphyllus* Wall. at 100 µg/mL against selected bacterial strains determined by the disc diffusion assay

Extract/ Fraction	Antibacterial assay				
	Zone of inhibition in mm (Mean $\pm$ SD) *				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>
L-MeOH	8 ± 0.5	20 ± 1.2	8.5 ± 0.51	7 ± 0.42	7 ± 0.42
L-n-Hex	8 ± 0.5	17.5 ± 1.05	8 ± 0.48	7 ± 0.42	8 ± 0.48
R-MeOH	9 ± 0.6	13.5 ± 0.81	9.5 ± 0.57	7 ± 0.42	9 ± 0.54
R-n-Hex	8 ± 0.5	13 ± 0.78	10 ± 0.6	7 ± 0.42	8 ± 0.48
S-MeOH	8 ± 0.5	17.6 ± 1.06	8.5 ± 0.51	8 ± 0.48	8 ± 0.48
S-n-Hex	8 ± 0.5	17 ± 1.02	10 ± 0.6	8 ± 0.48	8 ± 0.48
Roxithromycin	16 ± 0.96	16 ± 0.96	-	-	-
Cefixime	-	-	18.5 ± 1.1	25 ± 1.5	20 ± 1.2
DMSO	-	-	-	-	-

L-MeOH, leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH, shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. Results are summarized as mean $\pm$ SD of inhibition zones (mm). Roxithromycin and Cefixime were included as the reference antibiotics, while DMSO was included as the negative control.

Antifungal activity

The extracts showed limited and organism-dependent antifungal activity (Table 3). The greatest inhibition was observed against *Aspergillus fumigatus*. The roots *n*-hexane extract (R-n-Hex) and

shoots methanolic extract (S-MeOH) produced the largest inhibition zones, each measuring 15.0 ± 0.9 mm. Activity against the other tested fungi was weak or absent.

Table 3. Antifungal activity of *C. microphyllus* Wall. extracts prepared using methanol and *n*-hexane at 100 µg/mL against tested fungal strains, determined by the disc diffusion assay

Extract Names	Antifungal assay			
	Zone of inhibition in mm (Mean $\pm$ SD) *			
	<i>F. Solani</i>	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>A. niger</i>
L-MeOH	-	7 ± 0.42	7 ± 0.42	-
L-n-Hex	-	7 ± 0.42	9 ± 0.54	-
R-MeOH	-	7 ± 0.42	12 ± 0.72	-
R-n-Hex	-	-	15 ± 0.9	-
S-MeOH	-	7 ± 0.42	15 ± 0.9	-
S-n-Hex	-	-	12 ± 0.72	-
Clotrimazole	23 ± 1.38	24 ± 1.4	22 ± 1.32	25 ± 1.5
DMSO	-	-	-	-

L-MeOH, leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH, shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. Inhibition zones are reported as mean $\pm$ SD (mm). Clotrimazole was included as the positive standard, with DMSO serving as the negative control.

α -Amylase inhibition

The leaves *n*-hexane extract (L-n-Hex) showed the highest α -amylase inhibition, $52.5 \pm 2.1\%$, followed by the shoots *n*-hexane extract (S-n-Hex), $45.4 \pm$

1.8% (Figure 6). The roots *n*-hexane extract (R-n-Hex) also showed comparatively greater inhibition than the methanolic extracts. The shoots methanolic extract (S-MeOH) showed the lowest inhibition, $7.7 \pm 0.31\%$.

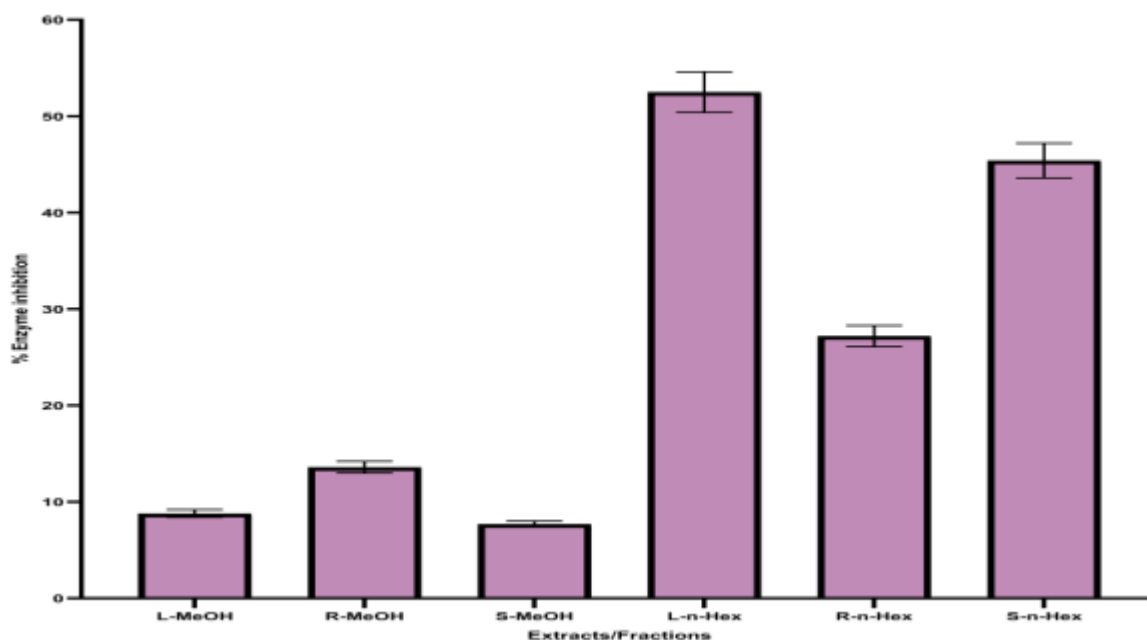


Figure 6. α -Amylase inhibition effect of crude extracts of *C. microphyllus* Wall. L-MeOH leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. Values are expressed as percentage enzyme inhibition and presented as mean $\pm$ SD (R-n-Hex) and leaves *n*-hexane extract (L-n-Hex) followed, with LC₅₀ values of 40.4 ± 1.0 and 46.9 ± 1.1 μ g/mL, respectively. In contrast, the methanolic extracts exhibited substantially higher LC₅₀ values, with L-MeOH showing the highest value, 148.6 ± 3.7 μ g/mL. Doxorubicin showed an LC₅₀ of 24.7 ± 0.6 μ g/mL.

Protein kinase inhibition
No extract tested exhibited significant protein kinase inhibition effect under the experimental conditions.

Brine shrimp lethality

The shoots *n*-hexane extract (Msn-Hex) showed the lowest LC₅₀ among the plant extracts, 36.7 ± 0.9 μ g/mL, indicating the greatest lethality in the brine shrimp assay (Table 4). The roots *n*-hexane extract

Table 4. Brine shrimp lethality of crude extracts of *C. microphyllus* Wall.

Extract Names	% Mortality (concentration: μ g/ml)				LC ₅₀ μ g/ml
	200 μ g/ml	100 μ g/ml	50 μ g/ml	25 μ g/ml	
L-MeOH	100 ± 2.5	50 ± 1.25	45 ± 1.12	30 ± 0.75	148.6 ± 3.7
R-MeOH	100 ± 2.5	52 ± 1.3	48 ± 1.2	35 ± 0.87	139.4 ± 3.4
S-MeOH	100 ± 2.5	51 ± 1.27	40 ± 1	30 ± 0.75	142.9 ± 3.5
L-n-Hex	100 ± 2.5	80 ± 2	50 ± 1.25	20 ± 0.5	46.9 ± 1.1
R-n-Hex	100 ± 2.5	70 ± 1.75	51 ± 1.27	25 ± 0.62	40.4 ± 1.0
S-n-Hex	100 ± 2.5	65 ± 1.62	53 ± 1.32	20 ± 0.5	36.7 ± 0.9
Doxorubicin	100 ± 2.5	80 ± 2.0	60 ± 1.5	50 ± 1.2	24.7 ± 0.6
DMSO	-	-	-	-	-

L-MeOH, leaves methanolic extract; R-MeOH, roots methanolic extract; S-MeOH, shoots methanolic extract; L-n-Hex, leaves *n*-hexane extract; R-n-Hex, roots *n*-hexane extract; S-n-Hex, shoots *n*-hexane extract. Results are reported as mean $\pm$ SD. LC₅₀, indicates the concentration causing 50% mortality. Doxorubicin served as the positive control, whereas DMSO was included as the negative control. “-” indicates no mortality observed.

Discussion

Phytochemical composition and antioxidant activity

The current study showed a prominent influence associated with plant part and the extraction solvent on the phytochemical profile of *C. microphyllus*. Methanol gave higher extraction yields than *n*-hexane, and the methanolic extracts generally contained higher phenolic and flavonoid contents. R-

MeOH had the highest TPC, whereas L-MeOH showed the highest TFC. The difference in distribution of TPC and TFC among the plant parts indicates that these constituents were not uniformly distributed within the plant. Similar differences in phytochemical composition according to plant part and solvent have been reported for *C. microphyllus* (Ali et al., 2021) found greater phenolic and flavonoid contents in the more polar fractions than in

the *n*-hexane fraction, while (Dadhwal et al., 2026) reported higher phytochemicals recovered from hydromethanolic and methanolic extracts than from aqueous extracts. The antioxidant assays performed in this study showed a consistent pattern, with L-MeOH giving the highest total antioxidant potential, ferric-reducing ability, and free-radical scavenging activity. The comparatively high phenolic and flavonoid contents of this extract occurred alongside its stronger antioxidant activity, suggesting that polyphenolic constituents may contribute to the observed response. This interpretation is consistent with work on several *Cotoneaster* species, in which antioxidant capacity was linked to total phenolics and, particularly, proanthocyanidins (Kicel et al., 2016; Ali et al., 2021). DPPH and ABTS scavenging activity was also reported in root and aerial extracts of *C. microphyllus*, with lower activity in the *n*-hexane fractions, while reporting stronger DPPH and FRAP activity in the more polar extracts (Dadhwal et al., 2026).

Taken together, the three antioxidant assays indicate greater antioxidant activity in the methanolic fractions, particularly L-MeOH. However, the present colorimetric assays do not recognize the compounds responsible for these effects. The LC-MS, GC-MS, and UHPLC characterization (Dadhwal et al., 2026) demonstrated considerable chemical diversity in *C. microphyllus*, supporting further compound-level investigation rather than attributing the observed activity to total phenolics or flavonoids alone.

Antimicrobial activity

The antimicrobial activity of *C. microphyllus* extracts varied among the test organisms and was generally selective. *Bacillus subtilis* recorded the strongest antibacterial response, with a considerable zone of inhibition seen for both methanolic and *n*-hexane extracts, whereas activity against the other bacterial strains was limited. Antifungal activity was not particularly considerable overall and was most evident against *Aspergillus fumigatus*. (Ali et al., 2021) Likewise, variable antibacterial and antifungal activity was reported among solvent fractions of *C. microphyllus*. The present findings (Ali et al., 2021) indicate that antimicrobial activity in this species varies with both extract fraction and target organism. Direct comparison of activity levels is difficult because plant material, extraction and fractionation procedures, extract concentrations, and test organisms vary among studies. Similar variability in antimicrobial activity has also been reported among other *Cotoneaster* species (Uysal et al., 2016)s. Overall, the present results demonstrate selective rather than broad-spectrum antimicrobial activity of the tested extracts.

Enzyme inhibition and cytotoxicity

The α -amylase inhibitory activity showed a pattern different from that of the antioxidant assays. The *n*-hexane extracts, particularly L-*n*-Hex and S-*n*-Hex,

showed the greatest inhibition, whereas the methanolic extracts were less active. This behavior of the extracts differs from several studies of other *Cotoneaster* species. (Zengin et al., 2014) reported α -amylase and α -glucosidase suppressing effects in *C. nummularia*, with methanolic extracts showing the strongest effect among the extracts tested. Uysal et al. (2016) also reported α -amylase and α -glucosidase inhibition in *C. integerrimus*, while (Les et al., 2017) detected enzyme-inhibitory activity in both polar and non-polar fruit extracts of *C. pannosus* (Kicel et al., 2022) further reported α -amylase inhibition in extracts of *C. integerrimus*, *C. zabelii* and *C. bullatus*, with activity varying among species and plant materials. The higher activity of the *n*-hexane fractions in the present study therefore represents a distinct extract-dependent response. Because enzyme inhibition was assessed using crude extracts, the constituents responsible cannot be identified from the present results and require further fractionation and chemical characterization. No appreciable protein kinase inhibition was detected under the conditions tested. The brine shrimp assay showed substantially greater lethality for the *n*-hexane extracts than for the methanolic extracts, with S-*n*-Hex producing the lowest LC₅₀ among the plant extracts. Neither (Ali et al., 2021) nor (Dadhwal et al., 2026) examined brine shrimp lethality in *C. microphyllus*, so direct comparison with those studies is not possible. The genus contains chemically diverse constituents, including phenolic compounds, procyanidins, triterpenes, and sterols, whose distribution varies among plant tissues and extracts (Kicel, 2020). The lower LC₅₀ values observed for the *n*-hexane fractions identify these extracts for further investigation, but brine shrimp lethality represents a preliminary toxicity response and does not establish anticancer activity. Validation in relevant mammalian cell models and chemical characterization of the active fractions are therefore required.

Conclusion

The present study revealed variation in the phytochemical profile and biological effects of *C. microphyllus* extracts depending on the extraction solvent and plant part. Methanolic extracts generally contained higher phenolic and flavonoid contents and showed stronger antioxidant activity, with the methanolic leaf extract showing the most pronounced antioxidant responses. In contrast, the *n*-hexane extracts showed greater α -amylase inhibition and brine shrimp lethality. Antimicrobial activity was selective, with the strongest responses observed against *Bacillus subtilis* and *Aspergillus fumigatus*. No appreciable protein kinase inhibition was detected under the conditions tested. These findings suggest that different extract fractions of *C. microphyllus* possess distinct biological profiles. Further work should focus on chemical profiling and fractionation of the most active extracts, followed by

identification of the compounds associated with antioxidant, α -amylase inhibitory, and cytotoxic activities. Enzyme-kinetic studies and validation in appropriate mammalian cell models would be useful for establishing the biological relevance of these activities.

References

- Abdel-Mageed, A. M., AlaaMustafa, H. A. R., & Elsheikh, S. Y. S. (2024). The Potential of Medicinal Plants with Bioactive Compounds as Antioxidants, Anti-Inflammatory and Immunomodulators: Review. *South Eastern European Journal of Public Health*, 1552–1567. <https://doi.org/10.70135/seejph.vi.2979>
- Alami, M. M., Guo, S., Mei, Z., Yang, G., & Wang, X. (2024). Environmental factors on secondary metabolism in medicinal plants: exploring accelerating factors. *Medicinal Plant Biology*, 3, e0024-0016. <https://doi.org/10.48130/mpb-0024-0016>
- Ali, M., Ullah, H., Bari, W. U., Ul Islam, N., Zahoor, M., Ullah, R., & Bari, A. (2021). Phytochemical isolation and biological screening of *Cotoneaster microphyllus*. *International Journal of Food Properties*, 24(1), 1318–1334. <https://doi.org/10.1080/10942912.2021.1963770>
- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A., & Omar, A. K. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>
- Babalola, O. O., Bridget, K., Oyubu, G., Waheed, S. A., Ajiboye, S. A., Fakayode, A. E., Edema, A. A., Chukwuma, U. G., Tiamiyu, R. T., Fakayode, A. J., Adebimpe, H. O., Onyeagba, K. D., Fakunle, C. O., Ottu, P. O., & Gabriel, S. (2025). Integrating phytochemicals and in silico methods for modern drug discovery: a comprehensive review. *Discover Chemistry*, 2, 297. <https://doi.org/10.1007/s44371-025-00373-y>
- Bibi, G. (2011). Antitumor, cytotoxic and antioxidant potential of *Aster thomsonii* extracts. *African Journal of Biotechnology*, 10(60), 12958–12964. <https://doi.org/10.5897/AJB11.2335>
- Chatterjee, S., Saha, P., Ganguly, P., & Patel, A. K. (2026). A Comprehensive Review on Network-Pharmacognosy-Based Decoding of Phytochemical Synergy in Complex Diseases. *Revista Brasileira de Farmacognosia*, 36, 55. <https://doi.org/10.1007/s43450-026-00765-w>
- Cotoneaster microphyllus* in Flora of China @ efloras.org. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200010834
- Cristea, A. M., Smeu, A., Cîmpeanu, I.-A., Iftode, A., Liga, S., Tchiakpe-Antal, D.-S., Vlad, D., Dehelean, C. A., & Iliescu, D. (2025). Biological effects of Rosaceae species in skin disorders—an up-to-date overview. *Plants*, 14(11), 1605. <https://doi.org/10.3390/plants14111605>
- Dadhwal, R., Sharma, S., Singh, S., Banerjee, R., Sharma, D., Thakur, A., Dulta, K., Singh, A., Bhardwaj, N., & Deghani, M. H. (2026). Integrated analysis of nutritional composition, phytochemical, and antioxidant activity in *Cotoneaster microphyllus* (Wall. ex Lindl.). *Scientific Reports*. <https://doi.org/10.1038/s41598-026-01234-5>
- Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296–302. <https://doi.org/10.1016/j.jfda.2013.11.001>
- Fatima, H., Khan, K., Zia, M., Ur-Rehman, T., Mirza, B., & Haq, I. (2015). Extraction optimization of medicinally important metabolites from *Datura innoxia* Mill.: an in vitro biological and phytochemical investigation. *BMC Complementary and Alternative Medicine*, 15, 376. <https://doi.org/10.1186/s12906-015-0891-1>
- Fatima, N., Zia, M., Rehman, R., Rizvi, Z. F., Ahmad, S., Mirza, B., & Chaudhary, M. F. (2009). Biological activities of *Rumex dentatus* L: Evaluation of methanol and hexane extracts. *African Journal of Biotechnology*, 8(24), 6945–6950. <https://doi.org/10.5897/AJB09.1246>
- Fayaz, F., Singh, K., Gairola, S., Ahmed, Z., & Shah, B. A. (2024). A Comprehensive Review on Phytochemistry and Pharmacology of *Rosa* Species (Rosaceae). *Current Topics in Medicinal Chemistry*, 24(4), 364–378. <https://doi.org/10.2174/0115680266274385231023075011>
- Harvey, A. L., Edrada-Ebel, R., & Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery*, 14(2), 111–129. <https://doi.org/10.1038/nrd4510>
- Hilal, B., Khan, M. M., & Fariduddin, Q. (2024). Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: phenolics, terpenes, and alkaloids. *Plant Physiology and Biochemistry*, 211, 108674. <https://doi.org/10.1016/j.plaphy.2024.108674>

- Jafri, L., Saleem, S., Ullah, N., & Mirza, B. (2017). In vitro assessment of antioxidant potential and determination of polyphenolic compounds of *Hedera nepalensis* K. Koch. *Arabian Journal of Chemistry*, **10**, S3699–S3706. <https://doi.org/10.1016/j.arabjc.2014.05.002>
- Jakimiuk, K., & Tomczyk, M. (2024). A review of the traditional uses, phytochemistry, pharmacology, and clinical evidence for the use of the genus *Alchemilla* (Rosaceae). *Journal of Ethnopharmacology*, **320**, 117439. <https://doi.org/10.1016/j.jep.2023.117439>
- Khramova, E., Kukushkina, T., Shaldaeva, T., Pshenichkina, Y., & Vysochina, G. (2020). Biologically Active Compounds and Antioxidant Activity of the Plants from CSBG SB RAS Collection of the Rosaceae Family (I). *BIO Web of Conferences*, **24**, 00038. <https://doi.org/10.1051/bioconf/20202400038>
- Kicel, A. (2020). An overview of the genus *Cotoneaster* (Rosaceae): phytochemistry, biological activity, and toxicology. *Antioxidants*, **9**(10), 1002. <https://doi.org/10.3390/antiox9101002>
- Kicel, A., Kolodziejczyk-Czepas, J., Owczarek, A., Marchelak, A., Sopinska, M., Ciszewski, P., Nowak, P., & Olszewska, M. A. (2018). Polyphenol-rich extracts from *Cotoneaster* leaves inhibit pro-inflammatory enzymes and protect human plasma components against oxidative stress in vitro. *Molecules*, **23**(10), 2472. <https://doi.org/10.3390/molecules23102472>
- Kicel, A., Magiera, A., Skrzywanek, M., Malczuk, M., & Olszewska, M. A. (2022). The inhibition of α -glucosidase, α -amylase and protein glycation by phenolic extracts of *Cotoneaster bullatus*, *Cotoneaster zabelii*, and *Cotoneaster integerrimus* leaves and fruits: Focus on anti-hyperglycemic activity and kinetic parameters. *Molecules*, **27**(20), 7081. <https://doi.org/10.3390/molecules27207081>
- Kicel, A., Michel, P., Owczarek, A., Marchelak, A., Żyżelewicz, D., Budryn, G., Oracz, J., & Olszewska, M. A. (2016). Phenolic profile and antioxidant potential of leaves from selected *Cotoneaster* Medik. species. *Molecules*, **21**(6), 688. <https://doi.org/10.3390/molecules21060688>
- Lautié, E., Russo, O., Ducrot, P., & Boutin, J. A. (2020). Unraveling plant natural chemical diversity for drug discovery purposes. *Frontiers in Pharmacology*, **11**, 397. <https://doi.org/10.3389/fphar.2020.00397>
- Les, F., López, V., Caprioli, G., Iannarelli, R., Fiorini, D., Innocenti, M., Bellumori, M., & Maggi, F. (2017). Chemical constituents, radical scavenging activity and enzyme inhibitory capacity of fruits from *Cotoneaster pannosus* Franch. *Food & Function*, **8**(5), 1775–1784. <https://doi.org/10.1039/C7FO00184B>
- Mashele, S. S. (2025). Phytochemicals as Multifunctional Agents: Antimicrobial, Enzyme Inhibitory, and Wound-Healing Potentials in the Era of Drug Resistance. *Natural Product Research*. <https://doi.org/10.1080/14786419.2025.2450000>
- Mihaylova, D., & Popova, A. (2026). Phytochemicals of Natural Products: Analysis and Biological Activities: 2nd Edition. *Horticulturae*, **12**(6), 676. <https://doi.org/10.3390/horticulturae12060676>
- Mo, X., Zhou, Y., Zhan, M., Zhang, Y., Liu, J., Quang, H., & Dong, L. (2025). A review of the traditional uses, phytochemistry, pharmacology and toxicity for the genus *Geum* (Rosaceae). *Fitoterapia*, **180**, 106333. <https://doi.org/10.1016/j.fitote.2024.106333>
- Newman, D. J., & Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of Natural Products*, **83**(3), 770–803. <https://doi.org/10.1021/acs.jnatprod.9b01285>
- Pant, P., Pandey, S., & Dall'Acqua, S. (2021). The Influence of Environmental Conditions on Secondary Metabolites in Medicinal Plants: A Literature Review. *Chemistry & Biodiversity*, **18**(11), e2100345. <https://doi.org/10.1002/cbdv.202100345>
- Reshi, Z. A., Ahmad, W., Lukatkin, A. S., & Javed, S. B. (2023). From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and in vitro approaches. *Metabolites*, **13**(8), 895. <https://doi.org/10.3390/metabo13080895>
- Royal Botanic Gardens, Kew. (2026). *Cotoneaster microphyllus* Wall. ex Lindl. *Plants of the World Online*. <http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:726359-1>
- Sathishkumar, K. (2025). Revitalising healthcare: the role of natural products in modern medicine. *Natural Product Research*, **39**, 3345–3347. <https://doi.org/10.1080/14786419.2024.2368751>
- Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—A review. *Journal of Functional Foods*, **18**, 820–897. <https://doi.org/10.1016/j.jff.2015.06.018>
- Skalicka-Woźniak, K. (2021). Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*, **20**, 1–2. <https://doi.org/10.1038/s41573-021-00000-x>
- Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human

- health. *Molecules*, **28**(4), 1845.
<https://doi.org/10.3390/molecules28041845>
- Uysal, A., Zengin, G., Mollica, A., Gunes, E., Locatelli, M., Yilmaz, T., & Aktumsek, A. (2016). Chemical and biological insights on *Cotoneaster integerrimus*: A new (-)-epicatechin source for food and medicinal applications. *Phytomedicine*, **23**(9), 979–988.
<https://doi.org/10.1016/j.phymed.2016.06.011>
- Xiao, Z., Storms, R., & Tsang, A. (2006). A quantitative starch–iodine method for measuring alpha-amylase and glucoamylase activities. *Analytical Biochemistry*, **351**(1), 146–148.
<https://doi.org/10.1016/j.ab.2006.01.036>
- Zengin, G., Uysal, A., Gunes, E., & Aktumsek, A. (2014). Survey of phytochemical composition and biological effects of three extracts from a wild plant (*Cotoneaster nummularia* Fisch. et Mey.): A potential source for functional food ingredients and drug formulations. *PLoS ONE*, **9**(11), e113527.
<https://doi.org/10.1371/journal.pone.0113527>



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Statements and Declarations

Author Contributions

Conceptualization,, KM, and KT; Formal analysis, MN, AA, SN, Methodology, KM, HS, MR.; Supervision , ZM, HS Writing—review and editing, KM, MN, KT,; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors confirm that they have no conflicts of interest.

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Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, the author(s) have not used any ChatGPT (OpenAI) tool.