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Biochemical composition, Phenolic and Flavonoid Contents, and Antioxidant Activity of *Actinodaphne pilosa* (lour.) Merr. From the Inland Sandy Areas of Hue City, Vietnam

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ABSTRACT

The biochemical composition and antioxidant capacity of *Actinodaphne pilosa* (Lour.) Merr., a medicinal species adapted to drought-prone inland sandy soils, were evaluated in root, stem, and leaf tissues. Biochemical constituents were quantified using standard colorimetric assays, and antioxidant activity was assessed using DPPH, FRAP, and catalase activity assays. Among the studied organs, stems exhibited the highest total phenolic (100.6 mg GAE/g DW) and flavonoid contents (75.88 mg QE/g DW). Stem extracts also showed superior antioxidant performance, with strong DPPH radical-scavenging activity (IC_{50} = 17.08 mg/mL), high reducing power (FRAP value of 178.03 µmol Fe^{2+}/g DW), and elevated catalase activity (147.2 U/mg protein). These findings indicate that the stem is a major contributor to the antioxidant capacity of *A. pilosa* and provide a scientific basis for further exploration of this species as a potential source of natural antioxidants under abiotic stress conditions.

Keywords: *Actinodaphne pilosa*, antioxidant capacity, catalase activity, flavonoids, sandy inland soil, total phenolic content

Introduction

Actinodaphne pilosa (Lauraceae) is widely distributed in tropical and subtropical regions[1 – 4], mainly in Southeast Asia, particularly Vietnam[3]. Although less studied than related species such as *A. macrophylla*, *A. pruinosa*[5], and *A. obovata*[3], it shows promising medicinal and economic potential due to its diverse biochemical profile. Essential oils from leaves and bark contain terpenoids, aldehydes, phenols, esters, and alcohols[2], including caryophyllene, caryophyllene oxide, spathulenol, and hinesol acetate, which contribute to antimicrobial, antifungal, anti-inflammatory, antioxidant, antidiabetic, and antitumoral activities[3]. In Vietnam, *A. pilosa* grows in the inland sandy soils of Phong Dien, Hue, characterized by high permeability, rapid heat absorption, and seasonal "sand drift," creating significant abiotic stress[6]. Such conditions can enhance the biosynthesis of secondary metabolites, including essential oils and phenolic compounds, increasing the biological efficacy of plant extracts[7–9]To date, most studies have focused on leaf essential oils, while information on nutritional composition and bioactive compounds in other plant parts remains limited[3,10]. This study therefore evaluates the nutritional composition, flavonoid and polyphenol contents, and antioxidant capacity of different tissues, providing a comprehensive understanding of the medicinal properties of this native species and supporting its potential applications and sustainable cultivation in Vietnam and Southeast Asia.

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Materials and Methods

Plant material and location

Actinodaphne pilosa belongs to the Lauraceae family as authenticated by Dr. Truong Thi Hieu Thao, Faculty of Biology, University of Education, Hue University, Hue, Viet Nam (Fig. 1). Mature roots, stems, and leaves of *A. pilosa* were collected in April 2025, during the flowering stage, from the sandy inland area of Phong Chuong Commune, Phong Dien Town, Hue City, Vietnam (16°38′49″N, 107°23′3″E; elevation 5–10 m). Sampling followed the protocols of Nguyen Nghia Thin (2006, 2008)[11,12]. Each specimen was labeled and detailed field observations–including color and morphology of roots, stems and leaves–were recorded to aid identification, as dried samples are difficult to recognize. Samples were sealed in plastic bags and transported for further analysis[11,12].

Preparation of extracts

After each sampling, root, stem, and leaf samples were rinsed thoroughly with distilled water and blotted dry with paper towels. The samples were then oven–dried at 40°C for 72 hours until a constant weight was achieved. The dried samples were ground into powder using an industrial electric blender (Superfine Powder Mill, MN–200B, Vietnam).

To prepare the crude extract, 200 g of the powdered sample was continuously shaken for 72 hours in 500 mL of 100% methanol using an orbital shaker (Benchmark Incu–Shaker). The solution was then filtered through Whatman No. 1 filter paper using a Buchner funnel and vacuum pump. The extract was concentrated using a rotary evaporator (DLAB RE 100–S, DLAB Scientific Co., Ltd) at 40°C.

The crude extracts were stored at 4°C in a refrigerator (Sharp SJ–X201E–SL, Thailand) until further use. All dried extract samples were reconstituted in methanol (1 mg/mL) to prepare stock solutions for antioxidant assays. The mixture was vortexed thoroughly, and the dissolved samples were used for analyses related to antioxidant activity and bioactive compounds in the study.

The standards and reagents

Analytical-grade reagents and solvents (≥98% purity) were obtained from Sigma-Aldrich (USA). The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and dimethyl sulfoxide (DMSO) used in antioxidant assays were purchased from Hi-Media

(India).

Moisture content (MC).
Following the procedures detailed in the AFNOR standard (NF–V03–402 1985) (AFNOR NF V03–402 1985)[13], moisture content was accurately quantified. Accurately weigh 5 g of fresh sample and dry at 100–105 °C for 2–3 hours. After drying, cool in a desiccator for 30 minutes and reweigh. Repeat the drying–cooling–weighing cycle until the weight difference between two successive measurements is less than 0.25%. The moisture content was calculated using the following formula:

$$MC\% = \left(\frac{m0 - m1}{m0}\right) \times 100$$

With: m_0 (g): Initial mass of the plant; m_1 (g): Mass after drying.
The result was expressed as a percentage of dry matter.

Lipid content
Lipid content was determined using the Soxhlet extraction method. This Soxhlet extraction procedure followed the method described by Hopkins et al. (2014)[14]. The extraction was performed using a Biohall Soxhlet apparatus (Germany), in which 2.5 g of dried and powdered sample was placed in a porous thimble and extracted with 85 mL of diethyl ether for 60 min in individual extraction flasks. The remaining solvent was allowed to evaporate for an additional 20 min, followed by drying in an oven at 105°C for 30 min to completely remove any residual solvent. The difference in sample weight before and after extraction was used to calculate the crude fat content according to Thiex et al. (2003)[15] and expressed as a percentage of dry sample weight.

Protein content
Protein content was determined using the Bradford method as described by Bradford (1976)[16]. Briefly, an aliquot of the sample extract was mixed with Coomassie Brilliant Blue G-250 reagent, and the absorbance was measured at 595 nm using a UV–Vis spectrophotometer. Bovine serum albumin (BSA) was used as the standard for the calibration curve. Protein concentration was calculated based on the standard curve and expressed as mg protein per g of dry sample weight.

Reducing sugar content. Reducing sugars were determined using the 3,5-dinitrosalicylic acid (DNSA) method. 1 mL of sample extract was mixed with 1 mL DNSA reagent, boiled for 5-10 minutes, cooled, and diluted with 8 mL distilled water. Absorbance was measured at 540 nm, and the reducing sugar content was calculated using a glucose standard curve:

$$\text{Reducing sugar (\%)} = \frac{C \times V \times 100}{m}$$

where: **C**: concentration from the glucose standard curve (mg/mL); **V**: total volume of the extract (mL); **m**: weight of the sample (mg).

Quantitation of bioactive compounds
Total phenolic content (TPC
The quantification of total phenolic content (TPC) was performed using the Folin–Ciocalteu colorimetric method[17]. Specifically, 20 µL of extract was mixed with 80 µL of 7.5% sodium carbonate (Na_2CO_3) solution, followed by the addition of 100 µL of 10% Folin–Ciocalteu reagent. The mixture was incubated in the dark at room temperature for 15 minutes. Absorbance was measured at 750 nm using a spectrophotometer (Thermo Scientific). Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g dry extract).

*Total flavonoid content (TFC***)**
The total flavonoid content was measured using an aluminum colorimetric method following Pintha et al. (2018)[18]. The sample was dissolved in 70% ethanol at a ratio of 1:20 (w/v). One milliliter of the extract was combined with 0.5 mL of 5% $NaNO_2$ and kept in the dark for 6 minutes. Then, 0.5 mL of 10% $AlCl_3$ was added, followed by gentle mixing and incubation for another 6 minutes. Next, 2 mL of 1.0 M NaOH was added, and the volume was adjusted to 10 mL with distilled water. The final mixture was thoroughly mixed and allowed to react for 15 minutes. The optical density was measured at 415 nm using a UV–Vis spectrophotometer (Thermo Scientific). The results were expressed as milligrams of catechin equivalents per gram of extract (mg CE/g dry extract).

Antioxidant activity
DPPH radical scavenging assay
The free radical scavenging activity was determined using the DPPH (2,2–diphenyl–1–picrylhydrazyl) assay with slight modifications[19]. Stock solutions of each sample (1 mg/mL in methanol) were serially diluted to final concentrations ranging from 1000 to 7.8 µg/mL. Then, 0.2 mL of each diluted sample was mixed with 3.8 mL of a 50 µM methanolic DPPH solution (1 mg/50 mL) and incubated at room temperature for 30 minutes in the dark. Absorbance was recorded at 517 nm using a spectrophotometer (Thermo Scientific). A control sample containing only DPPH solution (without extract or standard) was measured at 0 min.
The percentage of DPPH radical inhibition (I%) was calculated using the equation:
I% = [(A_blank − A_sample) / A_blank] × 100
where A_blank is the absorbance of the control and A_sample is the absorbance of the test solution. The IC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals, was determined from the dose–response curve and expressed as mean ± standard deviation of triplicate measurements.

Ferric reducing antioxidant power (FRAP) assay
A modified Benzie and Strain protocol was used to assess the FRAP–based antioxidant capacity of *A. pilosa* extracts[20]. In this assay, the FRAP working reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 40 mM TPTZ solution (2,4,6–tri(2–pyridyl)–s–triazine), and 20 mM ferric chloride solution in a volumetric ratio of 10:1:1. A total volume of 900 µL of this reagent was added to 30 µL of the extract sample, and the mixture was incubated in the dark for 30 minutes. Absorbance was then recorded at 593 nm using a spectrophotometer. All samples were analyzed in triplicate. The antioxidant activity was quantified using a calibration curve of $FeSO_4$, and results were expressed as µmol Fe^{2+} equivalents per gram of dry weight.

Catalase activity.
Catalase activity was measured using a $KMnO_4$ titration method. Enzyme extract was incubated with H_2O_2 at 30°C, and the reaction was stopped with H_2SO_4. The remaining H_2O_2 was quantified by titration with standardized $KMnO_4$. Catalase activity was then calculated based on the difference between sample and blank values and expressed per gram fresh weight or normalized to protein content for unit activity.

Statistical analysis
The data were analyzed using SPSS version 20.0 software and are presented as mean ± standard deviation (SD). Differences were considered statistically significant when $p < 0.05$, based on Duncan's multiple range test.

Results and Discussion
This is the first study to investigate the biochemical profile and antioxidant potential of *A. pilosa* (Lour.) Merr in the drought–prone inland sandy soils of Phong Dien district, Hue City, Viet Nam. The findings provide a comprehensive assessment of the species' metabolic characteristics, revealing the organ–specific distribution of primary metabolites and antioxidant activities. Among the plant parts, stems exhibited the most pronounced accumulation of nutrients and antioxidant capacity, underscoring their central role in storage, defense, and physiological adaptation to harsh edaphic conditions.

Biochemical Constituents in Different Plant Organs.
Moisture content in different organs of *A. pilosa* (Table 1) ranges from 9.8% to 15.8%, with the stem exhibiting the lowest value (9.8%). Except for the protein, the lipid and reducing sugar content from the stem of *A. pilosa* (13.60 g/100 g and 3.014 g/100 g, respectively) was higher than the leaf (12.13 g/100 g and 1.559 g/100 g, respectively) and

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root (5 g/100 g and 1.247 g/100 g, respectively) (Table 1). However, there was no statistically significant difference in protein content between the stem and the leaf ($p>0.05$).

Previous studies on *Actinodaphne* species have largely focused on essential oils and alkaloid content with antimicrobial or antioxidant effects[5, 10, 21, 22, 23], the macronutrient composition of *A. pilosa* remains poorly documented. Our data adds a novel dimension by revealing significant levels of proteins, lipids, and reducing sugars (Table 1), which are relevant not only for nutritional purposes but also for co - extraction of lipophilic bioactives. Compared with other Lauraceae members, the lipid content in the stem suggests greater potential for phytochemical extraction and functional utilization[24, 25, 26].

Biochemical variation among organs of *A. pilosa* also highlight adaptive strategies to survive in the drought–prone sandy soils of Central Viet Nam. Higher moisture content in leaves (15.80%) supports efficient photosynthesis and transpiration, ensuring hydration under xeric conditions. Conversely, the stem accumulates large amounts of lipids (13.60 g/100 g) and reducing sugars (3.014 g/100 g), which likely function as both energy reserves and osmoprotectants, enhancing tissue stability under water deficit. Similarly, other studies explored the roles of Non-structural carbohydrates (NSCs) in intensifying the osmotic stress responses under salinity[31] and the impacts on root carbohydrate storage under waterlogging stress[32]. In particular, soluble NSCs like fructose, glucose, sucrose, and sugar-alcohols (e.g., sorbitol, mannitol) are essential to act as osmoprotectants and maintain cell turgor under osmotic stress, e.g., salt and drought stress[33, 34]. These organ–specific allocations suggest that *A. pilosa* optimizes its metabolism to withstand harsh edaphic environments, consistent with adaptive strategies reported in other drought–tolerant plants[35, 36].

Table 1: Biochemical composition in various parts of *A. pilosa*.

Samples	Moisture (%)	Protein (%)	Lipid (%)
Root	11.67 ± 0.306^{b}	0.265 ± 0.014^{b}	5.00 ± 1.114^{b}
Stem	9.80 ± 0.200^{c}	0.403 ± 0.030^{a}	13.60 ± 1.400^{a}
Leaf	15.80 ± 0.529^{a}	0.407 ± 0.041^{a}	12.13 ± 1.234^{a}

The values are shown as mean ± SE. Distinct letters in each column denote significant differences determined by Duncan's test at $p < 0.05$.

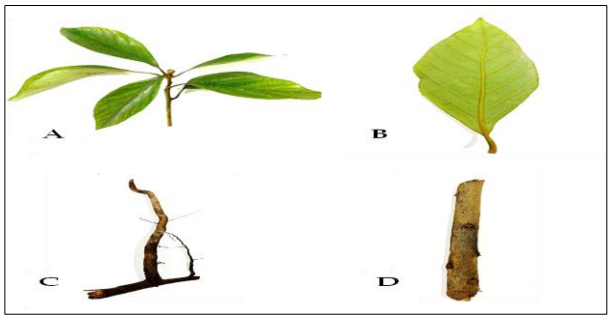

Figure 1: Various organs of mature *Actinodaphne pilosa* (Lour.) Merr plants. A, B: leaf; C: root; D: stem.

Antioxidant Assays

DPPH Radical-Scavenging Activity

The antioxidant potential of *Actinodaphne pilosa* extracts from root, stem, and leaf was assessed using the DPPH radical scavenging assay across concentrations ranging from 10 to 50 mg/mL (Fig. 2). All extracts exhibited clear dose–dependent activity, with similar inhibition rates (40–45%) at 10 mg/mL. However, at 50 mg/mL, the stem extract demonstrated significantly higher scavenging activity (94.40%) than the leaf (91.97%) and root (90.57%) extracts. Consistent with these results, the stem extract also exhibited the strongest antioxidant capacity, reflected by the lowest IC_{50} value (17.08 mg/mL), while the leaf and root extracts showed higher IC_{50} values ranging from 17.91 to 22.83 mg/mL (Fig. 3).

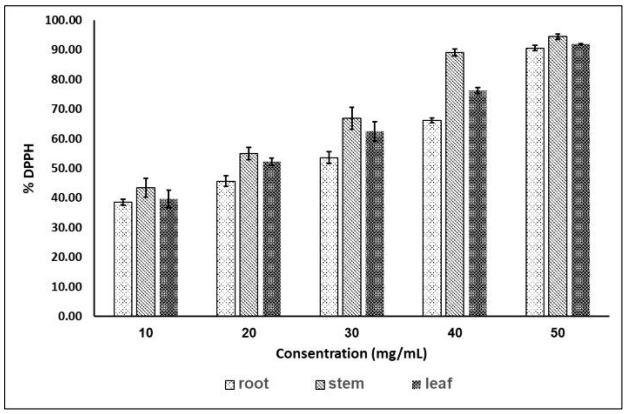

Figure 2: Percentage inhibition of DPPH free radical scavenging by the different parts extracts of *A. pilosa*. Different letters on a chart in each concentration of DPPH indicate statistically significant differences with $p < 0.05$ (Duncan's test).

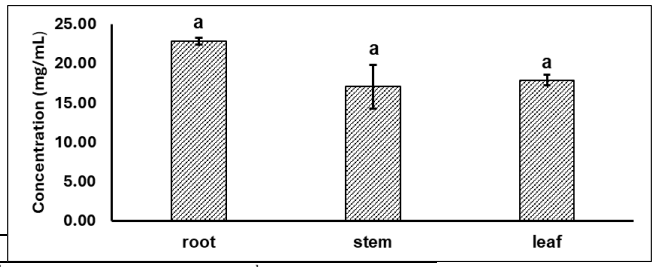

Figure 3: IC_{50} values of the extracts from different parts of *A. pilosa*. Values with different letters differ significantly at $p < 0.05$ (Duncan's test).

FRAP assay

Fig. 4 shows the FRAP values in different parts, with the highest level observed in stem extract (178.03 µmol Fe^{2+}/g DW) followed by root extract (170.09 µmol Fe^{2+}/g DW) and leaf extract (165.62 µmol Fe^{2+}/g DW).

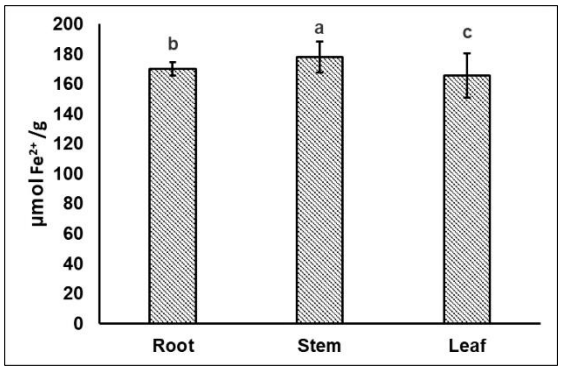

Figure 4: FRAP values (µmol Fe^{2+}/g) of root, stem, and leaf extracts of *A. pilosa*. Values with different letters differ significantly at $p < 0.05$ (Duncan's test).

Catalase activity (CAT)

The catalase activity in different organs of *A. pilosa* showed significant variation ($P < 0.05$) (Fig. 5). Among the organs analyzed, the stem exhibited the highest CAT activity (147.2 ± 1.96 U/mg protein), followed by the leaf (89.87 ± 2.31 U/mg protein), while the root displayed the lowest activity (72.49 ± 2.96 U/mg protein). This distribution suggests that the stem, being a major site of transport and metabolic activity, requires enhanced antioxidative protection, possibly to mitigate oxidative stress associated with higher reactive oxygen species levels.

The strong antioxidant activity observed in *A. pilosa* stem extracts (Fig. 3-5) appears to exceed previously reported values for well–known medicinal plants such as *Laurus nobilis* and *Pistacia lentiscus*[27], and aligns with notable antioxidant properties recorded in essential oils of *A. pruinosa*[5]. Additionally, leaf and root tissues of *A. pilosa* exhibited antioxidant profiles comparable to several reference species[2, 3], reaffirming the genus' potential as a source of radical–scavenging agents.

One striking observation is the elevated catalase activity detected in the stem (Fig. 5), which surpasses that reported for other Lauraceae species including *Litsea glutinosa* and *Cinnamomum camphora*[28, 29]. This suggests the existence of a robust enzymatic defense system in *A. pilosa*, potentially reflective of the stem's higher oxidative metabolism and exposure to environmental stressors. Lower catalase activity in the roots is consistent with known physiological trends, where aerial parts are generally equipped with stronger enzymatic antioxidant systems due to greater exposure to reactive oxygen species[30].

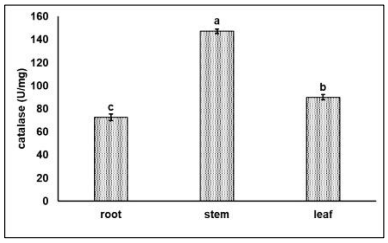

Figure 5: Catalase activity from extracts of different parts of *A. pilosa*. Values with different letters differ significantly at $p < 0.05$ (Duncan's test).

Bioactive metabolites

The total contents of phenolic (TPC) and flavonoid (TFC) compounds in different parts of *A. pilosa* are presented in Table 2, expressed as mean ± SD (mg GAE/g extract for TPC and mg CE/g extract for TFC).

Table 2: Content of bioactive compound in various parts of *A. pilosa*.

Samples	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg CE/g extract)
Root	28.17 ± 0.16^{c}	28.39 ± 0.49^{c}
Stem	100.6 ± 0.66^{a}	75.88 ± 0.12^{a}
Leaf	62.89 ± 0.32^{b}	48.00 ± 0.50^{b}

The values are shown as mean ± SE. Distinct letters in each column denote significant differences determined by Duncan's test at $p < 0.05$.

Total phenolic content

The stem exhibited the highest phenolic concentration (100.60 mg GAE/g extract), followed by the leaf (62.89 mg GAE/g extract) and root (28.17 mg GAE/g extract). Different superscript letters indicate statistically significant differences among samples. In relative terms, TPC in the stem was approximately 3.57-fold higher than in the root and 1.60-fold higher than in the leaf. The proportional contribution of each organ to the total TPC was ~52.49% (stem), ~32.81% (leaf), and ~14.70% (root).

Total flavonoid content.

A similar trend was observed for TFC, with the stem containing the highest concentration (75.88 mg CE/g extract), followed by the leaf (48.00 mg CE/g extract) and root (28.39 mg CE/g extract). The stem had TFC values about 2.67 times higher than the root and 1.58 times higher than the leaf. Relative contributions to the total TFC were ~49.83% (stem), ~31.52% (leaf), and ~18.64% (root).

These findings highlight the stem of *A. pilosa* as the primary reservoir of phenolic and flavonoid compounds, which may be associated with its structural and protective functions or tissue-specific metabolic regulation.

Secondary metabolite distribution patterns (Table 2) further support the functional role of the stem in stress resilience. The elevated accumulation of phenolics and flavonoids in stem tissues mirrors trends in other *Actinodaphne* and Lauraceae species, including *A. pruinosa* and *A. angustifolia*[5, 37]. Such accumulation is commonly observed in species growing under xeric conditions, where plants must mitigate oxidative stress arising from high light intensity, drought, and nutrient–poor soils.

In these environments, activation of the phenylpropanoid pathway enhances the biosynthesis of antioxidant metabolites such as phenolic acids and flavonoids, which effectively scavenge reactive oxygen species[38, 39]. Moreover, these compounds serve multifunctional roles in stress adaptation: flavonoids absorb UV radiation, reduce membrane peroxidation, and limit water loss[40, 41]; phenolic acids contribute to osmotic adjustment and cell wall reinforcement[42, 43, 44]. Certain flavonoids may also regulate key hormone signaling pathways–such as those involving abscisic acid or auxins–to mediate stomatal closure and enhance root growth, both of which are advantageous under drought conditions[45, 46].

Together, these results underscore the biochemical richness of *A. pilosa*, particularly in its stem and leaf tissues. Its unique antioxidant profile, comprising both enzymatic and non–enzymatic defenses, positions this species as a promising candidate for future applications in nutraceuticals, pharmaceuticals, and cosmeceuticals. Further studies should aim to isolate key bioactives, validate their mechanisms *in vivo*, and explore adaptive responses under simulated environmental stress to realize the full therapeutic potential of this underutilized medicinal plant.

Conclusion

This study demonstrates that the stem extract of *A. pilosa* possesses prominent biochemical and antioxidant properties compared to the leaf and root extracts. Among the tested fractions, the stem exhibited the highest total phenolic content, total flavonoid content, and the strongest antioxidant activities. These findings indicate that the stem is a promising source of bioactive metabolites with potential pharmaceutical or nutraceutical applications. Future studies should evaluate its antibacterial activity and explore sustainable cultivation models to fully harness the medicinal potential of this species.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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