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Qualitative and Quantitative Phytochemical analysis, Fluorescence Study and TLC of *Barleria prionitis* L. and *B. cristata* L. (Acanthaceae)

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ABSTRACT

The present study investigates the phytochemical profile, fluorescence characteristics, and chromatographic behaviour of powdered leaf and stem samples of Barleria prionitis L. and Barleria Cristata L., two ethnomedicinally important Acanthaceae species. Qualitative phytochemical screening using four solvent systems (aqueous, methanol, ethanol, and chloroform) revealed a rich presence of secondary metabolites, particularly alkaloids, terpenoids, phenolics, flavonoids, cardiac glycosides, reducing sugars, saponins, tannins, and steroids. Methanolic and ethanolic extracts exhibited the highest extraction efficiency, whereas aqueous and chloroform extracts showed selective solubility patterns. Notably, carbohydrates and proteins were largely absent across extracts, except for carbohydrates detected in B. prionitis leaf extracts. Quantitative estimation demonstrated that phenolics were the most abundant phytochemical in both species, with B. prionitis leaf showing the highest content (101.55 ± 0.34 mg/g), followed by B. cristata (78.3 ± 1.4 mg/g). Flavonoid concentrations were comparatively lower but consistently higher in leaf than in stem tissues. Fluorescence analysis under visible and UV light confirmed characteristic colour changes upon treatment with acids, alkalis, and oxidizing agents, supporting the presence of phenolics, flavonoids, tannins, and related metabolites. These distinctive fluorescence responses provide reliable pharmacognostic markers for authentication.TLC profiling of methanolic and ethanolic extracts revealed multiple spots in both species, with leaf extracts exhibiting more chemical diversity than stems. Several common Rf values indicate shared phytochemical constituents across the two taxa. Overall, the integrated phytochemical, fluorescence, and chromatographic findings highlight the chemical richness and diagnostic value of B. prionitis and B. cristata, supporting their traditional medicinal relevance and potential for future bioactive compound isolation.

Keywords: *Qualitative phytochemistry, Fluorescence analysis, TLC, Bioactive compounds.*

1. Introduction

Medicinal plants represent a rich reservoir of bioactive compounds that play a vital role in traditional healthcare systems and modern drug discovery. Members of the family Acanthaceae are widely recognized for their therapeutic applications, largely attributed to their diverse secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, glycosides, and steroids [1,2]. *Barleria prionitis* L. and *B. cristata* L. hold significant ethnomedicinal value across India and Southeast Asia, where they are traditionally used for treating respiratory ailments, inflammation, wounds, fever, gastrointestinal disorders, and liver abnormalities [3]. *Barleria prionitis* L., commonly known as Vajradanti, is documented for its anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, and wound-healing properties [3,4]. Phytochemical investigations of this species have previously revealed the presence of iridoid glycosides, phenolic acids, flavonoids, terpenoids, and steroids, which contribute to its pharmacological effects [5]. *Barleria cristata* L., popularly known as Philippine violet, also possesses notable medicinal qualities, including antioxidant, antidiabetic, antimicrobial, and analgesic potential, attributed to its diverse phytocompounds [6].

Despite their medicinal importance, comprehensive comparative studies on the phytochemical composition of their leaf and stem powders, especially using multiple solvent systems, remain limited.

Phytochemical screening is an essential preliminary step in identifying medicinally relevant plant constituents. The solubility of these compounds greatly depends on the polarity of the extraction solvents. Polar solvents like methanol and ethanol are known to extract a broader spectrum of phytochemicals, including phenolics, flavonoids, and glycosides, whereas non-polar solvents tend to extract terpenoids, steroids, and lipophilic components [7]. Evaluating extracts using different solvents therefore provides a clearer understanding of phytochemical diversity and distribution within plant tissues. Such analysis is fundamental for validating traditional uses and for guiding the isolation of pharmacologically active compounds.

Fluorescence analysis of crude plant drugs further serves as a rapid and reliable diagnostic tool in pharmacognosy. Many phytoconstituents either fluoresce naturally or exhibit fluorescence upon treatment with acidic, alkaline, or oxidizing reagents, producing characteristic colour changes under visible and ultraviolet light [8,9].

These fluorescence patterns create a diagnostic fingerprint that aids in crude drug identification, detection of adulteration, and quality control—an essential requirement in herbal standardization [10].

Thin-Layer Chromatography (TLC) is another indispensable technique used to separate complex mixtures of plant compounds. It provides insight into the number and nature of chemical constituents present in crude extracts and helps detect similarities across species by comparing retention factor (Rf) values [22,23]. TLC profiling is especially useful in chemotaxonomic studies and in preliminary selection of extracts for further chromatographic purification and phytochemical isolation.

Given the traditional significance and pharmacognostic potential of *Barleria prionitis* and *B. cristata*, the present study aimed to conduct a comparative analysis of their qualitative and quantitative phytochemical composition, fluorescence characteristics, and TLC profiles from leaf and stem powders using multiple solvent systems. Such an integrated evaluation not only strengthens authentication and quality control of these medicinal species but also provides a foundational basis for future phytopharmacological exploration.

2. Materials and Methods

2.1 Plant Material Collection and Authentication:

Fresh leaves and stems of *Barleria prionitis* L. and *Barleria cristata* L. were collected from (insert site; e.g., Akola district, Maharashtra) during the flowering season. Plant materials were authenticated by a qualified taxonomist, and voucher specimens were deposited in the departmental herbarium for future reference. The collected samples were washed, shade-dried for 10–15 days, and powdered using a mechanical grinder following standard pharmacognostic procedures [10].

2.2 Preparation of Plant Extracts:

Powdered leaf and stem samples (10 g each) were extracted separately using four solvent systems: aqueous, methanol, ethanol, and chloroform. Extracts were prepared by cold maceration for 48 hours with intermittent shaking, followed by filtration using Whatman No. 1 filter paper. Solvent systems were selected based on polarity gradient for effective recovery of a broad range of phytochemicals [2].

2.3 Qualitative Phytochemical Screening

Phytochemical tests for alkaloids, flavonoids, phenolics, tannins, terpenoids, saponins, steroids, proteins, and carbohydrates were performed using standard protocols[2, 13].

2.4 Quantitative Estimation of Major Phytochemicals

Total Phenolic Content (TPC)

TPC was estimated using the Folin–Ciocalteu method, with gallic acid as reference standard. Absorbance was recorded at 765 nm using a UV–Vis spectrophotometer [16,22].

Total Flavonoid Content (TFC)

TFC was determined using the aluminum chloride colorimetric assay, with quercetin as the standard. Absorbance was measured at 415 nm [14].

All values were calculated in mg/g dry weight and expressed as mean ± standard deviation.

2.5 Fluorescence Analysis

Fluorescence characteristics of powdered leaf and stem samples were studied following the method of [15,16].

2.6 Thin Layer Chromatography (TLC) Profiling:

TLC analysis was performed for methanolic and ethanolic extracts of both species following the protocol of Wagner and Bladt [11]. Both extracts were spotted onto silica gel 60 F254 plates and developed in solvent systems optimized for various phytochemicals. The mobile phase used was Petroleum ether: Acetone: Formic acid (70:20:10). Developed plates were visualized under daylight, UV 254 nm, UV 365 nm, and after spraying with anisaldehyde–sulfuric acid reagent. Rf values were recorded for each visible band.

3. Results

The present investigation evaluated the phytochemical richness, fluorescence characteristics, and chromatographic behaviour of leaf and stem samples of *Barleria prionitis* and *Barleria cristata*. Results obtained from qualitative and quantitative phytochemical screening demonstrated considerable variation in the presence and concentration of secondary metabolites across different solvent extracts. Fluorescence analysis further revealed distinct colour responses under visible and UV light, providing diagnostic features useful for crude drug identification. In addition, TLC profiling highlighted the diversity of phytoconstituents through varied numbers of spots and Rf values among extracts. These combined findings offer comprehensive insights into the chemical composition and pharmacognostic attributes of the two *Barleria* species.

3.1 Qualitative phytochemical analysis:

3.1.1Qualitative Phytochemical analysis of leaf powder extract:

The preliminary phytochemical screening of powdered leaf samples of the selected ethnomedicinal plants— *Barleria prionitis* L. and *Barleria cristata* L. was carried out using four different solvent extracts: aqueous (Aq. E), methanol (ME), ethanol (ET), and chloroform (CL). The results of the phytochemical analysis are summarized in Table-1.

The findings revealed that the selected species are rich in secondary metabolites, particularly alkaloids, terpenoids, phenolics, flavonoids, cardiac glycosides, reducing sugars, and steroids. These phytoconstituents were detected more consistently in the methanolic and ethanolic extracts compared to aqueous and chloroform extracts. Notably, carbohydrates and proteins & amino acids were absent in all solvent extracts across all species, with the exception of a few isolated observations.

In *Barleria prionitis* L., the aqueous extract (Aq. E) of leaf showed positive results for reducing sugars, saponins, tannins, flavonoids, phenolics, and carbohydrates. Both methanolic and ethanolic extracts exhibited the presence of cardiac glycosides, reducing sugars, saponins, flavonoids, phenolics, and carbohydrates. The chloroform extract of the leaf tested positive for terpenoids, reducing sugars, and steroids.

The powdered material of *Barleria cristata* L. showed the presence of cardiac glycosides, terpenoids, saponins, tannins, flavonoids, and steroids in the aqueous extract. The chloroform extract tested positive for terpenoids, reducing sugars, flavonoids, phenolics, and steroids, while the methanolic and ethanolic extracts exhibited the presence of all tested phytochemicals except carbohydrates and proteins & amino acids were present, except for reducing sugars, saponins, and flavonoids. The chloroform extract of the leaf revealed the presence of cardiac glycosides, terpenoids, reducing sugars, saponins, flavonoids, and steroids.

Among the species studied, *Barleria prionitis* L. showed a particularly high presence of terpenoids and phenolics in both leaf and stem, especially in methanolic and ethanolic extracts. Carbohydrates, proteins, and amino acidswere generally absent in all species and solvent extracts, with the exception of *B. prionitis* (Aq. E, ME, and ET extracts).

3.1.2. Preliminary phytochemical analysis of stem powder extract:

The preliminary phytochemical analysis of the stem powdered material of the selected ethnomedicinal plants—*Barleria prionitis* L., and *Barleria cristata* L., was conducted using four solvent systems: aqueous (Aq. E), methanol (ME), ethanol (ET), and chloroform (CL). The results are presented in Table 2.

The phytochemical screening revealed that all the selected plant species tested positive for terpenoids, reducing sugars (strong reaction), flavonoids, and steroids in all four extracts. Aqueous and ethanol extracts demonstrated a broader spectrum of phytochemical constituents, followed closely by methanol extracts, which were also rich in several bioactive compounds. Flavonoids were consistently detected in the aqueous, methanolic, and ethanolic extracts of all species. Steroids were present in all solvent extracts across all species. In contrast, tannins, carbohydrates, and proteins & amino acids were absent in all solvent extracts of both selected plants.

In *Barleria prionitis* L., the stem powder showed positive reactions for terpenoids, reducing sugars, saponins, flavonoids, phenolics, and steroids in aqueous extracts. The methanolic extract was positive for alkaloids, flavonoids, phenolics, steroids, and showed strong reactions for cardiac glycosides, terpenoids, and reducing sugars. Ethanol and chloroform extracts both tested positive for terpenoids, reducing sugars, and steroids.

In *Barleria cristata* L., the aqueous extract showed the presence of alkaloids, cardiac glycosides, reducing sugars, terpenoids, flavonoids, and steroids. The methanolic extract was positive for cardiac glycosides, terpenoids, saponins, flavonoids, and steroids. The ethanolic extract exhibited positivity for all tests except saponins, tannins, and phenolics. The chloroform extract demonstrated the presence of terpenoids, reducing sugars, saponins, and steroids.

Overall, terpenoids, reducing sugars, flavonoids, and steroids emerged as the most consistently detected phytochemicals across all stem extracts of the selected species. Methanolic and ethanolic solvents demonstrated higher extraction efficiency for multiple phytochemicals, whereas chloroform and aqueous extracts exhibited selective extraction patterns. The absence of tannins, carbohydrates, and proteins & amino acids in all extracts highlights a common chemical profile among the studied Acanthaceae species.

Table 1: Qualitative phytochemical analysis of leaf powder extract of B. prionitis andB. cristata L.

Sr. No	Plant Name	Solvent	Alkaloids	CardiacGlycosides	Terpenoids	ReducingSugar	Saponins	Tannins	Flavonoids	Phenolics	Steroids	Carbohydrates	Protein&Amino Acids
1.	*Barleria prionitis*L.	D.W	-	-	-	+	+	+	+	+	-	+	-
		Methanol	-	+	+	++	+	-	+	+	-	+	-
		Ethanol	+	+	+	+	+	-	+	+	-	+	-
		Chloroform	-	-	+	+	-	-	-	-	+	-	-
2.	*Barleria cristata*L.	D.W	-	++	++	-	+	+	+	-	++	-	-
		Methanol	+	++	+	+	+	+	+	+	+	-	-
		Ethanol	+	++	+	+	+	+	-	+	+	-	-
		Chloroform	-	-	+	+	-	-	+	+	+	-	-

(Aq.E = Aqueous extract, ME = Methanolic extract, ET = Ethanolic extract, CL = Chloroform extract)

Table 2 Qualitative phytochemical analysis of stem powder extract of B. prionitis and B. cristata L.

Sr. No	Plant Name	Solvent	Alkaloids	CardiacGlycosides	Terpenoids	ReducingSugar	Saponins	Tannins	Flavonoids	Phenolics	Steroids	Carbohydrates	Protein&AminoAcids
1.	*Barleria prionitis Linn.*	D.W.	-	-	++	++	+	-	+	+	++	-	-
		Methanol	+	++	++	++	+	-	+	+	+	-	-
		Ethanol	-	-	++	+	-	-	+	-	++	-	-
		Chloroform	-	-	+	+	-	-	-	-	+	-	-
2.	*Barleria cristata*	D.W.	+	+	+	+	-	-	+	-	+	-	-
		Methanol	-	+	+	-	+	-	+	-	+	-	-
		Ethanol	+	+	+	+	-	-	+	-	+	-	-
		Chloroform	-	-	+	+	+	-	-	-	+	-	-

(Aq.E = Aqueous extract, ME = Methanolic extract, ET = Ethanolic extract, CL = Chloroform extract)

3.2 Quantitative analysis of major phytochemicals:

The crude content of major phytochemical compounds in *Barleria prionitis* L and, *B. cristata* L., It has been found that among all four tested phytochemicals, selected plant showed higher level of phenolics followed by flavonoids. The observation found in both plants leaf extract showed higher content than stem in phenolics though same trend followed in stem powder.The content level of phenolics was significantly higher in leaf powder of *B. prionitis* leaf (101.55 ± 0.34) and less in *B. cristata* (78.3 ± 1.4), while the stem extract showed the same trend.As we see flavonoids content in leaf extract of *B. prionitis* (1.13 ± 0.46) is slight more than *B. cristata* (1.01 ± 0.50). The comparative account of quantitative analysis of these five plants is presented in (Table 3).

Table 3: Quantitative phytochemical analysis of powdered samples of B. prionitis and B. cristata L. (crude content in mg/g of dry sample)

Sr. No.	Phytochemicals / PlantName		Phenolics (mg/g)	Flavonoids (mg/g)
1	*BarleriaprionitisL.*	Leaf	101.55±0.34	1.13 ±0.46
		Stem	91.30±0.18	1.50 ±0.40
4	*BarleriacristataL.*	Leaf	78.3 ±1.4	1.01 ±0.50
		Stem	62.5 ±0.8	0.74 ±0.63

(Note: Results are the average of triplicate analyses.)

3.3 Fluorescence analysis of *B. prionitis* and *B. cristata* L. Leaf and Stem powder:

Both leaf and stem powders of *B. Prionitis* L. showed noticeable colour and fluorescence changes with different chemicals. Strong fluorescent green/yellow colours appeared under UV light, especially with NaOH, HCl 50%, and HNO_3, indicating phenolics and flavonoids. Acids like H_2SO_4 produced dark brown to black colours. $FeCl_3$ gave brown tones, confirming tannins. Leaf powder of *B. cristata* L. showed green to fluorescent yellow colours under sunlight. With water and acids, colours varied from yellow to moss green. Strong acids (H_2SO_4) produced blackish colours. NaOH and HNO_3 caused bright fluorescent reactions, indicating rich phenolic and flavonoid content. $FeCl_3$ produced brown, confirming tannins.

Table 4: A. Fluorescence analysis of B. prionitis L. Leaf and Stem powder

Reactions with chemicals	*BarleriaprionitisL.*					
	Leaf			Stem		
	Sunlight	UVlight- 254nm	UVlight- 365nm	Sunlight	UVlight- 254nm	UVlight- 365nm
Powderas such	Dull green	Green	Yellowish Green	Fluorescent yellow	Green	Dark green
Powder+ D.W	Light brown	Light Green	Light brown	Dull brown	Dull brown	Colorless
Powder+ HCL	Dark green	Dark Green	Dark brown	Dark brown	Dark brown	Dark brown
Powder+ HCL50%	Yellowish green	Fluorescent Green	Fluorescent yellow	Fluorescent brown	Colorless	Colorless
Powder+ H_2SO_4	Dark brown	Fluorescent brown	Dark brown	Dark black	Maroon	Deep brown
Powder + $H_2SO_4$50%	Dark green	Green	Brown	Dark brown	Dark brown	Deep brown
Powder+ NaOH	Light yellow	Yellowish green	Fluorescent yellow	Fluorescent Yellow	Fluorescent light green	Fluorescent green
Powder+ $FeCl_3$ 5%	Brown	Light brown	Dark brown	Dull brown	Fluorescent brown	Dark brown
Powder+ $HNO_3$50	Fluorescent yellow	Fluorescent green	Fluorescent yellow	Yellow	Fluorescent green	Fluorescent yellowish
Powder+ $NaNO_3$	Fluorescent brown	Fluorescent light	Light brown	Colorless	Colorless	Colorless
Hydroquinone	Light brown	Light brown	Dark brown	Dull brown	Dull green	Dark brown

Table 4: B. Fluorescence analysis of B. cristata L. Leaf and Stem powder

Reactions with chemicals	*Barleriacristata* L					
	Leaf			Leaf		
	Sunlight	UVlight- 254nm	UVlight- 365nm	Sunlight	UVlight- 254nm	UVlight- 365nm
Powderas such	Green	Creamson green	Lightish green	Green	Yellowish green	Fluorescent yellow
Powder+ D.W.	Light yellow	Brown	Moss green	Colorless	fluorescent gold	colorless
Powder+ HCL	Yellow green	Moss green	colorless	Brownish green	Gold brown	Moss green
Powder+ HCL50%	Fluorescent yellow	Yellow	Brown	cream	Sparkle Brownish	Greenish
Powder+ H_2SO_4	Bottle green	Brown	Black	Blackish brown	Black	Black
Powder + $H_2SO_4$50%	Green	Fluorescent green	Faintgreen	Dull brown	Light brown	Colorless
Powder+ NaOH	Light brown	Greenish brown	Fluorescent green	Light yellow	Yellowish green	Fluorescent yellow
Powder+ $FeCl_3$ 5%	Brown	Brown	Brown	Brown	Light brown	Dark brown
Powder+ $HNO_3$50	Light orange	Light orange	Moss green	Fluorescent yellow	Fluorescent green	Fluorescent yellow
Powder+ $NaNO_3$	Colorless	Muddy water	Light green	Fluorescent brown	Fluorescent light	Light brown
Hydroquinone	Dark brown	Brown	Black	Dark brown	Light brown	Dark brown

3.4 TL Canalysis of the crude powder extracts:

The TLC analysis was performed to identify the number of compounds extracted from the powder samples in their methanolic and ethanolic extract. The mobile phase used was Petroleum ether: Acetone: Formic acid (70:20:10).The methanolic extract of B. prionitis L. leaf and stem showed 5 and 5 spots respectively, whereas *B. Cristata* L. showed 5 and 3 spots respectively. The leaf extracts of both plants share three compounds in common (Rf value 0.13, 0.11 and 0.07). The account of TLC analysis of methanolic extract of selected plants are given below in (Table 5).

The ethanolic extract of all plants showed more spots as compare to methanolic extract. *B. Prionitis* leaf and stem showed 6, 5 spots whereas *B. Cristata* L. leaf and stem showed 6, 1, 7 and 5 spots respectively. In both selected plants leaf extracts showed more spots than stem. The leaf extracts of both plants shares two common compounds having RF value 0.28, 0.10, where the stem share only one compound common having RF value 0.12. The comparative account of TLC analysis of ethanolic extract of five plants are given below in (Table- 6).

Table 5: Rf values obtained from TLC analysis of powdered samples (Methanol extract)

***Barleriaprionitis*L.**				***Barleriacristata*L.**			
leaf		Stem		Leaf		Stem	
No. of Spot	RFvalue	No. ofspot	RFvalue	No. of spot	RF value	No. of spot	RF value
1	0.93	1	0.17	1	0.98	1	0.20
2	0.11	2	0.12	2	0.16	2	0.16
3	0.08	3	0.11	3	0.13	3	0.13
4	0.04	4	0.09	4	0.11	4	
5	0.01	5	0.08	5	0.07	5	
						6	

Table 6: Rf values obtained from TLC analysis of powdered samples (Ethanol extract)

***Barleriaprionitis*L.**				***Barleriacristata*L.**			
leaf		Stem		Leaf		Leaf	
No. of spot	RF value	No. of spot	No. of spot	No. of spot	No.of spot	No. of spot	RF value
1	0.90	1	0.91	1	0.91	1	0.12
2	0.37	2	0.30	2	0.28	2	
3	0.28	3	017	3	0.17	3	
4	0.17	4	0.12	4	0.12	4	
5	0.10	5	0.07	5	0.10	5	
6	0.08			6	0.07	6	

4. Discussion

The comparative analysis of extraction efficiency across solvents revealed methanol and ethanol to be superior in isolating the majority of chemical constituents from both leaf and stem tissues. This finding is consistent with earlier reports where polar solvents such as methanol and ethanol demonstrated higher efficiency in solubilizing alkaloids, glycosides, flavonoids, phenolics, and terpenoids compared to aqueous or chloroform solvents [7]. The stronger ability of alcohol-based solvents to penetrate plant cell walls and dissolve both moderately polar and polar constituents explains their consistent yield of positive phytochemical results in the present study.

The phytochemical screening confirmed the presence of major secondary metabolites, including alkaloids, terpenoids, phenolics, flavonoids, cardiac glycosides, reducing sugars, saponins, tannins and steroids. These classes of compounds are widely reported for their pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and cardioprotective effects [2,15,16]. The absence of carbohydrates, proteins, and amino acids in *B. cristata* highlights the dominance of secondary over primary metabolites in the studied taxa.

Among the investigated plants, *Barleria prionitis* exhibited a particularly high abundance of phenolics and terpenoids in both leaf and stem extracts, especially when methanol and ethanol were used as solvents. This is significant, as phenolic compounds and terpenoids are key contributors to the plant's traditional use to treat inflammatory disorders, wound healing, and hepatoprotection [18].

Fluorescence analysis of the powdered drug with different chemical reagents exhibited striking variations in coloration under visible and ultraviolet (UV) light. Such changes in fluorescence are attributed to the chemical nature of phytoconstituents, as many secondary metabolites fluoresce naturally or upon derivatization with reagents [8, 9]. The distinctive colors observed under UV light serve as a simple, rapid, and reliable pharmacognostic parameter for the identification and quality control of crude drugs [10]. The unusual variations in fluorescence response of the studied plants confirm the presence of diverse groups of phytochemicals and validate their diagnostic value in standardization studies [19]

The presence of diverse phytochemicals in all studied species underscores their therapeutic potential and validates their ethnomedicinal usage.

Flavonoids and phenolic compounds contribute significantly to antioxidant and anti-inflammatory effects, while terpenoids and steroids are associated with antimicrobial and cytotoxic activities [20,21].

The fluorescence and phytochemical screening results collectively provide a pharmacognostic fingerprint for the studied plants. Such fingerprints are crucial for crude drug authentication, prevention of adulteration, and for guiding future isolation and characterization of bioactive compounds [15,20].

5. Conclusion

The study confirms that *Barleria prionitis* and *Barleria cristata* are rich in key secondary metabolites, especially phenolics and flavonoids, with methanolic and ethanolic extracts showing highest yields. Distinct fluorescence reactions and diverse TLC profiles further support their pharmacognostic value. Overall, both species exhibit strong chemical potential, validating their traditional medicinal use and suitability for future bioactive compound isolation.

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