

Comparative LC-MS Profiling of two Linderniaceae Species

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ABSTRACT

Introduction: The presence of the phytochemical constituents has been reported from species of the Linderniaceae family. **Objectives:** The present study was designed to determine the bioactive compounds in the whole plant extracts of *Bonnaya ciliata* (Colsm.) Spreng. and *Torenia crustacea* (L.) Cham. and Schltdl. **Materials and Methods:** Phytochemical screening of these plants revealed the presence of several bioactive components. Liquid Chromatography Mass Spectrometry (LCMS) analysis of the ethanolic extracts was performed using LCMS equipment. **Results:** The phytochemical tests revealed the presence of several bioactive compounds. Utilizing qualitative analysis alongside advanced LC-MS profiling, we aimed to uncover the range of bioactive compounds these species contain. Our qualitative assessments revealed a rich array of secondary metabolites, including phenolic compounds, flavonoids, and glycosides, indicating significant bioactive potential. The LC-MS analysis further identified key constituents such as Aloesin, Luteolin-7-glucoside, Rhodiolside, and Kaempferol-3-O-glucoside, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. These findings highlight the therapeutic significance of *Bonnaya ciliata* (Colsm.) Spreng. and *Torenia crustacea* (L.) Cham. and Schltdl, suggesting their potential as sources of natural remedies. **Conclusion:** The findings suggest that by combining qualitative and quantitative approaches, this research provides a foundation for future studies aimed at developing plant-based solutions for health and wellness.

Keywords: Linderniaceae, Phytochemical, *Bonnaya ciliata*, *Torenia crustacea*, Plant-based drug discovery.

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INTRODUCTION

Secondary metabolites, including flavonoids, phenolic acids, and alkaloids, are integral to both ecological functions and human health applications.^[1] These compounds are well known for their diverse biological activities, such as antioxidant, antimicrobial, and anti-inflammatory effects, which help plants adapt to environmental stress and offer therapeutic potential for treating various human diseases.^[2] Despite their importance, plants from the Linderniaceae family, including *Torenia crustacea* (L.) Cham. and Schltdl and *Bonnaya ciliata* (Colsm.) Spreng. remain underexplored in medicinal research, especially regarding their full range of phytochemical diversity and bioactive potential.^[3]

Recent studies have begun to highlight the promise of *Bonnaya ciliata* (Colsm.) Spreng. in phytochemical research, noting the presence of bioactive compounds that could contribute to drug discovery and therapeutic treatments^[4] *Torenia crustacea* (L.) Cham. Also shows potential, but further investigation is needed to fully characterize its secondary metabolites.^[5] The genus

Bonnaya has been recognized for its considerable variation in leaf morphology, which not only aids in species identification but also provides insight into the ecological roles and medicinal uses of these plants.^[6] Furthermore, recent genetic studies on *Bonnaya* species, including plastid genome analyses, have provided valuable information on their evolutionary history, which may enhance their application in biotechnological and pharmacological studies.^[7]

Advancements in analytical techniques, such as Liquid Chromatography-Mass Spectrometry (LC-MS), have revolutionized the profiling of plant metabolites. LC-MS offers high sensitivity and precision, allowing for the detection of a wide range of compounds and the identification of novel therapeutic agents.^[8] In this study, LC-MS is used to examine the phytochemical profiles of *Torenia crustacea* (L.) Cham. and Schltdl and *Bonnaya ciliata* (Colsm.) Spreng. enabling the identification of both shared and unique metabolites. This comparative approach aims to uncover their potential therapeutic applications, explore their ecological roles, and provide deeper insights into their biosynthetic pathways. The ethnobotanical significance of *Torenia crustacea* (L.) Cham. and Schltdl and *Bonnaya ciliata* (Colsm.) Spreng. lies in their traditional use to treat wounds, skin disorders, fevers, and stress-related conditions, which is corroborated by LC-MS profiling that reveals



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a diverse range of bioactive compounds such as flavonoids, phenolic acids, and glycosides.^[9] These findings highlight their potential in antioxidant, anti-inflammatory, and antimicrobial applications. Moreover, the presence of unique metabolites like isorhamnetin-3,4'-diglucoside and rhodioloside further validates their historical use in folk medicine while paving the way for further exploration of their pharmacological and ecological roles.

With recent findings suggesting the importance of secondary metabolites like flavonoids and phenolic compounds in mitigating oxidative stress and promoting health.^[10] This research aims to contribute to the growing body of knowledge about the medicinal properties of Linderniaceae species. By elucidating the phytochemical profiles of *Torenia crustacea* (L.) Cham. and Schltld and *Bonnaya ciliata* (Colsm.) Spreng. We hope to identify new avenues for drug development and better understand the ecological functions of these plants.

MATERIALS AND METHODS

Collection of Plant Materials

Mature leaves of *Torenia crustacea* (L.) Cham. and Schltld and *Bonnaya ciliata* (Colsm.) Spreng. were collected from the Madayipara fields in Kannur district, Kerala, India. The plants were authenticated by Dr. M. U. Sharief, Botanical Survey of India, and voucher specimens were deposited at the Madras Herbarium.

Sample Preparation

The collected plant materials were washed, shade-dried, and powdered. Ethanol was used for solvent extraction using Soxhlet apparatus (Figure 1). The extracts were filtered, concentrated under reduced pressure, and prepared for LC-MS analysis.

LC-MS Analysis

Samples were analyzed using a Thermo Fisher Scientific LC-MS system. The sample preparation involved dissolving 10 mg of *Bonnaya ciliata* or *Torenia crustacea* in 1 mL of Tetrahydrofuran (THF) and diluting the mixture to a total volume of 10 mL using methanol. This solution was filtered before being injected into the chromatographic system. The Liquid Chromatography-Mass Spectrometry (LC-MS) analysis was performed with a Waters 1525 μ Binary Pump and a Xevo G2-XS QToF mass spectrometer (Waters, USA). The mobile phases consisted of 0.1% formic acid in water (Phase A) and acetonitrile (Phase B). A 10 μ L injection volume was used with a gradient elution program: initially, 95% A and 5% B, which gradually changed to 50% A and 50% B by 6 min, then to 5% A and 95% B at 12 min, before returning to the initial conditions at 18 min. The flow rate was maintained at 0.500 mL/min throughout the analysis. An Accucore C18 column (50 \times 4.6 mm, 5 μ m particle size, ThermoScientific) was employed for separation. The MS conditions included a capillary voltage of 3.0 kV, with a collision energy set at 20V, ramping from 30V to

90V. The source and desolvation temperatures were set at 150°C and 450°C, respectively, with cone and desolvation gas flows at 50 L/hr and 800 L/hr. Data processing was performed using MassLynx V4.1 software. The negative ion mode was applied for *Bonnaya ciliata* and *Torenia crustacea*. Data were processed using reference databases to identify compounds based on retention times and m/z values.^[11]

RESULTS

Phytochemical Profiles

Based on the LC-MS data provided, the chromatogram represents the analysis of *Bonnaya ciliata* (Colsm.) Spreng extracts, revealing the various peaks corresponding to different compounds. This method identifies metabolites by measuring their retention time (x-axis) and intensity (y-axis), indicating the presence and abundance of different secondary metabolites (Figure 2). The prominent peaks, particularly at retention times of 11.11 and 11.31 min, highlight major compounds in the extract, suggesting their possible therapeutic significance.

Notable peaks include those at retention times of 6.5 min, 12.2 min, and 15.8 min, which correspond to flavonoids such as luteolin-7-glucoside, phenolic acids like 5-caffeoylquinic acid, and alkaloids such as protopine, respectively. These peaks were identified based on their distinct m/z values and fragmentation patterns, indicating the presence of key bioactive compounds with significant therapeutic relevance. This LC-MS data highlights the complex phytochemical composition of *Torenia crustacea*, which warrants further investigation for its potential biological activities. The LC-MS analysis identified compounds across the 2 species includes phenolic acids, flavonoids, alkaloids, and coumarins.

Shared Phytochemicals

Both species exhibited compounds like kaempferol-3-O-glucoside, luteolin-7-glucoside indicating common biosynthetic pathways. These compounds are well-known for their antioxidant and anti-inflammatory activities, validating their roles in stress mitigation and microbial defense (Table 1). The detection of squamatic acid, a depside with potent antimicrobial properties, reflects their ecological adaptation to microbial challenges in their natural habitats.

Unique Phytochemicals

Bonnaya ciliata showed a flavonoid-dominant profile, with unique compounds such as isorhamnetin-3,4'-diglucoside and 7,3',4'-trihydroxyflavone, known for hepatoprotective, anti-inflammatory, and anti-cancer properties (Table 2). This suggests its therapeutic potential in managing liver diseases, oxidative stress, and inflammation.^[12]

Torenia crustacea exhibited a phenolic acid-rich profile, including 5-caffeoylquinic acid and rhodioloside, compounds associated

with neuroprotection, stress adaptation, and anti-diabetic effects (Table 3). These findings support its adaptogenic and cognitive enhancement potential.^[5]

Ecological Significance

The presence of these compounds underlines the adaptive strategies of these species to thrive in their environments, including defense against oxidative stress and microbial pathogens. The presence of depsides and alkaloids in *Torenia crustacea* aligns with its habitat's high microbial load, indicating an evolved antimicrobial defense.^[13]

Ethnobotanical Context

The identified compounds align closely with the traditional medicinal uses of these plants, including treatments for wounds, skin ailments, and stress-related conditions. This demonstrates a strong correlation between traditional knowledge and scientific validation. Therapeutic Potential: Flavonoids unique to *Bonnaya ciliata* make it a promising candidate for anti-inflammatory applications.^[14]

DISCUSSION

This study provides a detailed metabolomic comparison of two underexplored members of the Linderniaceae family *Torenia crustacea* (L.) Cham. and Schltdl and *Bonnaya ciliata* (Colsm.)

Spreng., using LC-MS. The results reveal a diverse array of secondary metabolites—primarily flavonoids, phenolic acids, glycosides, alkaloids, coumarins, tannins, and macrolides—that underscore both their ecological adaptations and pharmacological relevance (Figure 3).

The LC-MS chromatograms and compound identification demonstrate that both species share bioactive compounds such as kaempferol-3-O-glucoside and luteolin-7-glucoside, which are known for potent antioxidant and anti-inflammatory activities.^[15] The presence of these shared flavonoids suggests common biosynthetic pathways across Linderniaceae species that may play crucial roles in environmental stress response and defense against pathogens.^[16]

However, each species also exhibited unique metabolite signatures, reflecting species-specific adaptations and therapeutic potentials. In *Bonnaya ciliata*, the LC-MS profile revealed a flavonoid-dominant composition, with unique constituents such as isorhamnetin-3,4'-diglucoside, 7,3',4'-trihydroxyflavone, and scutellarein-7-glucuronide. These flavonoids are associated with hepatoprotective, anti-cancer, and anti-inflammatory properties, highlighting the plant's pharmacological versatility.^[17] Additionally, compounds like canavanine, amarogentin, vincamine indicate potential for antimicrobial, cognitive-enhancing, and anticancer applications.^[18] These compounds, visualized in Figure 3, emphasize *Bonnaya ciliata*

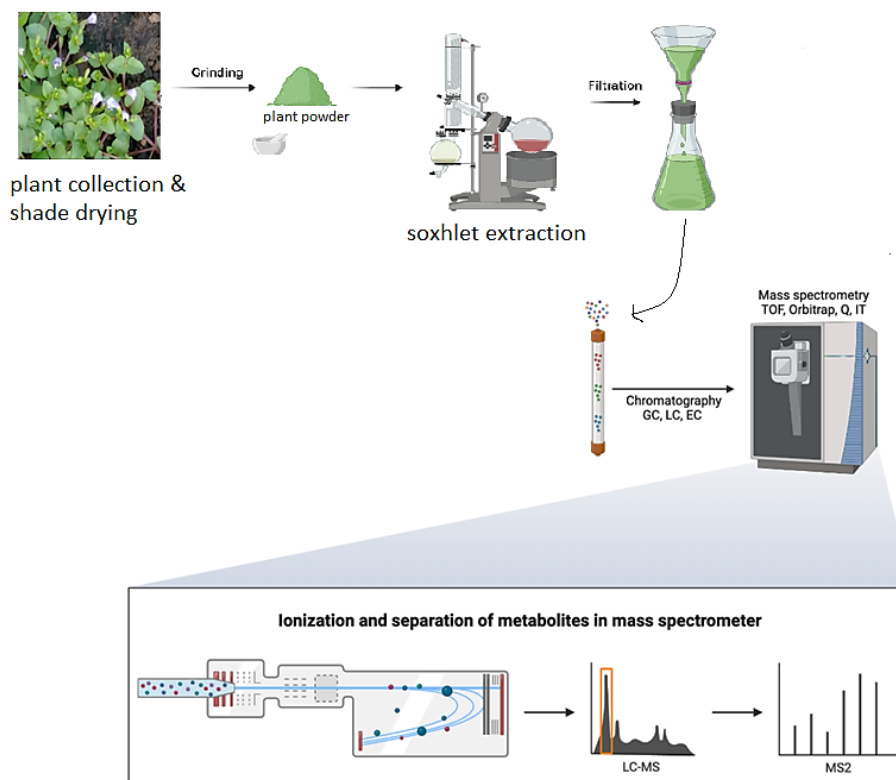


Figure 1: Extraction of phytocompounds for LCMS analysis: A comprehensive overview. Ona, S. (2022) BioRender. Ona, S. (2022).

Table 1: Summary of identified compounds from *Torenia crustacea* and their therapeutic relevance.

Compound	Significance	Therapeutic Potential	Class	Citation
Flavonoids and Derivatives				
Luteolin-7-glucoside	Flavonoid glycoside with antioxidant and anti-inflammatory properties.	Treats inflammatory diseases and oxidative stress.	Flavonoid-7-O-glycosides	[26]
Tricetin	Flavone with anti-inflammatory and anti-proliferative activities.	Inhibits cancer cell growth and oxidative stress.	Flavonoid	[27]
Procyanidin B2	Biflavonoid with free radical scavenging and cardiovascular protective properties.	Supports heart health and potential anti-cancer effects.	Flavonoid	[27]
Isosakuranin	Flavonoid glycoside with antioxidant properties.	Supports anti-inflammatory and hepatoprotective effects.	Flavonoid	[26]
3-Methylquercetin	Methylated flavonoid with strong antioxidant and anti-cancer properties.	Antioxidant and anti-cancer effects.	Flavonoid	[28]
Phenolic Compounds and Glycosides				
5-Caffeoylquinic acid	Major phenolic acid with antioxidant properties.	Neuroprotective, anti-diabetic, and anti-cancer potential.	Phenolic acid	[29]
Arbutin	Phenolic glycoside with depigmenting properties.	Used in skin lightening and treating hyperpigmentation.	Phenolic glycoside	[20]
Squamatic acid	Depside derivative with anti-inflammatory effects.	Potential use in inflammatory and immune-related disorders.	Phenolic compound	[30]
Alkaloids				
Protopine	Protopine alkaloid with antispasmodic, anti-inflammatory, and antimicrobial effects.	Used for spasms, inflammation, and infections.	Alkaloid	[31]
Rauwolscine	Yohimbine alkaloid with adrenergic. Receptor blocking properties.	Treats obesity, erectile dysfunction, and improves cognitive function.	Alkaloid	[32]
Coumarins				
Hymecromone (7-hydroxycoumarin)	Coumarin derivative with hepatoprotective and anti-spasmodic effects.	Used in liver disorders and bile regulation.	Coumarin	[33]
6,7-Dihydroxycoumarin	Coumarin with antioxidant and anti-cancer activity.	Effective against oxidative stress and cancer cells.	Coumarin	[34]
Tannins and Macrolides				
Thalsimine	Tannin derivative with antioxidant and antibacterial properties.	Useful in antibacterial and antioxidant therapies.	Tannin	[35]
(8E)-11,13-Dihydroxy-4-methyl-benzoxacyclododecine.	Macrolide analogue with antimicrobial properties.	Potential antimicrobial agent.	Macrolide	[36]

(Colsm.) Spreng., is a promise as a source of neuroprotective and antimicrobial agents.

Torenia crustacea, on the other hand, presented a phenolic acid-rich profile, particularly 5-caffeoylquinic acid, squamatic acid, and rhodioloside, each of which plays a role in neuroprotection, anti-diabetic activity, and adaptogenic effects.^[5] Table 1 summarizes the therapeutic relevance of key identified metabolites. Notably, tricetin, procyanidin B2, and 3-methylquercetin provide antioxidant, anti-proliferative, and cardioprotective functions, reinforcing the Species potential in chronic disease management.^[19] The detection of arbutin-a depigmenting agent-along with alkaloids like protopine and rauwolscine, supports traditional applications in treating skin ailments and cognitive disorders.^[20]

Ecologically, the presence of metabolites like squamatic acid, depsides, and multiple alkaloids suggests well-developed biochemical defenses against microbial stress, supporting their adaptability to diverse microhabitats. Such chemical profiles are indicative of evolved strategies for survival and resilience, particularly under environmental stress conditions.^[21]

Furthermore, these phytochemical findings strongly corroborate the ethnobotanical uses of both plants. Traditionally used for wound healing, fever reduction, and stress-related ailments, the compounds identified here-including flavonoids, phenolic glycosides, and coumarins-scientifically validate these applications.^[22] The presence of flavonoids with anti-inflammatory actions in *B. ciliata* and neuroactive phenolic acids in *T. crustacea* reflects a remarkable alignment between indigenous knowledge and modern phytochemical evidence.^[23]

This study also underscores the value of LC-MS-based metabolomics in natural product research.^[8] In conclusion, the comparative metabolomic data of *B. ciliata* and *T. crustacea* establish a compelling case for their inclusion in pharmacognostic investigations. While shared metabolites suggest fundamental roles in stress physiology and defense, the unique compounds identified in each species offer targeted therapeutic potentials-ranging from antioxidant and hepatoprotective to neuroprotective and antimicrobial activities.^[24] These findings advocate for further *in vivo* validation, bioassay-guided isolation, and exploration of synergistic effects, which could contribute to

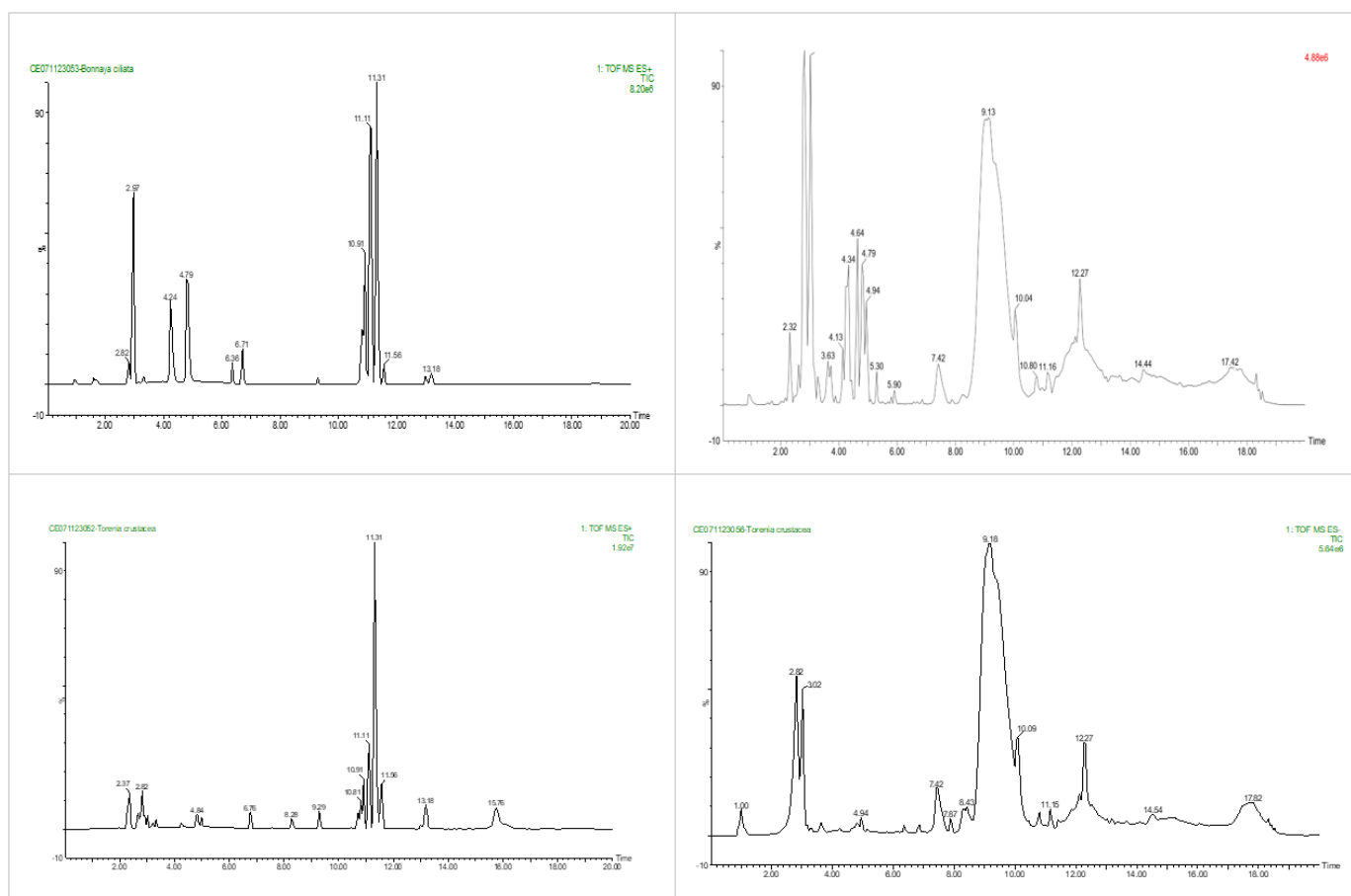


Figure 2: a) Positive mode LC-MS Chromatogram of *Bonneya ciliata* extract. b) Negative mode LC-MS Chromatogram of *Bonneya ciliata* extract. c) Positive mode LC-MS chromatogram of *Torenia crustacea* extract. d) Negative LC-MS chromatogram of *Torenia crustacea* extract. The chromatograms display prominent peaks at retention times highlighting the presence of bioactive compounds such as flavonoids, phenolic acids, and alkaloids, which are important for assessing the plants' therapeutic potential. The x-axis represents retention time (minutes), and the y-axis indicates compound intensity.

Table 2: Shared and Unique Compounds Identified in LC-MS Profiling.

Compound Name	Ontology	Species	Biological Relevance	Reference
Quinolin-4-yl(8-vinylquinuclidin-2-yl) methanol	Alkaloids	<i>Torenia crustacea</i> , <i>Bonnaya ciliata</i>	Antibacterial, Antifungal	[37]
Squamic acid	Depside	<i>Torenia crustacea</i> , <i>Bonnaya ciliata</i>	Antioxidant, Antimicrobial	[38]
Kaempferol-3-O-glucoside	Flavonoids	<i>Bonnaya ciliata</i> , <i>Torenia crustacea</i>	Anti-inflammatory	[39]
Aloesin	Phenolic glycosides	<i>Torenia crustacea</i>	Skin protectant, Anti-inflammatory	[40]
Luteolin-7-glucoside	Flavonoids	<i>Bonnaya ciliata</i>	Antioxidant, Anti-inflammatory	[26]
Rhodiolside	Phenolic glycosides	<i>Torenia crustacea</i>	Adaptogenic, Anti-stress	[41]
Ferulic acid	Phenolic acids	<i>Torenia crustacea</i>	Anti-inflammatory, Antimicrobial	[42]

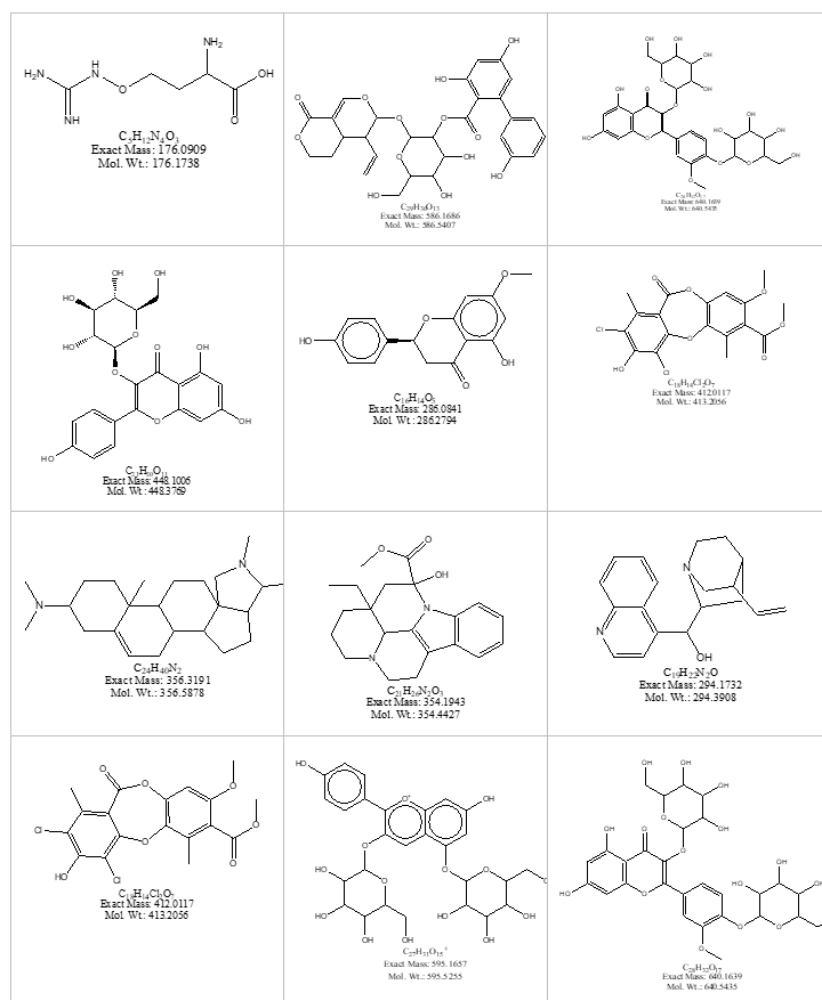


Figure 3: Some key phytoconstituents present in *Bonnaya ciliata* (Colsm.) Pennel. a) Canavanine b) Amarogentin c) Isorhamnetin 3,4'-diglucoside d) Kaempferol-3-O-glucoside e) Scutellarein-7-glucuronide f) Conessine g) Vincamine h) Quinolin-4-yl(8-vinylquinuclidin-2-yl)methanol, i) Gangaleoidin, j) Pelargonin, k) Isorhamnetin 3,4'-diglucoside, l) quercetin-3-O-rutinoside.

Table 3: LC-MS Analysis of *Torenia crustacea*.

Compound Name	RT (min)	Height	Area	Reference m/z	Formula	Ontology
5-Caffeoylquinic acid	1.71	4015	69365	353.0891	C ₁₆ H ₁₈ O ₉	Quinic acids and derivatives
Galocatechin	2.265	1153	16944	305.066	C ₁₅ H ₁₄ O ₇	Epigallocatechins
6,7-Dihydroxycoumarin	2.316	2617	59340	177.0185	C ₉ H ₆ O ₄	6,7-Dihydroxycoumarins
2'-Hydroxygenistein-7-O-glucoside	2.922	1680	26737	447.096	C ₂₁ H ₂₀ O ₁₀	Isoflavonoid O-glycosides
Phenolic glycoside (complex structure)	3.225	2925	52316	637.271	C ₂₇ H ₄₄ O ₁₇	Phenolic glycosides
Macrolide (complex structure)	3.326	1101	19993	289.108	C ₁₆ H ₁₈ O ₅	Macrolides and analogues
3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	4.235	23778	399722	285.034	C ₁₅ H ₁₀ O ₆	Flavonols
2-Hydroxy-4-(2-hydroxy-4-methoxy-6-propylbenzyloxy)-6-propylbenzoic acid	4.841	13259	184413	387.141	C ₂₁ H ₂₄ O ₇	Depsides and depsidones
(9Z,12E)-15,16-Dihydroxyoctadeca-9,12-dienoic acid	6.154	1046	14480	311.1764	C ₁₈ H ₃₂ O ₄	Linoleic acids and derivatives
5-Hydroxy-2,2-dimethyl-10-(2-methylbut-3-en-2-yl)pyrano[3,2-g]chromen-8-one	6.154	1091	15394	311.1866	C ₁₉ H ₂₀ O ₄	Linear pyranocoumarins
Flavonoid C-glycoside (complex structure)	8.326	166341	3682944	579.2896	C ₂₇ H ₃₂ O ₁₅	Flavonoid C-glycosides
3-Methylquercetin	9.084	9498	141292	297.2333	C ₁₆ H ₁₂ O ₇	3-O-Methylated flavonoids
Conessine	10.801	5165	189736	355.3119	C ₂₄ H ₄₀ N ₂	Conanine-type alkaloids
Diterpenoid (complex structure)	10.852	15297	14446	311.15	C ₁₅ H ₂₂ O ₄	Diterpenoids
Menthane monoterpene (complex structure)	11.104	7398	235022	325.1657	C ₁₇ H ₂₆ O ₆	Menthane monoterpene
Squamic acid	11.155	4791	103491	411.069	C ₁₉ H ₁₈ O ₈	Depsides and depsidones
Quinolin-4-yl(8-vinylquinuclidin-2-yl)methanol	12.266	145095	3909009	293.1659	C ₁₉ H ₂₂ N ₂	Cinchona alkaloids
Tryptophenolide	12.266	2390	74362	311.1653	C ₂₀ H ₂₄ O ₃	Oxosteroids
Menthane monoterpene (complex structure)	12.266	18959	910869	325.1657	C ₁₇ H ₂₆ O ₆	Menthane monoterpene
Rauwolfscine	12.266	44815	911233	353.187	C ₂₁ H ₂₆ N ₂	Yohimbine alkaloids
Dehydroabietic acid	12.316	2540	35045	299.236	C ₂₀ H ₂₈ O ₂	Diterpenoids
Coronardine	12.367	35575	675264	337.1937	C ₂₁ H ₂₆ N ₂	Ibogan-type alkaloids
Menthane monoterpene (complex structure)	13.63	12493	426152	339.1813	C ₁₈ H ₂₈ O ₆	Menthane monoterpene
2-Hydroxy-4-methoxy-3,5-bis(3-methylbut-2-enyl)-6-pentylbenzoic acid	14.438	6061	97753	373.2604	C ₂₃ H ₃₄ O ₄	p-Methoxybenzoic acids and derivatives
(6E,8Z,11Z,14Z)-5-Hydroxyicos-6,8,11,14-tetraenoic acid	14.842	2436	47713	327.2778	C ₂₀ H ₃₂ O ₃	Hydroxyeicosatetraenoic acids

Table 4: LC-MS Analysis of *Bonnaya ciliata*.

Name	RT (min)	Height	Area	Reference Formula	Ontology
Ursinoic Acid	0.952	7122	99589	C ₁₅ H ₁₆ O ₅	2,2-Dimethyl-1-benzopyrans
6,7-Dihydroxy-2H-chromen-2-one	2.316	1387	20344	C ₉ H ₆ O ₄	6,7-Dihydroxycoumarins
Isorhamnetin 3,4'-diglucoside	2.316	165031	2311629	C ₂₈ H ₃₂ O ₁₆	Flavonoid-3-O-glycosides
5,7-Dihydroxy-2-(4-hydroxyphenyl)-6,8-di-C-glucopyranosyl-4H-1-benzopyran-4-one	2.367	8807	126065	C ₂₆ H ₂₈ O ₁₄	Flavonoid 8-C-glycosides
Kaempferol-3-O-glucoside	2.417	1908	25084	C ₂₁ H ₂₀ O ₁₁	Flavonoid-3-O-glycosides
3-(4-Hydroxyphenyl)-7-hydroxy-6-methoxy-4H-1-benzopyran-4-one	2.468	1087	13692	C ₂₁ H ₂₀ O ₁₀	Isoflavonoid O-glycosides
Pelargonin	2.468	20489	278671	C ₂₇ H ₃₁ O ₁₆	Anthocyanidin-5-O-glycosides
Scutellarein-7-glucuronide	3.175	14197	189104	C ₂₁ H ₂₀ O ₁₂	Flavonoid-7-O-glucuronides
Procyanidin C1	3.175	25399	363410	C ₄₅ H ₃₈ O ₁₈	Proanthocyanidins
3-Hydroxy-4-methoxybenzoic acid	3.226	2431	31745	C ₈ H ₈ O ₄	Methoxybenzoic acids
Epicatechin-(4beta->8)-epicatechin 3'-O-gallate	3.226	13874	192509	C ₃₇ H ₃₀ O ₁₇	Proanthocyanidins
Apigenin-6-C-glucosyl-8-C-arabinoside	3.276	3598	51327	C ₂₁ H ₂₀ O ₁₀	Flavonoid C-glycosides
Malvidin 3,5-diglucoside	3.276	17808	240196	C ₂₉ H ₃₅ O ₁₇	Anthocyanidin-3,5-O-glycosides
Apigenin-6,8-di-C-arabinoside	3.327	3150	42893	C ₂₁ H ₂₀ O ₁₀	Flavonoid C-glycosides
5,7,4'-Trihydroxy-3',5'-dimethoxyflavone	3.327	4987	68926	C ₁₇ H ₁₄ O ₆	Methoxyflavones
(-)-Epicatechin gallate	3.428	19900	282387	C ₂₂ H ₁₈ O ₁₀	Catechin derivatives
Quercetin 3-O-(2"-p-coumaroyl) glucoside	3.428	7599	104421	C ₃₀ H ₂₆ O ₁₃	Flavonoid glycosides
Chrysoeriol 7-O-glucoside	3.478	1439	19804	C ₂₂ H ₂₂ O ₁₁	Flavonoid glycosides

the development of novel plant-based therapeutics rooted in the Linderniaceae lineage.

Limitation of the Study

The present study is limited by the lack of isolation and structural elucidation of individual phytochemicals detected through LC-MS analysis. Although the profiling reveals the presence of several bioactive compounds, their therapeutic potential can only be confirmed through further isolation and characterization. Techniques such as column chromatography should be employed in future studies to purify these constituents, which would provide a clearer understanding of their specific biological roles and pharmacological relevance.

CONCLUSION

This study offers a comprehensive comparative analysis of *Torenia crustacea* and *Bonnaya ciliata*, shedding light on their rich phytochemical diversity and therapeutic relevance. The shared metabolites between these species suggest the existence of common biosynthetic pathways, which can guide further research into the mechanisms underlying their medicinal properties. On the other hand, the identification of unique compounds in each species points to potential species-specific applications, further enhancing the appeal of these plants in the field of drug discovery. The findings highlight the promising potential of Linderniaceae species as sources of natural antioxidants, antimicrobials, and anti-inflammatory agents, which are critical for the development of novel therapeutic agents. These secondary metabolites are known for their ability to mitigate oxidative stress, combat microbial infections, and reduce inflammation, addressing key health challenges faced by modern society.^[25] As this study has

demonstrated, Linderniaceae species like *Bonnaya ciliata* (Table 4) and *Torenia crustacea* (Table 3) possess immense untapped potential for future pharmaceutical and biotechnological research. Further exploration of their phytochemical profiles, coupled with in-depth biological assays, could pave the way for new classes of drugs and therapies. Additionally, understanding the ecological roles these plants play, particularly in stress tolerance through their antioxidant and antimicrobial properties, contributes to a broader appreciation of their value beyond traditional uses.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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ABBREVIATIONS

LC-MS: Liquid Chromatography-Mass Spectrometry; **THF:** Tetrahydrofuran, **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **w/w:** Weight by Weight; **m/z:** Mass-to-Charge Ratio; **RT:** Retention Time; **UV:** Ultraviolet; **MS:** Mass Spectrometry; **QToF:** Quadrupole Time-of-Flight; **°C:** Degrees Celsius; **µL:** Microlitre; **µm:** Micrometre; **rpm:** Revolutions per Minute.

SUMMARY

This study employed advanced LC-MS techniques to identify and characterize bioactive compounds in the ethanolic extracts of *Bonnaya ciliata* (Colsm.) Spreng. and *Torenia crustacea* (L.) Cham. and Schltdl., two underexplored members of the Linderniaceae family. Phytochemical screening confirmed the presence of key secondary metabolites such as flavonoids, phenolic acids, glycosides, and alkaloids. LC-MS analysis revealed major constituents including luteolin-7-glucoside, kaempferol-3-O-glucoside, rhodiolide, and aloesin-compounds recognized for their antioxidant, anti-inflammatory, and antimicrobial activities. While both species shared common phytochemicals, *B. ciliata* exhibited a flavonoid-dominant profile featuring unique compounds like isorhamnetin-3,4'-diglucoside, whereas *T. crustacea* displayed a phenolic acid-rich composition, including 5-caffeoylquinic acid and depsides such as squamatic acid. These distinct chemical signatures suggest species-specific pharmacological potentials and ecological adaptations. The

findings support traditional medicinal uses and highlight the therapeutic promise of Linderniaceae species as sources of natural health-promoting agents.

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