Quantitative Comparison on Antioxidants, Bioactive Compounds and Scanning for Phytochemicals via LC-QTOF/MS of Three Lamiaceae Species

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Abstract

Herbal plants have long played a vital role in health care systems and are essential components in modern medical science, particularly plants in the Lamiaceae family, which are economically significant and diverse. This study aimed to comparatively analyze the content of antioxidants and bioactive compounds including phenolic compounds and flavonoids of the three plant species within the same family: Indian Borage (*Coleus amboinicus* Lour), Perilla (*Perilla frutescens* (L.) Britton), and Korean Perilla (*Perilla frutescens* var. *crispa*). The samples were prepared in the form of fresh and dried prior to the extraction using 95% (v/v) ethanol. The antioxidant activities were examined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods. Additionally, total phenolic and flavonoid contents were compared using TPC and TFC. Furthermore, the LC-QTOF/MS (Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry) technique was employed for the scanning of phytochemicals. Results indicated that the three plant species and different preparation methods significantly affected the content of antioxidants and bioactive compounds ($p < 0.05$) and affected the type and content of phytochemicals detected. Fresh *C. amboinicus* exhibited the highest total phenolic content (1.08 ± 0.03 mg GAE/g) and showed the most potent antioxidant activity across all assays (IC_{50} for DPPH = 143.71 mg/mL). While, drying of samples led to the decrease in the content of antioxidants and bioactive compounds in *C. amboinicus*. It conversely increased the total flavonoid content in Korean Perilla and Perilla, with dried Korean Perilla reaching the highest total flavonoid content (5.33 ± 1.08 mg QE/g). Results from the screening via LC-QTOF/MS revealed that rosmarinic acid, 4-Hydroxybenzoic acid and caffeic acid were the primary constituents in fresh *C. amboinicus*. Meanwhile, the drying process led to the detection of new compound groups, such as Luteolin and Naringenin in *C. amboinicus* These findings demonstrated that fresh Indian Borage was a superior source of antioxidants and bioactive compounds for further development in the pharmaceutical and functional food industries.

Introduction

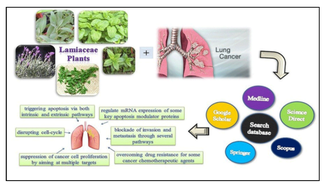


Figure 1 Multifaceted anti-cancer mechanisms of Lamiaceae species. (Aghdam & Hamedeyazdan, 2025)

Selected Plants

Coleus amboinicus Lour (Indian Borage)

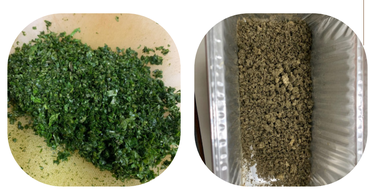
Perilla frutescens var. *crispa* (Korean Perilla)

Perilla frutescens (L.) Britton (Perilla)

Comparison of Sample Preparation Methods

Fresh vs Dried

Which method is better?

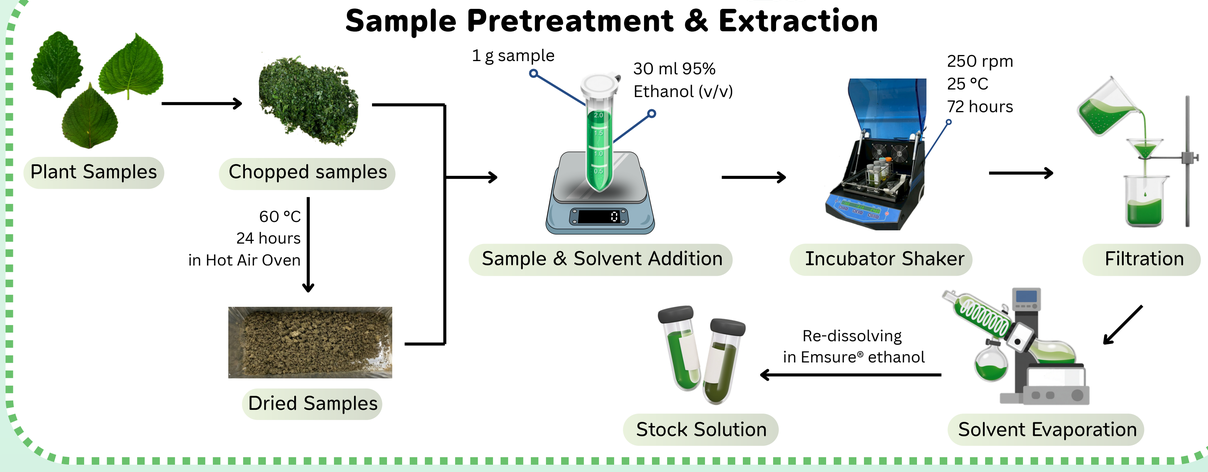


Objectives

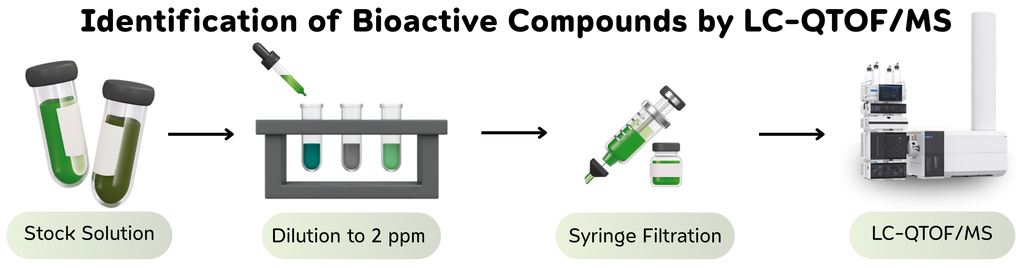
1. To compare antioxidant activities, total phenolic content and flavonoid content in Indian Borage, Korean Perilla, and Perilla leaves in fresh versus dried samples
2. To screen phytochemicals in the plant species using LC-QTOF/MS

Methodology

Sample Pretreatment & Extraction



Identification of Bioactive Compounds by LC-QTOF/MS



Analysis of Antioxidant Activities and Phytochemical Contents



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Results

Screening Results of the Extracts from Fresh and Dried Lamiaceae Leaves Using LC-QTOF/MS

Table 1: Screening Results of the Extracts from Fresh and Dried Indian Borage Leaves Using LC-QTOF/MS

Sample	Tentative identification	Formula	Mass	m/z	Score	Scan diff (Tgt, ppm)
C. amboinicus (Fresh)	Caffeic acid	$C_9H_8O_4$	180.042	179.0346	96.21	-1.67
	Rosmarinic acid	$C_{18}H_{16}O_8$	360.0838	359.0765	90.45	-2.08
	4-Hydroxybenzoic acid	$C_7H_6O_3$	138.0315	137.0242	91.1	-1.75
C. amboinicus (Dried)	Apigenin	$C_{15}H_{10}O_5$	270.0524	269.0451	99.23	-1.5
	Naringenin	$C_{15}H_{12}O_5$	272.0679	271.0607	98.52	-2.09
	4-Hydroxybenzoic acid	$C_7H_6O_3$	138.0316	137.0243	97.82	-0.61
	Caffeic acid	$C_9H_8O_4$	180.0421	179.0348	97.11	-0.92
	Luteolin	$C_{15}H_{10}O_6$	286.0473	285.04	94.68	-1.56

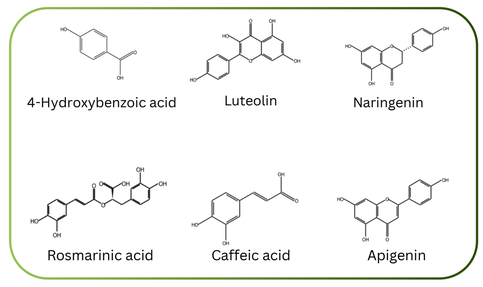


Figure 2 Identification of Antioxidant Bioactive Compounds in Lamiaceae Species via LC-QTOF/MS

Table 2: Screening Results of the Extracts from Fresh and Dried Korean Perilla Leaves Using LC-QTOF/MS

Sample	Tentative identification	Formula	Mass	m/z	Score	Scan diff (Tgt, ppm)
P. frutescens var. *crispa* (Fresh)	Apigenin	$C_{15}H_{10}O_5$	270.0526	269.0453	98.12	-0.66
P. frutescens var. *crispa* (Dried)	Apigenin	$C_{15}H_{10}O_5$	270.0524	269.0452	99.32	-1.45
	4-Hydroxybenzoic acid	$C_7H_6O_3$	138.0315	137.0244	97.46	-1.75
	Luteolin	$C_{15}H_{10}O_6$	286.0477	285.0402	84.76	-0.24

Table 3: Screening Results of the Extracts from Fresh and Dried Perilla Leaves Using LC-QTOF/MS

Sample	Tentative identification	Formula	Mass	m/z	Score	Scan diff (Tgt, ppm)
P. frutescens (Fresh)	Apigenin	$C_{15}H_{10}O_5$	270.0523	269.045	98.71	-1.9
	4-Hydroxybenzoic acid	$C_7H_6O_3$	138.0316	137.0243	87.3	-0.92
P. frutescens (Dried)	Apigenin	$C_{15}H_{10}O_5$	270.0524	269.0451	99.09	-1.64
	Luteolin	$C_{15}H_{10}O_6$	285.0396	286.0471	93.37	-0.35
	Rosmarinic acid	$C_{18}H_{16}O_8$	360.0839	359.0765	89.61	-1.77

LC-QTOF/MS Analysis identified Apigenin as a commonly found compound across all species. Notably, the drying process enhanced the phytochemical diversity, leading to the detection of Luteolin in all the dried samples and Naringenin specifically in dried *C. amboinicus*. These findings suggested that thermal treatment facilitated the released of complex flavonoids from the plant matrix.

Comparative Analysis of Antioxidant Activities and Phytochemical Contents in Fresh and Dried Lamiaceae Leaves

Table 4: Analysis of Antioxidant Activity (DPPH ABTS FRAP) in Indian Borage, Korean Perilla and Perilla Extracts

Sample	Preparation	DPPH		ABTS		FRAP
		%Inhibition	IC_{50} (mg/mL)	%Inhibition	IC_{50} (mg/mL)	mg AAE/g crude extract
C. amboinicus Lour	Fresh	81.59 ± 0.32[a]	143.71	68.37 ± 1.87[a]	762	166.38 ± 6.79[a]
	Dried	41.42 ± 4.82[b]	1670.68	28.19 ± 4.27[b]	40003.97	40.82 ± 0.72[b]
P. Frutescens var. Crispa	Fresh	11.71 ± 2.32[c]	IC*	14.88 ± 2.08[d]	IC*	28.94 ± 1.65[c]
	Dried	17.24 ± 2.68[c]	IC*	16.60 ± 0.13[d]	IC*	24.02 ± 3.56[c]
P. Frutescens	Fresh	18.78 ± 1.76[c]	IC*	16.96 ± 0.47[cd]	IC*	11.95 ± 0.19[d]
	Dried	18.61 ± 3.89[c]	IC*	22.56 ± 0.40[bc]	IC*	23.44 ± 1.77[c]

Different letters (a–d) in the same column indicate significant differences ($p < 0.05$) by Tukey's test. Each value is mean SD (n = 3).
IC: Inconclusive (Inhibition < 50% at the highest concentration tested).

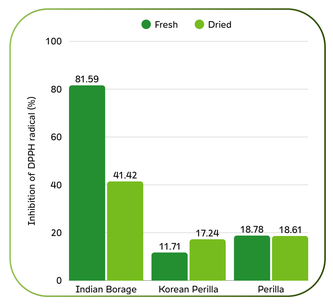


Figure 3 DPPH Radical Inhibition (%) of Fresh and Dried Lamiaceae Extracts.

Table 5: Analysis of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in Indian Borage, Korean Perilla and Perilla Extracts

Sample	Preparation	Total Phenolics (mg GAE/g, crude extrat)	Total Flavonoids (mg QE/g, crude extract)
C. amboinicus	Fresh	1.08 ± 0.03[a]	3.89 ± 0.38[ab]
	Dried	0.39 ± 0.01[b]	1.71 ± 0.55[c]
P. frutescens var. *crispa*	Fresh	0.16 ± 0.01[d]	2.06 ± 0.45[c]
	Dried	0.14 ± 0.01[d]	5.33 ± 1.08[a]
P. frutescens	Fresh	0.16 ± 0.00[d]	3.19 ± 0.76[bc]
	Dried	0.27 ± 0.05[c]	4.18 ± 0.36[ab]

Different letters (a–d) in the same column indicate significant differences ($p < 0.05$) by Tukey's test. Each value is mean SD (n = 3).

Fresh Indian Borage exhibited the highest total phenolic content (TPC) of 1.08 ± 0.03 mg GAE/g and the most potent antioxidant activity, with a DPPH IC_{50} of 143.71 mg/mL and FRAP value of 166.38 ± 6.79 mg AAE/g.

Drying significantly decreased these values in Indian Borage but increased the total flavonoid content (TFC) in Korean Perilla and Perilla leaves, with dried Korean Perilla reaching the highest TFC at 5.33 ± 1.08 mg QE/g. In contrast, both Perilla species showed relatively low antioxidant activities for both preparation methods.

Conclusion

Fresh Indian Borage (*Coleus amboinicus* Lour) exhibited the highest total phenolic content and antioxidant activity across all assays. While drying reduced the total phenolic content in Indian Borage, it conversely increased the total flavonoid content in Korean Perilla and Perilla leaves. These findings provided a crucial information for developing future high-value pharmaceuticals and functional food products.

Acknowledgment

This study was made possible through the generous support from RIHES, Chiang Mai University, specifically the Environmental and Occupational Health Sciences Research Unit, alongside the Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University.

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