Determination of Antioxidant Activities, Total Flavonoid and Phenolic Contents and Identification of Bioactive Compounds by LC-QTOF/MS of Zingiberaceae Plants Cultivated in Northern Thailand.

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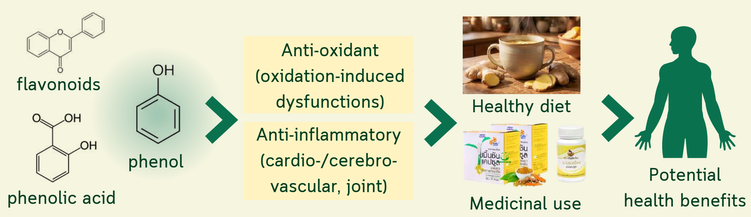
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Abstract

Plants in the Zingiberaceae family are widely recognized as rich sources of phenolic and flavonoid compounds that contribute substantially to antioxidant activity. This study aimed to evaluate the antioxidant activity, total phenolic and flavonoid contents, and phytochemical profiles of four medicinal species: *Boesenbergia rotunda, Curcuma comosa, Zingiber montanum*, and *Curcuma longa* with particular emphasis on the influence of sample preparation (fresh and oven-dried). Plant materials were extracted using 95% ethanol. Antioxidant potential was assessed using DPPH, ABTS, and FRAP assays, along with determination of total phenolic and flavonoid contents. Phytochemical constituents were characterized by LC-QTOF/MS analysis. The findings revealed that *C. longa* exhibited the highest antioxidant activity, followed by *Z. montanum*, *B. rotunda*, and *C. comosa,* respectively. Oven-dried *C. longa* showed the highest FRAP value (130.32 ± 0.76 mg AAE/g) and the greatest total flavonoid content (7.50 ± 0.45 µg QE/g). In contrast, fresh *Z. montanum* demonstrated the highest total phenolic content (1.04 ± 0.03 mg GAE/g). LC-QTOF/MS profiling identified several major constituents, including dehydrocurdione and embelin. Additionally, other bioactive compounds were detected in some species, such as naringenin in dried *B. rotunda* and 6**β**,7**β**-dihydroxykaurenoic acid in dried *C. comosa*. Collectively, these results demonstrate that sample preparation significantly affects phytochemical yield and antioxidant performance. This study provides fundamental support for the standardized development and potential commercialization of herbal extracts derived from Zingiberaceae species.

Introduction



- Zingiberaceae plants are widely used in traditional medicine and cuisine in Southeast Asia.
- Several species contain abundant phenolic and flavonoid compounds with antioxidant properties.
- Natural antioxidants from medicinal plants are of increasing interest due to their potential role in preventing oxidative stress-related diseases.

Objectives

This study aimed to determine the antioxidant activities, total phenolic and flavonoid contents, and identify bioactive compounds using LC-QTOF/MS in Zingiberaceae plants cultivated in Northern Thailand.

Specific objective:

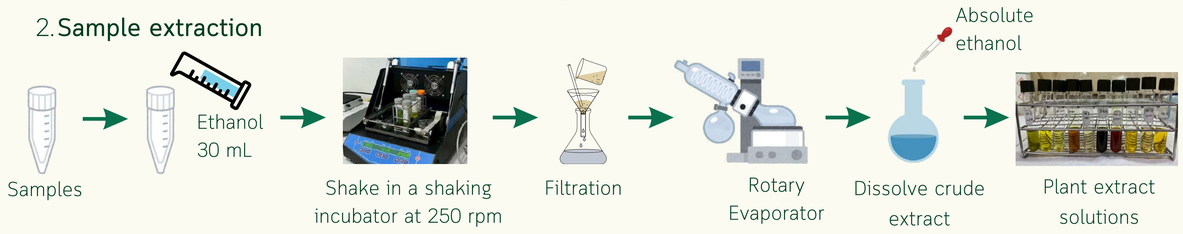
- To evaluate antioxidant activity of selected Zingiberaceae plant
- To determine TPC and TFC in fresh and oven-dried Zingiberaceae extracts with 95% ethanol
- To identify bioactive compounds using LC-QTOF/MS

Methodology

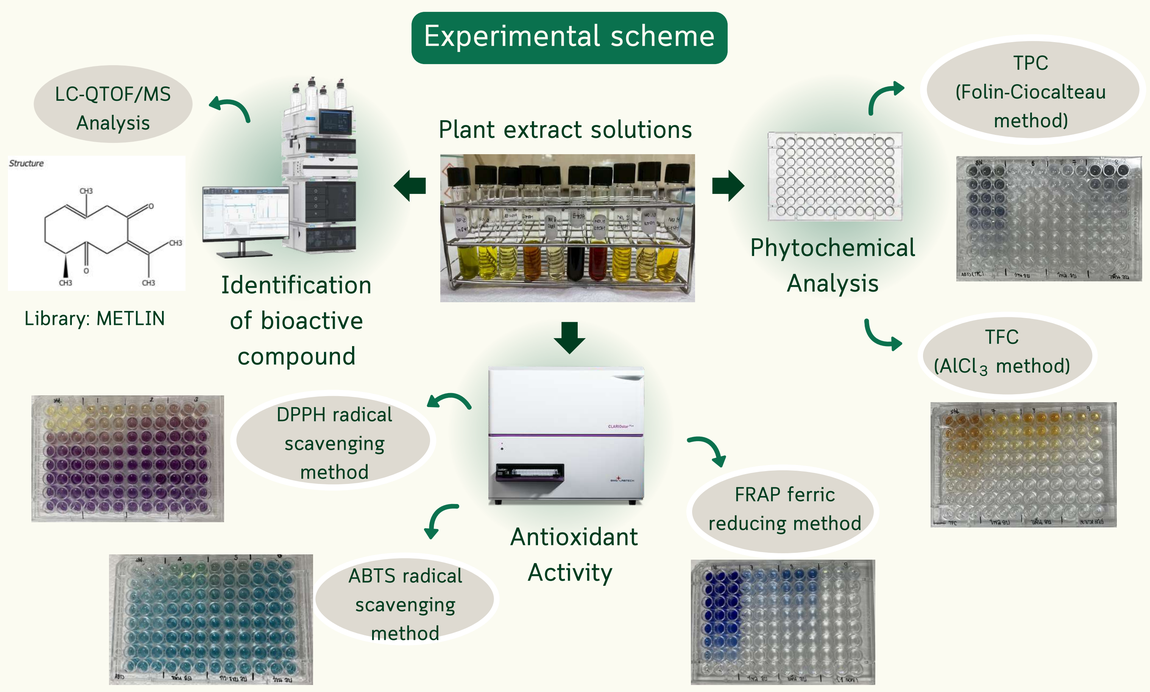
1. Preparation of samples



2. Sample extraction



Experimental scheme



Acknowledgement

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Result & Discussion

• Antioxidant Activities

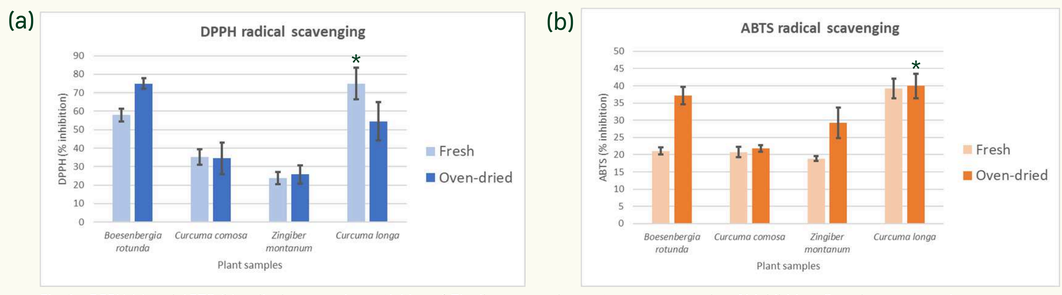


Fig. 1 - DPPH (a) and ABTS (b) radical scavenging activities of Zingiberaceae plant extracts expressed as % inhibition. Sample concentration was 1 mg/mL. Values are presented as mean ± SD (n = 3). Higher % inhibition indicates stronger antioxidant activity.

- Antioxidant activity varied among the studied Zingiberaceae species. In the DPPH assay, oven-dried *Curcuma longa* showed the highest radical scavenging activity, followed by *Boesenbergia rotunda*, while *Zingiber montanum* showed the lowest activity.
- Similarly, oven-dried samples showed higher radical scavenging activity than fresh samples in the ABTS assay.

Table 1 Ferric Reducing Antioxidant Power (FRAP) (mgAAE/g, crude extract)

Sample	Condition	FRAP (mgAAE/g, crude extract)
Boesenbergia rotunda	Fresh	1.05±0.58
	Dried	34.61±1.14
Curcuma comosa	Fresh	21.78±0.43
	Dried	23.85±2.20
Zingiber montanum	Fresh	26.38±0.57
	Dried	46.55±0.38
Curcuma longa	Fresh	92.32±3.95 *
	Dried	130.32±0.76 *

* The concentration of samples used for the assay was 1 mg/mL. Values are expressed as mean ± SD (n = 3).

- *Curcuma longa,* particularly the oven-dried sample, exhibited the highest ferric reducing antioxidant power among all tested plants, suggesting that drying may enhance the concentration of antioxidant compounds.

• Antioxidant Contents

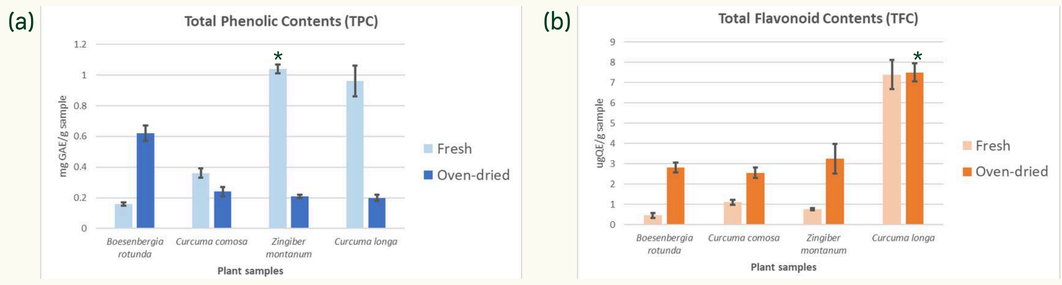


Fig. 2 - Total phenolic content (TPC) expressed as mg GAE/g sample (a) and total flavonoid content (TFC) expressed as µg QE/g sample (b) of Zingiberaceae plant extracts. Values are presented as mean ± SD (n = 3).

- Fresh *Zingiber montanum* showed the highest total phenolic content (TPC), indicating a high abundance of phenolic compounds in this species.
- *Curcuma longa*, particularly the oven-dried sample, exhibited the highest total flavonoid content (TFC), suggesting that drying may enhance the concentration or extractability of flavonoid compounds.

• LC-QTOF/MS Identification of Bioactive Compounds

Major compounds

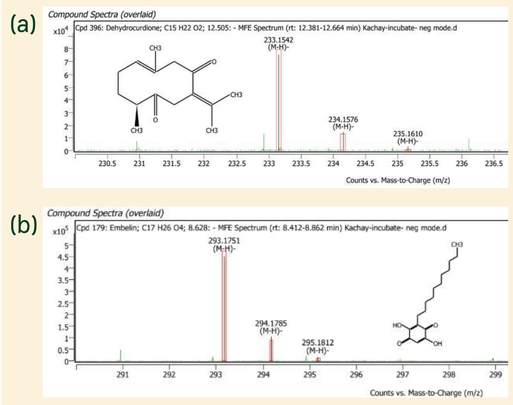


Fig. 3 - LC-QTOF/MS mass spectrum of Zingiberaceae plant extracts showing the detected compounds **Dehydrocurdione** (a) and **Embelin** (b) were consistently detected across most samples

Unique compounds

Table 2 Chemical structures, plant sources, and metabolite classes of the identified compounds

Compound	structure	Mass	Plant source	Metabolite class
Naringenin		272.0677	*Boesenbergia rotunda*	Flavonoid (Flavanone)
1,2,3-Trihydroxybenzene		126.0316	*Boesenbergia rotunda*	Phenolic compound
6**β**,7**β**-Dihydroxykaurenoic acid		334.2136	*Curcuma comosa*	Terpenoid (Diterpenoid)

- LC-QTOF/MS analysis revealed several bioactive compounds in the Zingiberaceae extracts. **Dehydrocurdione** and **Embelin** were consistently detected in most plant samples and were considered the major compounds.
- In addition to the major compounds, several unique compounds were identified in specific plant species, including **naringenin** and **1,2,3-trihydroxybenzene** in *Boesenbergia rotunda* and **6β,7β-dihydroxykaurenoic acid** in *Curcuma comosa*.
- The identified compounds belong mainly to **flavonoid**, **phenolic**, and **terpenoid** classes, which are known to contribute to antioxidant activity in Zingiberaceae plants.

Conclusion

- Antioxidant activities varied among the studied Zingiberaceae species and were influenced by sample preparation. Oven-dried samples generally exhibited higher antioxidant activities than fresh samples.
- *Curcuma longa* showed the strongest antioxidant capacity, particularly in the oven-dried sample, which also exhibited the highest ferric reducing antioxidant power and total flavonoid content.
- LC-QTOF/MS analysis identified several bioactive compounds, including **dehydrocurdione**, **embelin**, **naringenin**, **1,2,3-Trihydroxybenzene** and **6β,7β-dihydroxykaurenoic acid**, which may contribute to the antioxidant activity of the plant extracts.