Comparatie study of rosmarinic acid content and antioxidant activity of four Lamiaceae species extracts in Vietnam

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Abstract

The Lamiaceae (formerly Labiatae), known as the mint family, is a popular herbal plant in Vietnam and worldwide. This study aimed to compare the rosmarinic acid content of four plant extracts from the Lamiaceae, including Elsholtzia ciliata (Thunb.) Hyland., Mentha piperita L., Ocimum basilicum L., Plectranthus amboinicus (Lour.) Spreng. Ground materials were extracted on a magnetic stirrer at 90^{0}C for 60 minutes. The filtered extracts were checked for quantitative phytochemical analysis, rosmarinic acid content using a spectrophotometer and antioxidant activity. The quantitative result showed that four Lamiaceae species extracts had a lot of polyphenols (dark green precipitate) and flavonoids (yellow precipitate).

The rosmarinic acid content in four species was 4.87-15.42 mg/g DW. These results also showed a strong correlation between rosmarinic acid concentration and IC_{50} value (r = -0.87) in which rosmarinic acid production and antioxidant activity of Mentha piperita L. were highest. Our results suggest that Mentha piperita L. and others may be a good source of rosmarinic acid, an antioxidant compound.

Keywords: Antioxidant activity, Extract, Lamiaceae, Rosmarinic acid.

Introduction

The Lamiaceae (formerly Labiatae), also known as the mint family, is the sixth-largest plant family, with more than 230 genera and 7000 species[18,29]. This family is known in Vietnam by approximately 40 genera and 143 species. Many species of this family produce essential oils and secondary compounds used as medicine, vegetables and spices or to make drinks e.g. *Elsholtzia ciliata* (Thunb.) Hyland., mint (*Mentha piperita* L.,), basil (*Ocimum basilicum* L.), country borage (*Plectranthus amboinicus* (Lour.) Spreng.)[32]. Plants belonging to the Lamiaceae family have been reported to have potent antioxidant activity and they are the most widely utilized and commercialized. Plants' biological activity is due to phenolic compounds, particularly rosmarinic acid[1,5,17].

Rosmarinic acid, an ester of caffeic acid and 3,4-dihydroxy phenyl lactic acid, was isolated from *Rosmarinus officinalis*. It can be found among various Boraginaceae and Lamiaceae plants[23]. Rosmarinic acid has been shown to exhibit biological activities *in vitro* such as high antioxidant[24,34], anti-inflammatory[26], antibacterial activity[2], anti-virus[14,30], risk reduction of Alzheimer's disease[10], anti-tumor[12,13] etc. Nowadays, rosmarinic acid is used extensively in the pharmaceutical, cosmetic and food sectors. As a result, finding plants that contain a high concentration of rosmarinic acid, is essential for providing new sources. This study aimed to investigate and to compare rosmarinic acid content and antioxidant activity in extracts from the dried leaves of eight medical plants. The relationship between the results could provide insight into rosmarinic acid's antioxidant action in Vietnamese spices from the Lamiaceae family.

Material and Methods

Collection of plants: Four species (*Ocimum basilicum* L, *Mentha piperita* L., *Elsholtzia ciliata* (Thunb.) Hyland., *Plectranthus amboinicus (Lour.)* Spreng.) were grown in Phu Chanh district, Binh Duong province, Vietnam. These plants were brought to the laboratory of Thu Dau Mot University and washed to remove leaves that were yellow and pest-infested (Figure 1). The leaves are dried at 50^{0}C, crushed and shifted through a 1 mm sieve to achieve sample homogeneity. The powders were stored in a desiccator at room temperature before extraction (Figure 2).

Preparation of extracts: The ground plant sample (1 g) was extracted with 50% ethanol (40 mL) in a 100 mL Erlenmeyer flask on a magnetic stirrer heater at 70^{0}C for 1 hour. The mixtures were filtered and the clear liquid was collected to conduct qualitative phytochemical analysis, to measure rosmarinic acid content and to test antioxidant activity.

Qualitative phytochemical analysis of plant extracts: To identify flavonoids, 1 mL of the extract and 1 mL of 10% $Pb(CH_3COO)_2$ were added to a test tube. A yellow precipitate formed, indicating the flavonoids' presence[27]. To identify polyphenols, 1 mL of the extract combined with 1 mL of water and 1-2 drops of 5% $FeCl_3$. A dark green precipitate was formed indicating the presence of polyphenols[27].

Rosmarinic acid determination: The rosmarinic acid content was evaluated by observing a bright yellow reaction when rosmarinic acid and zirconium (IV) oxide chloride formed a combination. Mix 200 µL extract solutions with 4.6 mL ethanol and 200 µL zirconium oxide chloride solution (0.5 M).

Figure 1: Four Lamiaceae species in Binh Duong, Vietnam. A: *Elsholtzia ciliata* (Thunb.) Hyland.; B: *Mentha piperita* L.; C: *Ocimum basilicum* L.; D: *Plectranthus amboinicus* (Lour.) Spreng.

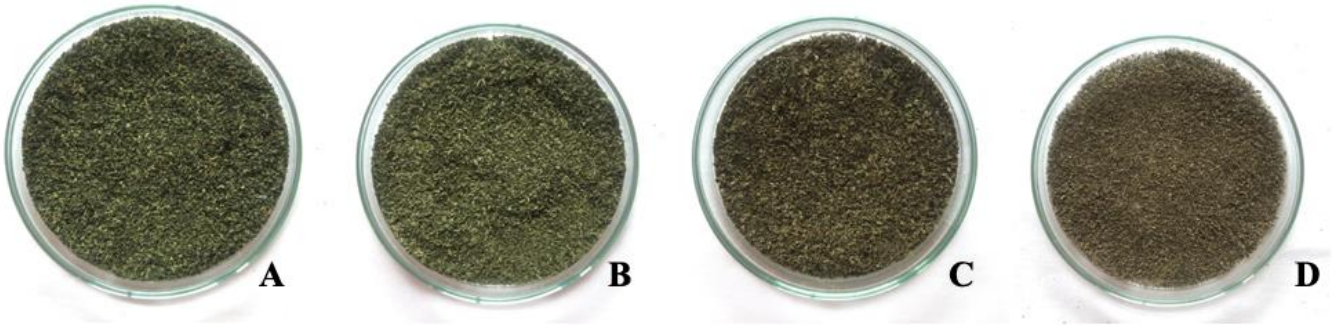

Figure 2: Dried powders from four Lamiaceae species in Binh Duong, Vietnam. A: *Elsholtzia ciliata* (Thunb.) Hyland.; B: *Mentha piperita* L.; C: *Ocimum basilicum* L.; D: *Plectranthus amboinicus* (Lour.) Spreng

Table 1
The presence of flavonoids and polyphenols in four Lamiaceae species extracts in Vietnam

Extract sample	Qualitative reaction	
	Flavonoids	Polyphenols
Elsholtzia ciliata (Thunb.) Hyland.	+	+
Mentha piperita L.	+	+
Ocimum basilicum L.	+	+
Plectranthus amboinicus (Lour.) Spreng.	+	+

(+) presence

After five minutes, the absorbance of the reaction mixture at 362 nm was measured using a spectrophotometer[22].

Antioxidant activity test: The extracts were evaporated at 50^0C until dry. The extract was dissolved in 100% alcohol at concentrations of 4000, 3000, 2000, 1000 and 500 µg/mL. A total of 3.5 mL ethanol and 0.5 mL of DPPH ethanolic solution (0.6 mM) was added to 0.5 mL of each extracts to various concentrations (500-4000 µg/mL). The mixture was incubated in the dark at 37^0C for 30 min and measuring of absorbance was made using a spetrophotometer at 517 nm. The percentage of inhibition of DPPH free radicals was determined as follows:

$$\% \text{ Inhibition} = \left[1 - \frac{\text{Abs(sample)}}{\text{OD (control)}}\right] \times 100.$$

The IC_{50} value (µg/mL of extract) is the concentration that inhibits 50% of DPPH free radicals[11].

Statistical analysis: The averages of three measurements were calculated, together with their standard deviations (SD). Microsoft Excel and Statgraphics Centurion XV software were used for statistical analysis.

Results

Qualitative phytochemical analysis of plant extracts: According to table 1 and figure 3, qualitative phytochemical analysis of the extracts showed the presence of flavonoids and polyphenols in the leaf extracts from 4 species of the Lamiaceae family.

Compare rosmarinic acid content of eight species belong to the Lamiaceae: Figure 4 showed that among the four analyzed plants belonging to the Lamiaceae family, the highest rosmarinic acid content was found in *Mentha piperita* L. (15.42 mg/g DW). Next, rosmarinic acid was relatively high in *Ocimum basilicum* L, reaching 12.10 mg/g DW. Rosmarinic acid was low in *Plectranthus amboinicus* (Lour.) Spreng. and *Elsholtzia ciliata* (Thunb.) Hyland, reaching 5.06 and 4.87 mg/g DW respectively.

Antioxidant activity of eight species belonging to the Lamiaceae: The extracts produced yellowish solutions on a

purple backdrop, indicating antioxidant activity in the DPPH experiment. The extract of *Mentha piperita* L. had the highest inhibition of DPPH free radicals, reaching 86.21% at a concentration of 4000 µg/mL. Following, extracts of *Ocimum basilicum* L., *Plectranthus amboinicus* (Lour.) Spreng and *Elsholtzia ciliata* (Thunb.) Hyland inhibited DPPH free radicals by 55.54%, 44.94% and 25.85% at 4000 µg/mL respectively (Figure 5).

From the linear regression equation (Table 2), the IC_{50} value of the extracts can be deduced. *Mentha piperita* L. had the best antioxidant activity among the four samples with the lowest IC_{50} value (795.87 µg/mL) and *Elsholtzia ciliata* (Thunb.) Hyland had the lowest antioxidant activity with IC_{50} value reaching 8133.14 µg/mL.

Discussion

Most genera of the Lamiaceae family are rich in terpenoids and contain large quantities of compounds such as glycosides, flavonoids and phenolic acids[19]. The qualitative analysis results in this study showed that the extracts all contained flavonoid and phenolic compounds. Several studies have shown that flavonoids and polyphenols are detected in significant quantities in the plants *Elsholtzia ciliata* (Thunb.) Hyland.[6,33], *Mentha piperita* L.[7], *Ocimum basilicum* L.[9] and *Plectranthus amboinicus* (Lour.) Spreng.[20,25]

Regarding rosmarinic acid analysis, *Mentha piperita* L. had the highest rosmarinic acid content and the lowest was found in *Elsholtzia ciliata* (Thunb.) Hyland. According to the report of Shekarchi et al[28], rosmarinic acid content in *Mentha piperita* L. in Iran reached 28.20 mg/g DW[28], which was higher than rosmarinic acid content in this study.

On the contrary, RA contents in *Ocimum basilicum* L. (5.97 mg/g DW) and in *Plectranthus amboinicus* (Lour.) Spreng. (2.79 mg/g DW) in Thailand[3] were lower than in *Ocimum basilicum* L. and *Plectranthus amboinicus* (Lour.) Spreng. in Binh Duong province, Vietnam. Similarly, *Elsholtzia ciliata* (Thunb.) Hyland. (0.115 mg/g DW) in Lithuania[25] had a lower rosmarinic acid content than *Elsholtzia ciliata* (Thunb.) Hyland in this trial.

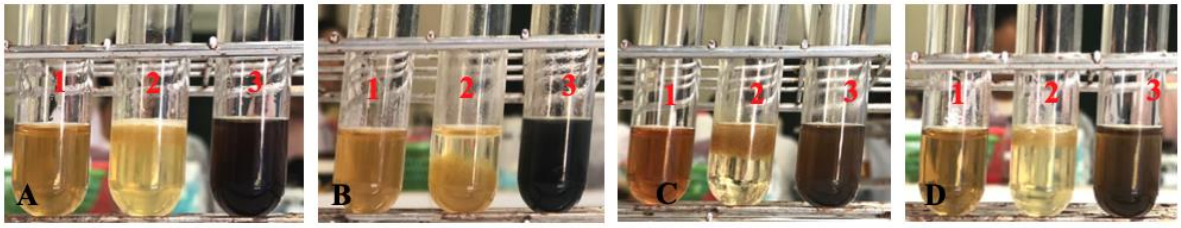

Figure 3: Qualitative reaction of flavonoids and polyphenols in four Lamiaceae species extracts in Vietnam.
A: *Elsholtzia ciliata* (Thunb.) Hyland.; B: *Mentha piperita* L.;
C: *Ocimum basilicum* L.; D: *Plectranthus amboinicus* (Lour.) Spreng.
Note: (1) Sample before reaction; (2) Qualitative reaction of flavonoids; (3) Qualitative reaction of polyphenols

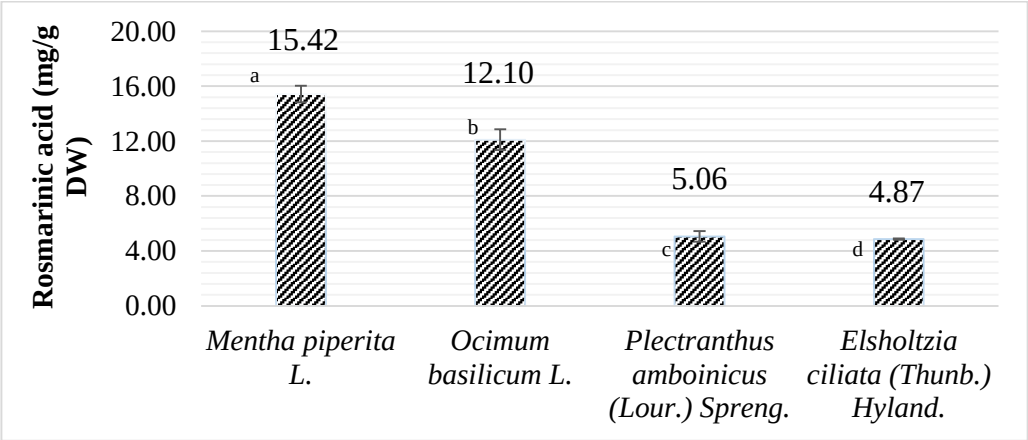

Figure 4: Rosmarinic acid content of four species belong to the Lamiaceae

Table 2
IC_{50} values of four plant extracts for DPPH free radical scavenging activity

Extract sample	Linear regression equation	IC_{50} (µg/mL)
Mentha piperita L.	y = 0.0126x + 39.972 (R^2 = 0.9344)	795.87
Ocimum basilicum L.	y = 0.0127 + 10.738 (R^2 = 0.9053)	3091.50
Plectranthus amboinicus (Lour.) Spreng.	y = 0.0105x + 4.2191 (R^2 = 0.9857)	4360.09
Elsholtzia ciliata (Tliunb.) Hyland.	y = 0.0059x + 2.0145 (R^2 = 0.9445)	8133.14

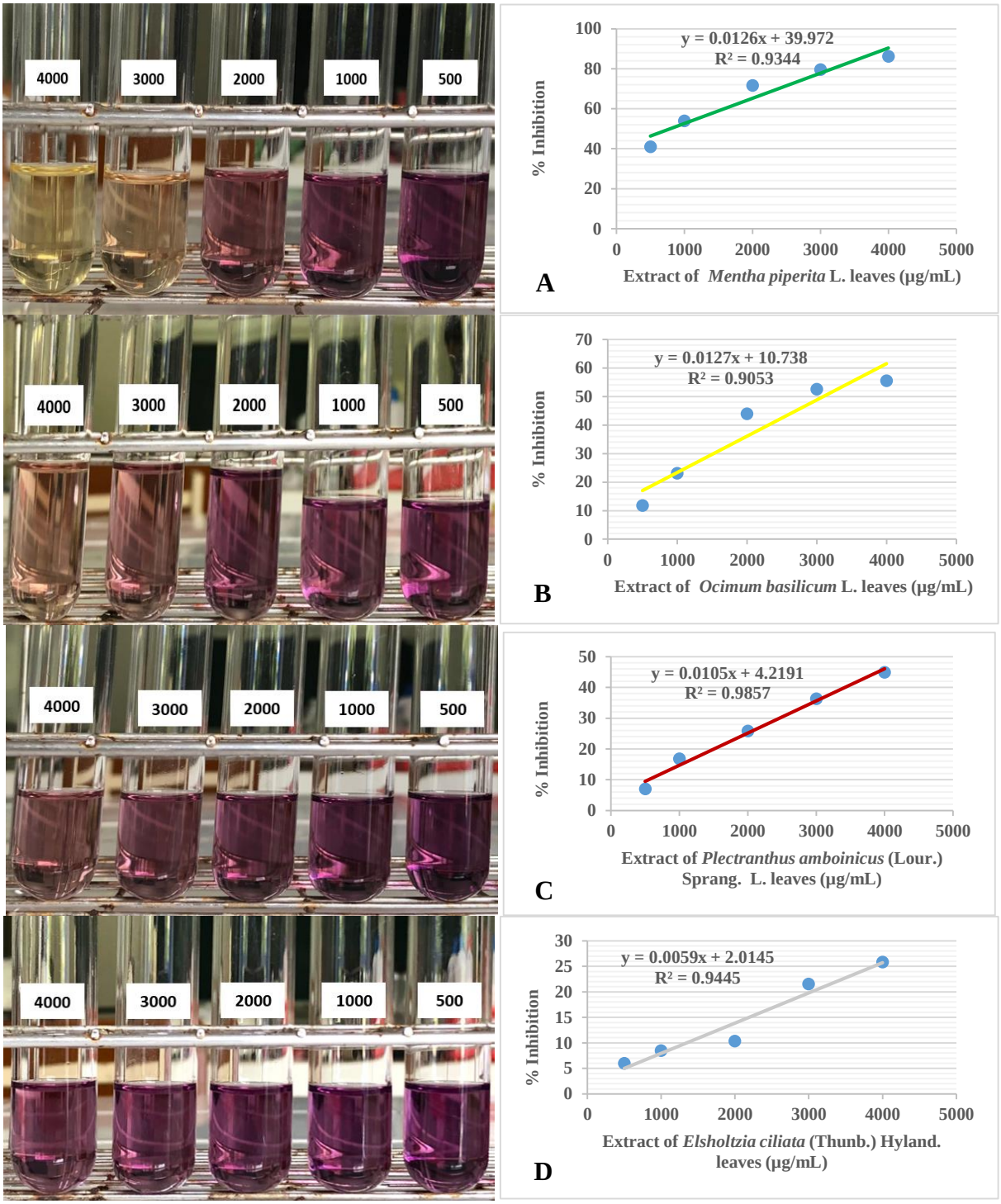

Figure 5: DPPH color changes and inhibition percentage of DPPH free radical by the extracts: A: *Mentha piperita* L.; B: *Ocimum basilicum* L.; C: *Plectranthus amboinicus* (Lour.) Spreng.; D: *Elsholtzia ciliata* (Thunb.) Hyland

So, analyzing the plants that are growing in each Nation, is necessary to determine the best source of rosmarinic acid because it is known that a variety of factors such as soil and climatic conditions, plant ontogenesis phases, harvest and plant storage and extraction method, influence plant's rosmarinic acid content[28].

Assays for DPPH radical scavenging are frequently used to assess a compound's antioxidant capacity. The DPPH free radical absorbs most when it is purple and at a wavelength of 517 nm. The antioxidant and DPPH react, reducing DPPH to DPPH-H and changing its hue from purple to yellow[31]. In this experiment, the antioxidant capacity of the extracts depended on the plant species and extract concentration. Compared with other plant species, such as extracts of *Pseuderanthemum palatiferum* (Nees) radlk (IC_{50} = 3429 µg/mL)[21], *Portulaca oleracea* L. (IC_{50} = 2835.33 µg/mL)[16], *Mentha piperita* L. (IC_{50} = 1130 µg/mL)[15], *Ocimum basilicum* L. in Portugal (IC_{50} = 3990 µg/mL)[4], the extracts from Lamiaceae species had equivalent or higher antioxidant

activity, except for the extract of *Elsholtzia ciliata* (Tliunb.) Hyland. Rosmarinic acid contents of the tested plants showed a good negative correlation with the IC_{50} values (r = - 0.87). This means that rosmarinic acid contributes to the antioxidant activity of four extracts measured by DPPH assay. Many results about similar correlations between total phenolic content, especially rosmarinic acid and the antioxidant activity of various species belonging to the Lamiaceae family were found[2,5].

Conclusion

The study showed that the four extracts had rosmarinic acid contents ranging from 4.87 to 15.42 mg/g DW, of which, *Mentha piperita* L. had the highest RA content (15.42 mg/g DW). The extract samples all had good antioxidant activity, especially *Mentha piperita* L., which had the highest antioxidant capacity with IC_{50} of 795.87 mg/L. In addition, the qualitative analysis also showed that the extracts contained many polyphenols and flavonoids. Therefore, these compounds have the potential to be used in food, pharmaceuticals and cosmetics.

Acknowledgement

We thank members of the Institute of Engineering and Technology, Thu Dau Mot University for helping with the experiments and technical assistance.

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(Received 28th February 2025, accepted 08th May 2025)