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Effect of various factors on rosmarinic acid content of water mint (*Mentha aquatica*)

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Abstract

Water mint (*Mentha aquatica*) is well-known as a popular herbal plant in Viet Nam. Rosmarinic acid (RA) is a phenolic compound found in many genus of *Mentha* and has numerous biological activities. Its applications are wide from the food industry to cosmetics. The purpose of this study was to evaluate the effects of different plant parts, drying, various solvent to sample ratios (1:20, 1:30, 1:40, 1:50, 1:60, and 1:70 (w/v)) and extraction temperature (30, 50, 70, and 90°C) on RA content of water mint. Ground materials of fresh and dried leaves, fresh and dried whole plants (each for 1 g) were extracted with water. The extraction was performed on magnetic stirrer. The extract was passed through filter paper retaining the clear solution for the quantitative and RA analysis. RA content was determined by using a spectrophotometer. The dried whole plants, a solvent to sample ratio of 1:50 (w/v), extraction temperature of 70°C, and extraction time of 30 minutes were the best conditions for RA content from water mint.

Keywords: extraction, rosmarinic acid, solvent to sample ratio, temperature, water mint.
Abbreviations: MC_ moisture content; RA_rosmarinic acid; W_w_wet weight; W_d_dry weight; w/v_weight/volume.

Introduction

Rosmarinic acid (RA) is an ester of caffeic acid and 3,4-dihydroxy phenyl lactic acid first isolated from the leaves of rosemary (*Rosmarinus officinalis* L.). In some previous researches, RA has much higher antioxidant activity compared with vitamin E. It has *in vitro* properties such as anti-HIV-1, anti-bacterial, anti-oxidant, and anti-cancer activity. *In vivo* studies have also shown that RA has anti-allergic, antithrombotic, and anti-cancer properties (Shekarchi et al., 2012). Today, RA is extracted from herbal species widely used in the pharmaceutical, cosmetic, and food industries.

The genus *Mentha* belongs to the family Lamiaceae, which is a group of aromatic and medicinal plants, contains many essential oils in the epidermal glands and many bioactive compounds such as phenolics, tannins, terpenes, terpenoids, quinones, coumarins, flavonoids, alkaloids, sterols, and saponins (El Hassani, 2020).

Water mint (*Mentha aquatica*) belongs to the genus of *Mentha*, which is a herbaceous plant with green or purple stems, tetragonal shape, and variably hairy to almost hairless (Do Ngoc Dai et al., 2015). Leaves have a broad blade, a concave base, a convex middle with auxiliary veins like puff pastry, grow in opposite pairs, have serrated edges, many essential oil sacs, and short petioles. It usually grows on the shallow edges of rivers, streams, and rivers, forming dense growth patches (Schanzer et al., 2012). The taste of water mint at first is warm, fragrant, and bitter, but the aftertaste brings a cool feeling. The extract is used as an antispasmodic or cold medicine. They are also used to treat fainting, flatulence, biliary tract disease, gout, hepatitis, and neurological disorder (Safaiee et al., 2019).

The extracts of water mint have performed anti-hemolytic and anti-oxidant activities (Ebrahimzadeh et al., 2010). In addition, the phenolic compounds with anti-bacterial were displayed in water mint. The main phenolic compounds extracted from the water mint were RA, luteolin-7-O-glucoside and eriocitrin (Salmanian et al., 2013). In another study, RA, which was considered the major phenolic compound of water mint in Portugal was 68 µg/mg (Pereira et al., 2019). Besides, the content of RA in water mint was ranked 4th with 24.6 mg/g after *Mentha spicata, Mentha Piperita*, and *Mentha longifolia* (Ćavar et al., 2021).

The approach we have used in this study aims to determine the RA content in water mint by using the UV-vis method. The impact of the solvent ratio and temperature on RA content in water mint were studied to determine the best conditions for extraction.

Results and discussion

Qualitative analysis of various biologically active compounds in water mint

The purpose of the experiment was to investigate the qualitative analysis of various bioactive compounds in the extract of leaves and whole parts of a plant. Table 1 and Fig 2 showed the presence of biologically active compounds in water mint included: alkaloid, carbohydrate, flavonoid, and phenolic. Based on the previous studies, species of the genus *Mentha* contained various components, including flavonoids and phenolic acids (Ćavar Zeljković et al., 2021) Similarly, Bahadori et al. (2018) showed that ethanol extract of *Mentha longifolia* contained 16 compounds (10 phenolic acids and 6 flavonoids). The sinapic acid and RA were the most predominant compounds in both of the samples. Kapp et al. (2020) collected leaves of cultivated and wild-grown plants (33 *Mentha* spp. species) and determined the polyphenol compounds in the water extracts. The results indicated that 32 compounds were identified and the most abundant phenolic compound in the extracts was RA. In addition, mint was also reported to contain sugars, alkaloids in its extract (Brahmi et al., 2017).

The comparative experiment of RA content in different plant parts of water mint by using drying method

Because of the heterogeneity of plants, it is necessary to determine the quality of medicinal and food plants. Many studies have demonstrated the qualitative and quantitative composition of the active ingredient in different species, varieties, parts of the plant, and between different plants of the same species (Pudziuvelyte et al., 2020). Moreover, RA was identified as the phenolic compound in water mint (Brahmi et al., 2017). Therefore; its retention is particularly important during the drying method.

In this study, the determination of RA content on fresh and dried parts of water mint was performed (Fig 3). Results showed that different plant parts significantly affect the RA content of water mint ($p < 0.05$). In detail, the highest RA content was observed in dried leaves (dried at 50°C in 5 hours) with 11.73 mg/g, while the lowest was observed in a fresh whole plant (2.35 mg/g). When the stems were extracted with the leaves (whole plant samples), the amount of RA was reduced. In some previous researches, the drying process has been studied, which proves that its plays an important role in preservation plant and helps to obtain more biologically active compounds (Kankara et al., 2014; Xing et al., 2017; Abdullah et al., 2012).

The same, Al-Juhaimi and Ghafoor (2011) reported the highest amounts of the total phenolic contents from extracts obtained from the dried leaf of *Mentha arvensis* leaves, followed by *Petroselinum crispum* leaves and *Coriandrum sativum*) leaves. However, there were non-significant differences in the phenolic contents of extracts obtained from dried stems of these herbs. Bittner et al. (2019) also analyzed and compared the leaves and rhizomes of *Mentha piperita* from the side of phenolic compounds. They identified that the main phenolic compound in the rhizomes was RA. Although its content was three times lower than that in leaves, rhizomes can also be considered as a potential source for the pharmaceutical and food industry. Next, according to Pudziuvelyte et al. (2020), the content of phenolics was statistically significantly higher in the whole herb, leaf, and flower extracts than stem extract of *Elsholtzia ciliata* (Thunb.) Hyl. The results of the cited studies showed a similar trend as in our study that the leaf extracts have higher phenolic compound contents than the stem extracts. This difference may be due to different concentrations of these compounds and RA in particular in different parts of the plant, the solvent used, the extraction conditions, and the quality of the raw plant materials.

In Table 2, there was no significant change between RA content between dried leaves and stems when based on a percent of dry weight, and RA content was calculated on a dried whole plants basis, so using dried whole plants was the economic factor in this study.

The effect of solvent to sample ratio on RA content in water mint

In this study, the effect of solvent to sample at different ratios from 1:20 to 1:70 (w/v) on RA was researched. In Table 3, the RA content was dependent on the ratios of solvent. Overall, an increase in the volume of water in solution has a positive influence on the extraction efficiency of RA and the content of RA was maximized at 1:60 (w/v) ratio, then followed by a slight drop with further increases of the solvent to sample ratio at 1:70 (w/v). For example, RA content increased from ratio 1:20 to 1:60 (w/v) (from 10.25 to 17.76 mg/g, respectively) and decreased at ratio 1:70 (w/v) with 16.95 mg/g. Besides, a significant influence of ratio (1:20, 1:30, 1:40, and 1:50 (w/v)) was observed, while there was no significant change in the RA content results among the ratio 1:50, 1:60, and 1:70 (w/v). The result showed RA content increased with the increase of the solid-to-liquid ratio until reaching an optimum level.

According to LSD test, there was no statistically significant difference between the ratios 1:50 and 1:60 (w/v). Thus, a solvent ratio of 1:50 (w/v) was selected because of the economic aspects (solvent and power consumption for extraction and concentration).

The effect of extraction temperature on RA content in water mint

The impact of different extraction temperatures (30°C, 50°C, 70°C, and 90°C) on RA content was investigated. Table 4 showed RA content was significantly affected by temperature extraction. In detail, RA content at 70°C and 90°C was significantly higher than those extracted at 30°C and 50°C. In some previous researches (Kankara et al., 2014; Dent et al., 2013; Elboughdiri, N., 2018), increasing temperatures promoted extraction by enhancing both solubility and diffusion coefficients from a solid to liquid matrix of polyphenols. Moreover, elevated temperatures also increased cell membrane permeability because of the breakdown of cellular constituents; hence, the polyphenols were free to be extracted. High temperatures were, however, not always suitable for extracting all sorts of phenolic compounds as they could lead to the loss of antioxidant activity. As such, only samples with higher proportions of thermostable compounds should be extracted at high temperatures (Kankara et al., 2014).

Similarly, the polyphenol content obtained from the extract of *Salvia officinalis* L. at 60°C for 30 minutes was higher than that at 90°C due to the enlargement in solubility and diffusion coefficients at 60°C in ethanol and acetone extracts.

Table 1. The presence of biologically active compounds in water mint.

Chemical Constituents	Fresh sample		Dried sample	
	Leaves	Whole plant	Leaves	Whole plant
Alkaloid	+	+	+	+
Carbohydrate	+	+	+	+
Flavonoid	+	+	+	+
Phenolic	+	+	+	+

Fig 1. The Vietnamese water mint (*Mentha aquatica*)

Table 2. The weight percent of plant parts and RA content calculated by a dry whole plant of water mint.

Plant parts	Percent of dry weight (%)	RA content (mg/g of whole plant)
Dried leaves	$97.52^{a} \pm 0.45$	12.24 ± 0.70^{a}
Dried stems	$2.48^{b} \pm 0.45$	10.83 ± 0.54^{a}

The means with different letters within the same column are statistically different (p<0.05).

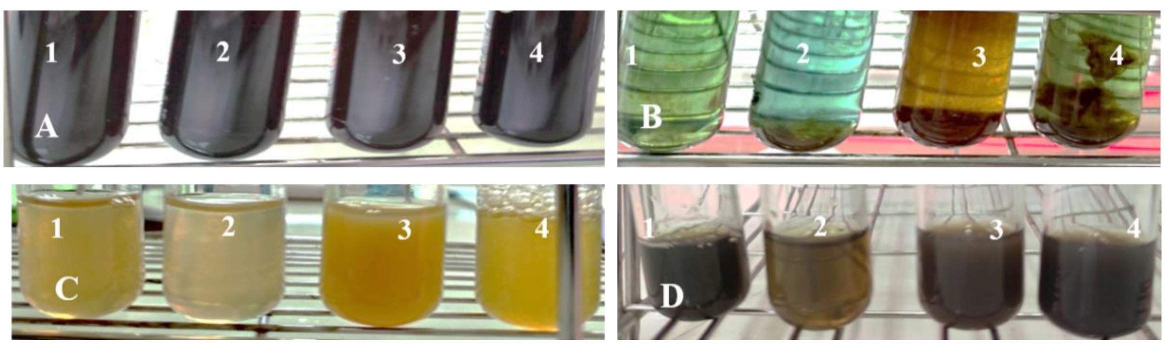

Fig 2. Qualitative analysis of various biologically active compounds in water mint (*Mentha aquatica*): A: Alkaloid; B: Carbohydrate; C: Flavonoid; D: Phenolic. Note: (1) fresh leaves; (2) fresh whole plants; (3) dried leaves; and (4) dried whole plants.

Table 3. The effect of solvent ratios on RA content in water mint.

Solvent to sample ratios (w/v)	RA content (mg/g)
1:20	10.25 ± 0.42^{a}
1:30	13.67 ± 0.57^{b}
1:40	15.54 ± 0.66^{c}
1:50	17.35 ± 1.48^{d}
1:60	17.76 ± 1.12^{d}
1:70	16.95 ± 0.95^{cd}

The means with different letters within the same column are statistically different (p<0.05).

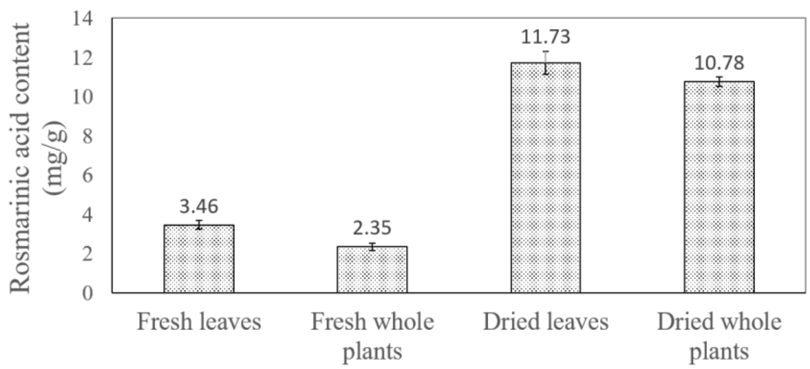

Fig 3. RA contents in the different plants parts extracts of water mint (*Mentha aquatica*) expressed in term of UV-vis.

Table 4. The effect of extraction temperature on RA content in water mint.

Temperature (^oC)	RA content (mg/g)
30	13.23 ± 0.26^a
50	13.83 ± 0.55^a
70	16.68 ± 0.47^b
90	16.66 ± 1.27^b

The means with different letters within the same column are statistically different ($p<0.05$).

When the temperature was higher than 60°C, phenolic compounds may be decomposed by hydrolysis, internal redox reaction, and polymerization (Dent et al., 2013). According to Safaiee et al. (2019), the content of phenolic compounds in extracts from water mint increased with increasing temperature. The suitable extraction temperature was 60°C.
Besides, there was no significant change between the temperature at 70°C and 90°C in Table 4. Therefore, the temperature extraction at 70°C was considered as the best condition with RA content at 16.68 mg/g.

Materials and methods

Plant materials

Water mint (*Mentha aquatica*) was grown and harvested in Binh Duong province, Vietnam in May 2021, and transported to the laboratory at Thu Dau Mot University. Water mint was washed to remove dust and dehumidified (Fig 1).

Methods

Determination of moisture content in the sample

The water content is determined by removing moisture and then by measuring weight loss (Nielsen, 2010).
In our study, we aimed to reduce the moisture content in a sample by approximately 5%. Five-gram raw samples were placed in a wide flask and put in the oven at 50°C. The sample was measured every 1 hour until the moisture content reached 5%. The moisture content is computed as follow: $\%\, MC = \frac{Ww-Wd}{Ww} x100.$
The result showed that after 5 hours of drying, the moisture content was approximately 5%.

Qualitative analysis of various biologically active compounds in water mint

The aerial plant parts including leaves, whole plants (leaves and stems) were collected. The fresh and dried samples were extracted for the qualitative analysis. The samples were ground using a laboratory mortar and pestle. After that, 1 g of the ground sample was added to 20 mL of water in a 100mL Erlenmeyer flask then covered for 30 min by shaking at 70°C. The mixture was filtered using filter paper.
To test for the presence of alkaloid, 2 mL of the extract was transferred into a test tube where 3-4 drops Bouchardat reagent was added. Formation of red brown color designated the presence of alkaloid.
To test for the presence of carbohydrate, 5 mL of the extract was transferred into a test tube where 1 mL Fehling A ($CuSO_4$) + 1 mL Fehling B ($KNaC_4H_4O_6.4H_2O$) were added. Formation of the brick red precipitation at the bottom of test tube indicated the presence of carbohydrate.
To test for the presence of flavonoid, 1 mL of the extract was transferred into a test tube where 1 mL $Pb(CH_3COO)_2$ 10% was added. Formation of the yellow color indicated the presence of flavonoid.
To test for the presence of phenolic, 2 mL of the extract was added to 2 mL H_2O and 1-2 drops $FeCl_3$ 1% in a test tube. Formation of green color indicated the presence of phenolic (Richa Saxena et al., 2014).

The comparative experiment of RA content in different plant parts of water mint by using drying method

Ground materials of fresh and dried leaves, fresh and dried whole plants (each for 1 g) were extracted with 20 mL of water (w/v). The extraction was performed by shaking at 70°C for 30 minutes. Finally, the extract was passed through filter paper and was used directly to analyze RA content.

The effect of solvent to sample ratio on RA content in water mint

One-gram samples were prepared then extracted with water at 70°C for 30 minutes. The solvent to sample ratio between water mint and water was based on weight/volume (w/v). The ratios were performed from 1:20 to 1:70 (w/v). The extract was filtered and was used directly to determine RA content.

The effect of temperature on RA content in water mint

One-gram samples were prepared then extracted with a suitable solvent to sample ratio for 30 minutes. The temperature was conducted for different periods (30°C, 50°C, 70°C, and 90°C). The liquid was filtered and was used directly for the estimation of RA content.

Determination of RA content by spectrophotometric test

RA content was determined based on a complex bright yellow reaction between RA and Zirconium (IV) oxide chloride at the wavelength of 362 nm on a spectrophotometer (Öztürk et al., 2010). The RA content was calculated using a standard graph with RA (Sigma-Aldrich) concentration from 5 to 25 µM.

Statistical analysis

All experimental data showed the mean ± SD with three replications. The data were analyzed by ANOVA in Statgraphics Centurion XV software with a significant difference of a 5% level according to LSD test.

Conclusion

Products from water mint are a useful functional food with a promising source of RA content. In this study, different plant parts and drying**,** solvent ratio, and extraction temperature were evaluated to identify optimum extraction conditions for RA. The results showed that RA content can be achieved using whole plants (dried at 50°C for 5 hours to reduce the moisture content under 5%), the solvent to sample ratio of 1:50 (w/v) at 70°C for 30 minutes. In conclusion, water mint is cheap and good for health by their bioactivities compound. The results of this study could be the first step for health products in the food, cosmetic, and pharmaceutical industry.

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