Physico-Chemical Characteristics of *Rosmarinus officinalis* L. Essential Oils Grown in Lam Dong Province, Vietnam

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Composition of rosemary essential oil largely depends on the geographical position of the cultivated plant and conditions of the extraction process. In this study, fresh rosemary leaves were used for extraction of essential oil by hydrodistillation and evaluation of chemical compositions and physico-chemical characteristics of the obtained oil were performed. The yield of essential oil was 1.0 %. The physico-chemical parameters showed specific gravity (0.8978 g/cm³), acid index (1.122 mg KOH/g), ester index (15.708 mg KOH/g) and refractive index (1.464). Twenty three components were identified in *Rosmarinus officinalis* L. oil. The major components were α -pinene (35.54 %), eucalyptol (20.902 %), camphene (4.384 %), bicyclo[3.1.1]hept-3-en-2-one (7.794 %), caryophyllene (1.225 %), *endo*-borneol (4.147 %) and bornyl acetate (4.065 %). Present study unveiled differences in the chemical composition of Vietnamese rosemary oil comparing with similar studies carried out in other countries.

Keywords: *Rosmarinus officinalis* L., Essential oils, GC-MS.

INTRODUCTION

Natural preservatives have been receiving a great deal of consumer attention. However, the safety aspect of preservative has not been studied thoroughly. Moreover, essential oils and extracts obtained from different plants have become increasingly popular and catching great scientific interest. The antimicrobial activity of essential oils from various plant materials including clove, cinnamon and garlic against microorganisms in food, as well as their applications in different fields have been examined and reviewed [1-6].

Rosmarinus officinalis L. belongs to the Lamiaceae family, which is cultivated mainly in different countries such as Italy, Spain, France, etc. [2-5]. Rosemary is one of spices and medi-

cinal herbs adding to different kinds of foods to enhance the flavour and organoleptic properties. Rosemary has a large quantity of essential oil (upto 1 %) with the highest antioxidant activity [7-10]. Rosemary essential oil exhibits powerful antimutagenic, antibacterial, anticancer and antifungal properties. Previous study reported that rosemary essential oils play an important role in food preservation, aromatherapy and fragrance industries [11]. In recent years, cultivation of rosemary have been receiving a great deal of public attention due to the essential oil extracted from its flowers and leaves (about 1.0-2.5 % essential oil) [12]. It has been reported that chemical composition of the essential oils of *R. officinalis* varies depending on the different areas in the world [13-15]. The variability of quantitative and qualitative composition of the essential oil depends

on not only intrinsic features but also extrinsic. Chromatography coupled with mass spectrometry (GC-MS) methods could help identifying different constituent of the essential oil in case of adulterated oil [15]. Since essential oils tend to be unstable in the presence of heat, light, moisture and the preservation process plays a crucial role in maintaining essential oils.

Hydrodistillation is a prevailing technique isolation of essential oils from plant materials due to the simplicity of installations and ease of performing. Previous study demonstrated that steam distillation method is feasibly utilizable for extraction of essential oils from lavender and artemisia leaves. Moreover, Yildirim *et al.* [16] adopted hydrodistillation and steam distillation for isolation of *Teucrium orientale* L. var. *orientale* essential oil. Based on steam distillation, Cassel and Vargas [17] optimized the yield of citronella essential oil extraction. Previous study revealed that microwave assisted hydrodistillation was more efficient in terms of extraction time and energy consumption [18]. There have been many studies attempting oil extraction by hydrodistillation to solve the problem of separation efficiency, composition and utility of essential oils [19].

In this study, using GC-MS method, the effect of seasonal variation on *Rosmarinus officinalis* essential oil are examined. The chemical profiles of essential oil from rosemary cultivated in Lam Dong province were also analyzed to compare with other studies.

EXPERIMENTAL

The samples of *Rosmarinus officinalis* L. were harvested from Lam Dong province, Vietnam. The materials consist of young leaves, branches of rosemary and hand-picked rosemary flowers. Rosemary branches are cut into pieces with length of about 15 cm from the tip. This size is proven to be optimal to achieve high essential oil content.

Extraction of essential oil of *Rosmarinus officinalis* L.:

Hydrodistillation method was conducted using water as the solvent to extract essential oils in a PQ-Agri apparatus. Briefly, 13 kg dry leaves were boiled with 42 L water at 102 °C in 4 h.

Physico-chemical analyses: Some basic physical and chemical parameters of the raw materials have been identified including acid index (TCVN 8450: 2010), sensory index (TCVN 8460: 2010) and density of essential oils. The experiments were repeated three times. Acid index is defined as the number of milligrams of KOH needed to neutralize free acids in 1 g of fat. The soap index is the number of milligrams of KOH needed to neutralize all free acids and acid combined as esters in 1 g of fat.

Gas chromatography-mass spectrometry (GC-MS): A GC-MS is applied to investigate the composition of essential oils of all samples. Briefly, 25 µL of essential oil was mixed in 1 mL *n*-hexane. Instrument used was GC Agilent 6890N, MS 5973 inert with HP5-MS column, head column pressure 9.3 psi. GC-MS system was performed following conditions: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 µL; injection temperature 250 °C; oven temperature progress included an initial hold at 50 °C for 2 min, then increased by 2 °C/min to 80 °C, and increased by 5 °C/min to 150 °C, continue rising to 200 °C at 10 °C/min and rise to 300 °C at 20 °C/min for 5 min.

RESULTS AND DISCUSSION

The essential oil was obtained by steam distillation of rosemary plant, resulting the yield of 1.0 %, which is expressed in 130 mL/13 kg of dry matter. In comparison with those of other studies, the yield of essential oil of *Rosmarinus officinalis* is higher than that by Bekkara *et al.* [20] and Derwich [21], achieving 0.8 and 0.54 %, respectively. In order to assess the quality of rosemary essential oils, we proceed to identify some of their characteristic physico-chemical. The results are shown in Table-1.

TABLE-1
ORGANOLEPTIC CHARACTERISTICS OF ESSENTIAL OILS OF *Rosmarinus officinalis* FROM VIETNAM AND COMPARISON WITH KABYLIA AREA (BOUIRA, ALGERIA)

Organoleptic characteristics	Essential oil	
	Vietnamese	Kabylia, Algeria
Aspect	Liquid	Liquid
Colour	Yellow clear	Yellow clear
Odour	Specific, α -pinene	Specific, Camphore
Density at 20 °C	0.8978	0.91
Acid index	1.122	6.1
Ester index	15.708	24.32
Rotator power	+2	+9°
Refractive index	+1.464	+1.469

Density is a main characteristic index for essential oil, which depends on its chemical composition. The density of rosemary essential oil was 0.8978 g/cm³. The acid index indicates free acid content, which could be indicative of newly extracted or preserved essential oils. In Table-1, acid number of rosemary oil was less than 2, suggesting that the obtained oil is conservable.

The ester index illustrates the fatty acid content associated with glycerin. The ester in the essential oils plays an important role in taste of essential oils. Table-2 shows the retention time and the percentage of separated constituents. There are 23 different components in the oil composition as shown in Fig. 1. The GC spectra analysis of rosemary oil composition showed that two components whose peaks situated at 7.449 and 12.071 min showed high abundance. The peaks of constituents at points 7.4, 7.983, 12.071 and 21.849 were located separately from each other and relatively large in intensity, proving that these are high-content constituents in essential oils. The remaining constituents have relatively low intensity indicated that the content of such components in essential oils is negligible. On the other hand, there are some constituents whose retention times are located closely with each other indicated that they can be the isomers of each other, exemplified by components with the peaks of 11.287 and 11.737; or components with peaks of 21.064, 21.305 and 21.849. The most abundant constituent in rosemary oil was shown to be α -pinene (35.54 %), followed by eucalyptol (20.902 %), camphene (4.384 %), bicyclo[3.1.1]-hept-3-en-2-one (7.794 %), caryophyllene (1.225 %), *endo*-borneol (4.147 %), bornyl acetate (4.065 %) and β -myrcene (1.011 %). Other components were found in much lower quantity (< 2 %). In terms of retention time, in steam distillation process, α -pinene is mostly obtained in the first 7 min. Camphene, *trans*-verbenol and β -myrcene share a similar pattern between

TABLE-2
CHEMICAL CONSTITUENTS OF
Rosmarinus officinalis ESSENTIAL OIL

Compound	R.T.	Concentration (%)
α -Pinene	7.449	35.54
Camphene	7.983	4.384
<i>trans</i> -Verbenol	8.234	0.694
Bicyclo[3.1.1]heptane	9.217	2.45
β -Myrcene	10.074	1.011
1,3-Cyclohexadiene	11.287	0.615
Benzene	11.737	0.562
Eucalyptol	12.071	20.902
1,4-Cyclohexadiene	13.671	1.291
Cyclohexene	15.407	0.914
1,6-Octadien-3-ol	16.306	2.358
Bicyclo[2.2.1]heptan-2-one	18.597	3.025
Endo-borneol	19.799	4.147
Bicyclo[3.1.1]heptan-3-one	19.935	0.472
3-Cyclohexen-1-ol	20.155	0.873
<i>trans</i> - <i>p</i> -Mentha-1(7),	20.385	1.237
3-Cyclohexene-1-methanol	21.064	2.137
Bicyclo[3.1.1]hept-2-ene-2-methanol	21.305	0.588
Bicyclo[3.1.1]hept-3-en-2-one	21.849	7.794
2,6-Octadien-1-ol	23.825	2.485
Bornyl acetate	24.86	4.065
Caryophyllene	29.137	1.225
Caryophyllene oxide	33.32	0.237

Fig. 1. Chromatogram of *Rosmarinus officinalis* L. essential oil

7 and 10 min. The bottom position belongs to caryophyllene oxide at 33.32 min.

Previous study demonstrated that the steam distillation is reliant to phenomena of transfer of oil at the surface of plant [22]. The chemical compositions of essential oils are reliant to different factors including seasons, soil structure, texture, and geographical location [23,26]. It is also revealed that the major component of rosemary essential oils in Iran was α -pinene which is similar to present result [27]. In another recent study, 45 components were classified, in which the major components were camphor (8.6 %), 1,8-cineole (54.6 %) and α -pinene (6.8 %) [28].

Diab *et al.* [29] identified that the major component, α -pinene reached a maximum level at flowering period [29]. Fig. 2 compares the amount of α -pinene in *Rosmarinus*

Fig. 2. Variation of α -pinene in *Rosmarinus officinalis* L. essential oil in different countries

officinalis essential oils reported in various studies conducted in several countries. The amount of α -pinene in *R. officinalis* in Iran takes a lead (43.9 %), followed by France (35.8 %) and Vietnam (35.54 %). Besides, the amount of α -pinene in *R. officinalis* in Spain achieved at 24.7 %, which was 12 % higher than that of Morocco. The bottom position belongs to Turkey (2.83 %). The composition of the oils is significantly sensitive to climatic conditions, leading to compositional variations within the common species of *Rosmarinus officinalis*. The antimicrobial properties of essential oils from the leaves of *R. officinalis* is primarily determined by content of α -pinene.

Conclusion

The extraction of the essential oils extracted from fresh *Rosmarinus officinalis* L. leaves harvested in Lam Dong province, Vietnam is conducted. The essential oil of *Rosmarinus officinalis* (1.0 %) was obtained by hydrodistillation. The physico-chemical properties of the oil were averaged at 0.8978 g/cm³ for specific gravity, acid index (1.122 mg KOH/g), ester index (15.708 mg KOH/g), refractive index (1.464). Moreover, the essential oil of fresh rosemary obtained using hydrodistillation was analyzed by GC-MS. The results of phytochemical screening of essential oils revealed the presence of α -pinene, eucalyptol, camphene, caryophyllene, *endo*-borneol, bornyl acetate and β -myrcene as major compounds in *R. officinalis* essential oil. Moreover, these components were shown to be different in content depending on the locations and chemotypes in which the plant material grows. This study aids to improve the useful value of rosemary as well as effectively exploiting the economic potential it brings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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