Egypt. J. Chem. **Vol. 66**, No. SI: 13 pp. 1583 - 1587 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Comparison of Chemical Composition of Essential Oil of Rosemary (*Rosmarinus Officinalis* L.) Obtained By Three Extraction Methods: Hydrodistillation, Steam Distillation, and Microwave-Assisted Hydrodistillation

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Abstract

Rosemary essential oil is one of the most popular essential oils and has many health benefits. Rosemary essential oil is becoming increasingly important for its useful antioxidant activities or as a source of fragrance in cosmetics and foods. The present study conducted to evaluate the efficiency of essential oil recovery, qualitative and quantitative chemical components in rosemary essential oil in Lam Dong province. Three extraction methods Steam distillation (SD), Microwave-assisted hydrodistillation (MAHD) and Hydrodistillation (HD) were used for essential oil isolation and determination by gas chromatography and mass spectrometry. Essential oil yield was obtained by HD (0.9ml/g), SD (0.73ml/g), MAHD (1.4ml/g) method. 35 compounds were identified with the typical main components α-Pinene (18.317%, 23.455%, 25.667%), Eucalyptol (20.269%, 15.24%, 16.924%), Verbenone (11.56%, 10.111%, 11.881%), Bornyl acetate (8.116%, 10.111%, 7.516%),… Comparison of three extraction techniques of essential oils gives a clear difference in content and active ingredients based on analytical results.

Keywords: Rosmarinus officinalis L, GC-MS, Steam distillation, Microwave-assisted hydrodistillation and Hydrodistillation

1. Introduction

Rosemary (Rosmarinus officinalis L.) is a native of the Mediterranean region, formerly grown in Southern Europe, Western Asia, and North Africa. In Vietnam, trees are imported and planted in some central and southern provinces. It is the most exploited wild-growing species due to its essential oil value but also due to its strong phenol and antioxidant content [1], [2]. In our country, rosemary was introduced to Vietnam around 2007 and is grown in many central and southern provinces. Rosemary, scientifically known as Rosmarinus officinalis, is a species of flowering plant in the family Lamiaceae [3]. Rosemary is a small, pleasant-smelling shrub that is used in food, traditional medicine, and as a cosmetic flavoring. Essential oils are mainly in the leaves and flowers, the best quality is in the leaves. Several methods are used to extract essential oils such as CO2 supercritical extraction, Ultrasound-assisted extraction, Hydrodistillation, Cold pressing, Steam distillation, and Microwave-assisted hydrodistillation [4]–[8]. Essential oils are a complex mixture of several compounds such as hydrocarbons, alcohols, esters, and aldehydes, with high activity capable of inhibiting many types of bacteria or deodorizing in the air [9], [10]. The performance and quality of the essential oil depend on the influence of the test method and the presence of chemical components in the essential oil. In a few published studies, the main components in rosemary essential oil are mainly 1,8-cineole, α-pinene, camphene, β-myrcene, borneol, and camphor, etc [2], [11], [12]. Variation in qualitative composition and quantification of essential oils can also be influenced by environmental factors, such as climate, genetics,

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EJCHEM use only: Receive Date: 05 June 2021; Revise Date: 08 December 2021; Accept Date: 18 August 2023
DOI: 10.21608/EJCHEM.2023.79194.3885
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and age of plants or cultivation conditions [5], [13]. This is a fairly new plant species in Vietnam. Currently, there is not much research on it. Therefore, with abundant and available raw materials in Vietnam, we used this material to investigate the performance and chemical composition of rosemary and compare their composition with the essential oil of rosemary in other regions or countries. In this study, three laboratory-scale methods: Steam distillation , Microwave-assisted hydrodistillation and Hydrodistillation were used to extract rosemary essential oil.

2. Materials and methods

2.1. Plant material

Fresh rosemary is purchased at Lam Ha, Lam Dong, Vietnam, harvested every 3 months in dry weather. After being transported to the laboratory, the raw materials are treated, cleaned of sandy soil and removed damaged branches. The materials used for extraction will be removed from the branches, and the leaves will be used for extraction. The remaining ingredients will be put in sealed bags and stored in the refrigerator.

2.2. Essential oil extraction process

The rosemary leaves after processing are extracted essential oil based on three methods of Steam distillation, Microwave-assisted hydrodistillation and Hydrodistillation. Rosemary leaves are weighed to a specified weight before being placed in the raw material flask. Set the required temperature parameters for each experiment and conduct distillation. The distillation unit is fixed at the heating temperature. The distillation time is calculated from the first drop of liquid obtained after passing through the condenser. After passing through the condenser, the mixture of essential oils and distilled water is extracted to obtain essential oils. The essential oil obtained after extraction still has the presence of less water, so it is anhydrous with pure Na_2SO_4 salt, then filtered to obtain the pure essential oil.

Hydrodistillation: 200g of rosemary leaves were imported into a 1000ml flask. Distillation takes place for 3 hours when the first drop of liquid appears, heated to 120ºC.

Steam distillation: 1500 mL of water was added to the flask and 300g of rosemary leaves were placed in a separate 1000 mL flask. A flask containing water is heated on a stove with a heating layer. Distillation stops when the volume of the essential oil remains constant, i.e. there is no difference in successive readings of the volumes.

Microwave-assisted hydrodistillation: Performs direct microwave-assisted distillation in a water solvent. The input weight for each sample was 100g of rosemary fixed at 1:1 ratio. 450W power according to the microwave and extracting time is 50 minutes.

2.3. GC-MS Analysis of Essential oil

Gas chromatography-mass spectrometry (GC/MS) analysis was used to determine the chemical composition of essential oil samples, 25 L of the essential oil obtained from the optimized process was mixed in 1.0 mL of n-hexane and reduced. water with Na2SO4. The instrument is GC Agilent 6890 N (Agilent Technologies, Santa Clara, CA, USA), MS 5973 inert, HP5-MS column, column head pressure 9.3 psi. GC-MS was obtained under the following conditions: Helium carrier gas; flow rate 1.0 mL/min; division 1: 100; injection volume 1.0 µL; spray temperature 250 ºC. From initial hold, dilute approximately 10 times and rerun with 40°C heat program for 2 min, then increase 20°C/min to 60°C for 5 min, increment by 5°C/min to 150°C, increments 10°C/min to 200°C, increments 20°C/min to 300°C holds for 5 min.

3. Results and discussion

3.1. The yield of rosemary essential oil

The rosemary essential oil yield obtained by the three methods of Steam distillation, Microwave-assisted hydrodistillation and Hydrodistillation is shown in Figure 1. For the HD method, this yield peaked after 3 h of distillation of 0.9 ml/g fresh ingredients. After 3 hours, the amount of essential oil was almost extracted. Microwave-assisted distillation can heat up quickly, saving time. When the material is heated quickly, it will break the essential oil bag in the material, and the essential oil will follow the steam through the condenser. The yield obtained was 1.4

ml/g of fresh material. Compared with the SD method, the yield was only 0.73 ml/g fresh material.

Previous studies have also shown that the extraction of essential oils by different methods will yield different concentrations of essential oils. This is explained by the influence of conditions such as too long heating time, hydrolysis will occur, the ester components in the essential oil are denatured to produce acids and alcohols. In addition, the amount of water used must be appropriate, if the amount of water is too much, it will not be beneficial to the water-soluble ingredients, on the contrary, if the amount of water is too little, the ingredients will easily burn. In 2014, Zeynab Mohkami's research group used raw materials from the Zabol region (Iran) extracted by steam distillation method, yielding 1.3% (dry material) [14]. In another publication in the field of extraction, Itmad Awad Elhassan et al. (2014) conducted experiments on dried rosemary leaf samples in Khartoum North (Sudan) area, the yield was 3% by hydrodistillation method [15]. With raw materials collected from the area Fez (Morocco), Majda Elyemni's research group (2019) conducted the extraction of essential oils from two methods of microwave-assisted hydrodistillation and Clevenger hydrodistillation, the results obtained between the two methods without any difference is about 1.35% ± 0.04% [16].

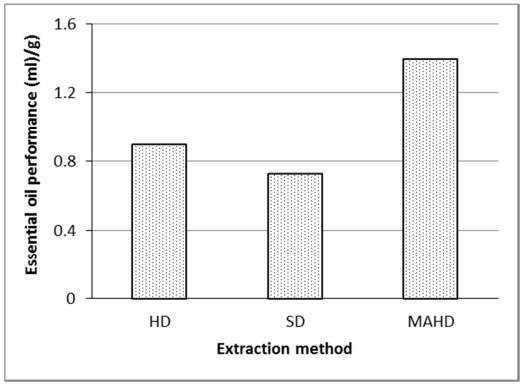

Figure 1: The extraction efficiency of rosemary essential oil by 3 methods (HD, SD, MAHD)

3.2 Identification of compounds

All chemical compositions, percentages of each compound, and the retention times (RT) of the evaluated and analyzed essential oils are given in Tables 1 and Figure 2, components eluted according to retention times and listed in their order from the HP5-MS column, the GC-MS analysis combined with retention indices was performed for the qualitative and quantitative determination of the compounds. Figure 2, the characteristic peaks for the main compounds in the essential oil are present during the retention time from 5 to 30 minutes of the elution process. The results showed that 35 compounds were identified in the three methods MAHD, SD, and HD, accounting for 96% - 100% of the composition in essential oils. The predominant constituents in rosemary essential oil are α-Pinene (18.317%, 23.455%, 25.667%), Eucalyptol (20.269%, 15.24%, 16.924%), Verbenone (11.56%, 10.111%, 11.881%). , Bornyl acetate (8.116%, 10.111%, 7.516%), respectively. Camphene (3.567%, 3.623%, 2.859%), β-Pinene (2.159%, 2.687%, 2.374%), Limonene (2.233%, 4.166%, 2.632%), Linalool (2.979%, 2.769%, 2.297) %), Camphor (4.476%, 2.792%, 3.545%), Borneol (4.807%, 3.823%, 4.001%), α-Terpineol (3.008%, 1.969%, 2.495%), Caryophyllene (2.045%, 4.635%, 2.245%) were also determined, along with some components with a content of ≤ 1%. The difference between the three extraction methods is reflected in the presence of several chemical components such as MAHD (Dehydrosabinene, cis-Sabinene hydrate, Chrysanthenone), SD (α-Thujene, α-Phellandrene, o-Cymol, Geranyl) acetate), HD (β-Phellandrene). Basically, the content of oxygenated compounds in MAHD is higher than in HD and SD, while the concentrations of monoterpene hydrocarbons in MAHD are lower than in HD, because the polar components will be influenced by microwave and temperature rises very quickly, being drawn out by the steam [17]. On the other hand, oxygenated compounds are more valuable in contributing to the aroma of essential oils than monoterpenes hydrocarbon compounds[7].

The chemical composition of rosemary essential oil is very complex, always changes with the time of plant growth or also changes with climatic conditions of the weather. In the part of the tree, the essential oil content is also different, so it is necessary to determine the time of collection to give the highest amount of essential oil and the best quality. In 2011 S. K. Tavassoli et al. used GC and GC/MS to analyze rosemary essential oil, 50 chemical components were determined with the main components such as α-terpineol (4.30%), α-pinene (9.87%), borneol (5.61%), camphene (5.58%), β-pinene (6.10%), camphor (12.35%) and 1.8-cineole (23.14%) [18]. Thirty-six compounds, which have been identified in rosemary essential oil, account for 99.9% of the total essential oil. The main constituents of rosemary essential oil are camphene (12.78%), 1,8-cineole (23.56%), β pinene (12.3%), camphor (12.55%), caryophyllene oxide (5.02%) and γ-terpinene (7.56%) with materials from the Sfax region, Tunisia,

performed by the research group of Marwa Jardak (2017) [12].

In 2018 Emel Dıraz Yıldırım et al. used rosemary sources in all four seasons (spring, summer, autumn, and winter) to conduct essential oil extraction. The results of GC-MS analysis indicate the diversity of the main components with seasonal variation and the presence of compounds in rosemary essential oil. Specifically, 27 components were identified, the main components being 1.8 cineole (41.25% - 45.96%), α-pinene (9.28% - 11.22%), isoborneol (11) ,96% - 14.89%) and α-terpineol (4.65% -8.41%) [19]. In 2017 K. Hannour et al. studied the chemical composition and application of rosemary essential oil as a fumigant to kill Phthorimaea operculella, with the material areas in the Middle Atlas and Loukkos (Morocco). Both essential oils are mainly composed of monoterpene compounds, the main components found in both oils are 1,8-cineole (46.23 and 17%), camphor (17.29 and 21.33%), β-pinene (5. 0.62% and 8.58%), camphene (2.63% and 7.44%), α-pinene (1.85% and 9.19%), respectively [20].

Table 1: Chemical composition of rosemary essential oil by GC-MS

Compounds	MAHD (%)	SD (%)	HD (%)
Tricyclene	-	0.132	0.23
α-Thujene	-	0.27	-
α-Pinene	**18.317**	**23.455**	**25.667**
Camphene	**3.567**	**3.623**	**2.859**
2,4(10)-Thujadiene	0.228	0.658	-
Dehydrosabinene	0.418	-	-
β-Pinene	**2.159**	**2.687**	**2.374**
β-Myrcene	0.686	1.204	0.808
α-Phellandrene	-	0.321	-
β-Phellandrene	-	-	0.662
α-Terpinene	0.519	0.929	-
o-Cymol	-	0.618	-
Limonene	**2.233**	**4.166**	**2.632**
Eucalyptol	**20.269**	**15.24**	**16.924**
γ-Terpinene	1.203	2.062	1.441
cis-Sabinene hydrate	0.541	-	-
Terpinolene	0.807	1.509	0.914
Linalool	**2.979**	**2.769**	**2.297**
Chrysanthenone	0.503	-	-
Camphor	**4.476**	**2.792**	**3.545**
Pinocarvone	0.288	0.279	-
Borneol	**4.807**	**3.823**	**4.001**
Isocamphopinone	1.236	0.998	0.999
Terpinen-4-ol	1.708	1.372	1.725
α-Terpineol	**3.008**	**1.969**	**2.495**
Myrtenol	0.632	0.496	0.54
Verbenone	**11.56**	**10.111**	**11.881**
Geraniol	3.989	4.098	3.179
Bornyl acetate	**8.116**	**10.111**	**7.516**
Myrtenyl acetate	0.745	1.162	0.604
Geranyl acetate	-	0.458	-
Methyleugenol	0.745	1.162	0.604
Caryophyllene	2.045	4.635	2.245
Humulene	0.273	0.734	0.324
Caryophyllene oxide	0.52	0.762	0.498

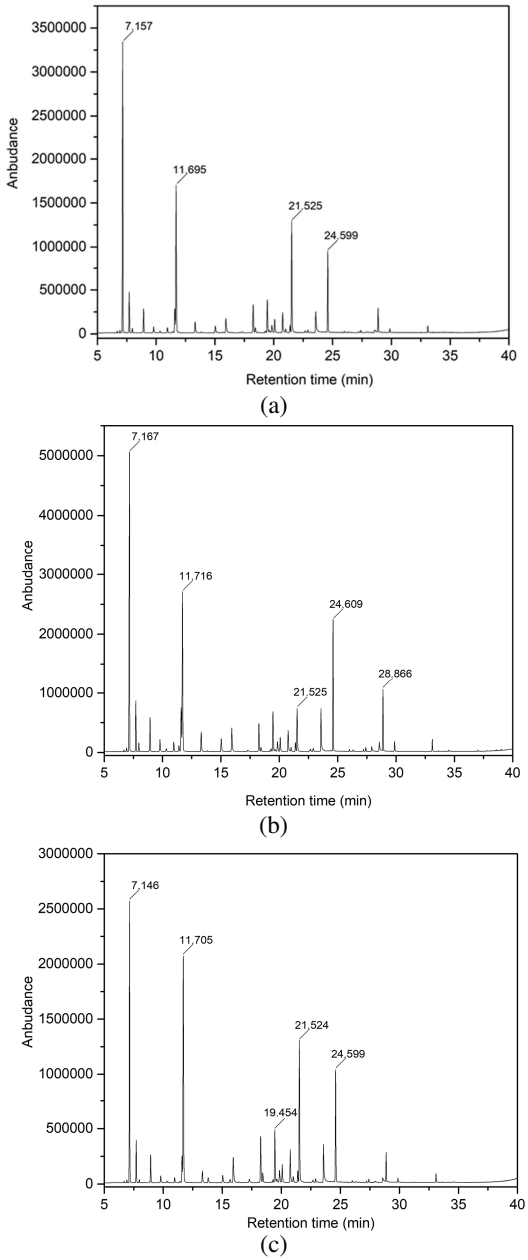

Figure 2: Chromatography of rosemary essential oil extracted by 3 methods: (a) HD, (b) SD, (c) MAHD

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