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Original Article

Comparison Extraction Methods of Essential oils of *Rosmarinus officinalis* L. in Iran By Microwave Assisted Water Distillation; Water Distillation and Steam Distillation

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Abstract

Rosmarinus officinalis L. is a perennial herb that belongs to the Lamiaceae family. It is used as a food flavouring agent, and well known medicinally for its powerful antimutagenic, antibacterial and chemopreventive properties. The most applicable method for extraction of essential oil is water distillation (WD) and steam distillation (SD). It is a traditional technique which is used in most industrious companies. The essential oil from the leaves rosemary obtained by Microwave assisted water distillation (MAWD) on 440W, 770W and 1100W has been compared with those obtained by conventional WD and SD. The total yield of the volatile fractions obtained through WD was 1.30%, SD was 0.54% and MAWD in 440W was 0.45%, 770 W was 0.50% and 1100 W was 0.55%, respectively. Essential oils samples were analyzed by GC and GC-MS, the oils revealed the presence of 28 to 35 compounds in the essential oils obtained through HD, SD and MAWD, respectively. Main components in WD method were camphene 33.08%, γ-terpinene 8.62% and verbenene 8.57%, in SD were camphene 31.71%, WD, γ-terpinene 8.92%, and verbenene 8.74%, and by Microwave-assisted water-distillation (MAWD) on 440W were linalool acetate 28.67% cis-sabinene hydrate acetate 20.59% and terpin-4-ol 11.56%, on 770W were camphene 15.88%, γ-terpinene 14.61% and cis-sabinene hydrate acetate 10.28%, and on 1100W were camphene 28.22%, γ-terpinene 13.66% and β-pinene 8.42%, respectively.

Keywords: Microwave assisted water distillation (MAWD), Water distillation (WD), Steam distillation (SD), Essential oil, Rosemary

Introduction

Rosemary (*Rosmarinus officinalis* L.) is a perennial herb with fragrant evergreen needle-like leaves. It is native to the Flore of Iran region and it has been cultivated for a long time. It belongs to the Lamaiaceae family, which comprises up to 200 genera and about 3500 species. Besides the therapeutical application, the essential oil is widely applied in the cosmetic industry producing various Cologne waters, bathing essences, and as a component of disinfectants and insecticides [1]. The leaves are evergreen, with dense short woolly hairs. Rosemary has been a significant herb since antiquity, although rosemary is more familiar to contemporary westerners as a kitchen herb used to add a spicy or slightly medicinal flavor to some foods, it was traditionally used as an antiseptic, astringent, and food preservative before the invention of refrigeration. Rosemary's antioxidant properties are still used to extend the shelf life of prepared foods Rosemary is also known medicinally for its powerful antioxidant activity [2].

Rosemary is also known medicinally for its powerful antioxidant activity and antibacterial

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properties and as a chemopreventive agent [3], Today, essential oil of rosemary is widely used in the cosmetic industry producing various bathing essences, hair lotions and shampoos its antibacterial and antimutagenic properties, and as a chemopreventive agent [4]. Traditional methods used for extraction of essential oil were water-distillation (WD) or steam distillation. Essential oils are well known to be thermally sensitive and vulnerable to chemical changes [5-8]. Losses of some volatile compounds, low extraction efficiency, toxic solvent residue in the extract may be encountered using these extraction methods [9-11]. Microwave-assisted hydro-distallation combines rapid heating in the microwave field with the traditional solvent extraction. This enables significant time-saving, so the extraction can be completed in meter of minutes [12]. Microwave-assisted solvent extraction [13] appeared to be particularly attractive for isolation of essential oil from rosemary. Tigrine-Kordjani *et al.,* [14] developed a microwave assisted distillation (MAD) with free solvent for laboratory scale applications in the extraction of essential oils from different kinds of aromatic plants. Sui *et al.* [15], have worked on an efficient microwave pretreatment (MP) method to maintain quality of postharvest rosemary leaves and observed that MP could be a good method for extracting essential oil and maintaining quality in rosemary and other aromatic herbs.

Essential oils are isolated using a number of methods such as steam distillation (SD), water-distillation (WD), organic solvent extraction, microwave assisted distillation (MAD), microwave hydro-diffusion and gravity (MHG), high pressure solvent extraction (HPSE), supercritical CO2 extraction (SCE), ultrasonic extraction (UE) and solvent free microwave extraction (SFME).

However, the properties of the essential oils extracted through these methods have been found to vary depending on the method used. Rosemary (R. officinalis L.) is a perennial herb that belongs to the *Lamiaceae* family. It is used as a food flavouring agent and known medicinally for its powerful antimutagenic properties, antibacterial and as a chemopreventive agent [3]. The plant is also known for its powerful antioxidant activity [2]. The essential oil of R. officinalis has usually been isolated by traditional hydrodistillation, steam distillation or organic solvent extraction. Losses and degradation of some volatile compounds due to long extraction times, degradation of unsaturated or ester compounds through thermal or hydrolytic effects are the principal disadvantages of these extraction methods [16,17].

In this paper, the essential oil from rosemary obtained by microwave hydro-distillation has been compared with those obtained by conventional water-distillation (WD) and steam distillation (SD). Then the quality and quantity of essential oil, cost, energy consumption and safety environmental consideration of two methods was studied. Also different parameter on essential oil extraction quantity and quality was checked. The aim of this research is finding an optimum method for extraction of essential oil.

Material and Methods

Leaves of the cultivated plants of rosemary (*R. officinalis* L.) were collected during June–July 2017 from the Research Institute of Forests and Ranglands. Plant specimen determined by Iranian Botanical Garden (IBG) staff. The initial moisture of leaves was 60.2%.

Water Distillation (WD)

100 g of dried leaves of *Rosmarinus officinalis* L. were submitted to water distillation with a Clevenger-type apparatus [18], (Clevenger, 1928, Maisonneuve, 1996) according to the European Pharmacopoeia, and extracted with 1 liter of water for 180min (until no more essential oil was obtained). The essential oil was collected, dried under anhydrous sodium sulphate yielding 1.30% and stored at 4 °C until used.

Steam Distillation (SD)

100 g of dried leaves of *Rosmarinus officinalis* L. were submitted to Steam distillation and extracted with 2 liters water for 120 min (until no more essential oil was obtained). The essential oil was collected, dried under anhydrous sodium sulphate, yielding 0.54% and stored at 4 °C until used.

Microwave Assisted Water Distillation (MAWD)

The home-made microwave extraction apparatus is illustrated in Fig. 1. Microwave assisted water distillation (MAWD) was purchased from Feller Germany microwave apparatus Model MW 420 GS. The multimode microwave reactor has a rated voltage 220-240V ~ 50-60 Hz, rated input power Microwave 1550 W, rated output power Microwave 1100 W, rated input power (Grill)

1200-1400 W, oven capacity 42 L, turntable diameter 345 mm, external dimensions (LxWxH) 553 x 465 x 326 mm. MAWD was carried out with 1oog of dried aerial parts of *Rosmarinus officinalis* L. were water-distilled with 500 mL of water by microwave energy at (three different wats 40, 70 and 100 W). The extraction oil was performed at atmospheric pressure for 40 W 30 min and for 70 and 100 W 20 min. The essential oils were dried over anhydrous sodium sulphate yielding for 40 W0.45%, 70 W 0.50% and 100 W was 0.55% and samples stored in the dark at 4˚C until analysis, then the essential oils were identified by GC and GC/MS.

Gas Chromatography

GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a split/non-split injector and a flame ionization detector (FID) at 250°C. N_2 was used as a carrier gas He, 0.5ml/min. and a DB-5 column type was utilized as the capillary (50m x 0.2 mm, film thickness 0.32 µm). Temperature within the column for 3 min was retained at 60 °C, after that the column was heated at a rate of 5 °C/min, until it reached at 220 °C and maintained in this condition for 5 min. The split ratio was 1:100.

Gas Chromatography - Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph which was coupled to a Saturn II ion trap detector was used. The column was the same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier potential is 2000 V.

Fig. 1 Microwave Water distillation apparatus used in this study.

Most constituents were identified by comparison of their GC Kovats retention indices (RI), determined with reference to a homologous series of C_7- C_{25} *n*-alkanes and with those of authentic standards available in the authors' laboratory. Identification was confirmed when possible by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology, Adams and Wiley libraries) and with mass spectra literature data (Adams, 2017). [19-21]. Component relative concentrations were obtained directly from GC peak areas obtained with GC-FID.

Results

Essential oils from aerial parts of *Rosmarinus officinalis* L. were obtained by conventional Water distillation (WD), Steam distillation (SD) and microwave Assisted water distillation (MAWD). The oils were investigated by capillary GC and GC/MS. In total, 37 constituents were identified. All these compounds were characterized by comparing their mass spectra and their retention indices with those of our own library. The retention indices of the oils, yields, extraction times, extract constituents and their relative percentages are listed in Table 1. Main components in WD method were camphene 33.08%, γ-terpinene 8.62% and verbenene 8.57%, in SD was camphene 31.71%, WD, γ-terpinene 8.92%, and verbenene 8.74%, and by Microwave water distillation (MWD) on 440W were linalool acetate 28.67% cis-sabinene hydrate acetate 20.59% and terpin-4-ol 11.56%, on 770W were camphene 15.88%, γ-terpinene 14.61% and cis-sabinene hydrate acetate 10.28%, and on 1100W were camphene 28.22%, γ-terpinene 13.66% and β-pinene 8.42%, respectively. Akhbari,M. and *et al*. 2018, studeid on Optimization of microwave assisted extraction of essential oils from Iranian *Rosmarinus officinalis* L. using RSM; Optimal conditions for obtaining the maximum extraction yield in the microwave assisted method were predicted as follows: extraction time of 85 min, microwave power of 888 W, and water volume to plant mass ratio of 0.5 ml/g.

The extraction yield at these predicted conditions was computed as 0.7756%.

Table 1 Results of three different methods of extracting Oils from *Rosmarinus officinalis* L.

Compounds name	R.I.	W.D.	S.D.	MAWD 440 W	MAWD 770 W	MAWD 1100W
α- pinene	938	0.37	0.31	-	-	0.25
Camphene	944	33.08	31.71	-	15.88	28.22
Verbenene	962	8.57	8.74	-	3.67	6.04
Sabinene	971	0.30	0.22	0.61	0.62	0.45
β- pinene	976	7.68	9.47	0.38	8.00	8.42
α- phellandrene	1002	0.82	0.91	0.69	1.44	1.48
1,8-cineole	1038	0.41	0.59	-	-	-
(E)- β-ocimene	1048	1.44	2.72	-	1.26	1.33
n-pentyl isobutyrate	1053	4.95	7.88	-	3.07	3.90
γ- terpinene	1060	8.62	8.92	1.28	14.61	13.66
Benzyl formate	1077	0.25	0.47	-	0.19	0.20
Linalool	1106	2.60	3.19	0.18	0.29	0.20
6-champhenol	1108	-		5.35	4.22	2.81
Trans – thujone	1115	-	0.31	0.10	0.14	-
α- campholenal	1126	0.70	0.91	0.49	0.51	0.35
Camphor	1147	0.45	0.40	1.02	0.85	0.60
Borneol	1167	-		1.30	0.43	0.27
Terpin-4-ol	1177	7.72	5.42	11.56	10.27	7.61
Verbenone	1205	1.34	1.22	1.57	1.98	1.42
Octanol acetate	1208	-	-	1.56	-	-
Cis- sabinene hydrate acetate	1219	4.85	3.03	20.59	10.28	7.08
Neo-iso-dihydro carveol	1224	0.80	0.68	2.84	2.18	1.45
Isobornyl formate	1232	1.32	0.61	4.87	2.28	1.70
Myrtenyl acetate	1235	0.97	0.46	3.65	1.60	1.13
Linalool acetate	1256	6.72	0.91	28.67	9.79	7.50
Ethyl salicylate	1267	-	-	1.14	0.80	0.38
γ –terpinen-7-al	1287	0.74	0.32	-	1.31	0.87
2-undecanone	1290	-	-	3.05	-	-
n-tridecane	1300	0.90	0.32	3.44	1.35	1.00
n-nonanol acetate	1312	3.60	6.01	1.80	1.34	0.66
Terpin-4-ol acetate	1345	-	0.21	0.16	-	-
Geranyl acetate	1382	-	0.19	0.30	-	-
α-cedrene	1409	-	0.35	0.13	-	-
(E)-caryophyllene	1425	-	-	0.24	0.28	-
γ-curcumene	1470	0.67	2.68	0.48	0.64	0.70
α-cadinol	1654	-	-	1.36	0.38	0.18
(Z, Z)-farnesol	1716	-	-	0.37	-	-
Percentage of oil	-	1.30	0.54	0.45	0.50	0.55

R.I. = retention indices on DB-5 column
W.D. = water distillation
S.D. = Steam distillation
MAWD = Microwave Assisted Water Distillation

The qualities of the obtained essential oils under designed experiments were optimized based on total contents of four major compounds (α-pinene, 1,8-cineole, camphor and verbenone) which determined by gas chromatography equipped with mass spectroscopy (GC–MS).

The highest essential oil quality (55.87%) was obtained at extraction time of 68 min; microwave irradiation power of 700 W; and water volume to plant mass ratio of zero [22]. Moradi,S. and *et al*. 2018, studied on Microwave-Assisted Hydro-Distillation of Essential Oil from Rosemary: Comparison with Traditional Distillation, In this research, MAHD of essential oils from the aerial parts (leaves) of rosemary (*Rosmarinus officinalis L.*) was studied and the results were compared with those of the conventional HD in terms of extraction time, extraction efficiency, chemical composition, quality of the essential oils and cost of the operation. Microwave hydro-distillation was superior in terms of saving energy and extraction time (30 *min*, compared to 90 *min* in HD).

Chromatography was used for quantity analysis of the essential oils composition. Quality of essential oil improved in MAHD method due to an increase of 17% in oxygenated compounds [23]. Fadel, O. and *et al*. 2011, studied on volatile compounds of *Rosmarinus eriocalyx* samples were extracted by steam-hydro-distillation (HD) and microwave-assisted hydro-distillation (MAH). GC-MS and GC-FID analysis of the oils revealed the presence of 22 and 26 compounds in the essential oils obtained through HD and MAH, respectively. The total yield of the volatile fractions obtained through HD and MAH was 1.21% and 1.47%, respectively. The two oils contained the same dominant components: camphor (35.92% HD; 35.33% MAH), camphene (19.74%; 17.07%), a-pinene (14.53%; 12.87%), and 1,8-cineol (6.52%; 6.73%). Higher amounts of oxygenated monoterpenes such as borneol, 1,8-cineol, isobornyl acetate, a-terpeneol, caryophyllene oxide (13.59%) were present in the oil of MAH in comparison with HD (12.36%). However, HD oil contained more monoterpene hydrocarbons such as tricyclene, a-pinene, camphene, o-cymene and limonene (39.04%) than MAH extracted oil (34.45%) [24].

Discussion

Water distillation (WD), steam distillation (SD) and microwave Assisted water distillation (MAWD) techniques have been compared, for the extraction of essential oil from leaves of *Rosmarinus officinalis* L. This microwave extraction methods offers important advantages over traditional water-distillation and steam distillation, shorter extraction times for 440 W 30 min and for 770 and 1100 W 20 min against 3 h for hydro-distillation and steam distillation, better yields for MWD with 440 W 0.45% and for 770 W 0.50% and 1100 W 0.55% for WD 1.30% and SD 0.54%, respectively. Environmentally friendly lower cost and the possibility for a better reproduction of natural aroma of the *Rosmarinus officinalis* L. essential oil than teradintional distillation like Water distillation (WD), steam distillation (SD) essential oils.

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