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CHARACTERISTICS OF ROSEMARY ESSENTIAL OIL OBTAINED BY HYDRODISTILLATION AND STEAM DISTILLATION

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Abstract: Rosemary (*Rosmarinus officinalis* L.) is mainly found in yards and gardens. Still, for several years, attempts have been made to cultivate it in the experimental field of the Institute of Rose and Essential Oil Crops, Kazanlak. The present study aimed to compare essential oils obtained by hydrodistillation and steam distillation from vegetatively propagated rosemary of Iranian origin. The chemical composition of the essential oils was identified by GC/MS analysis. The major components (over 2 %) were the monoterpene hydrocarbons α-pinene (41.09 and 51.53 %) and verbenone (9.40 and 1.13 %), and the monoterpene oxygen derivatives 1,8-cineole (10.22 and 14.24 %), camphor (9.27 and 5.05 %), bornyl acetate (5.67 and 4.05 %), and borneol (5.34 and 2.63 %). The antioxidant activity of both oils was also determined by two methods, DPPH (0.653 and 0.616 mM TE·mL^{-1}) and ABTS (0.630 and 0.605 mM TE·mL^{-1}). The antioxidant activity exhibited is the basis for including the oils in various food and cosmetic products, subject to further studies.

Keywords: *antioxidant activity, chemical composition, rosemary essential oil*

INTRODUCTION

The representatives of the Lamiaceae are very widespread in Bulgaria; most of them are cultivated plants, and others are wild. The essential oils obtained from them are widely used in medicine, pharmacy, perfumery and cosmetics [1 – 3].

Rosemary (*Rosmarinus officinalis* L.) is found in yards and gardens, but today, it is cultivated in the field of the Institute of Roses, Essential and Medical Plants, Kazanlak, Bulgaria. Various studies are being conducted with plants of different origins to obtain sustainable crops with high essential oil content.

It is known that different aromatic substances have been identified in the composition of rosemary essential oil. Major constituents are 1,8-cineole (from 17 to 69 %), α-pinene (from 9 to 27 %), camphor (from 5 to 43 %), limonene (from 7.3 to 54 %), bornyl acetate (from 0.5 to 18 %), verbenone (from 1 to 27 %), borneol (from 2 to 7 %), etc. [1 – 3]. Their amount varies depending on the soil and climatic conditions of the plant's cultivation [4 – 6].

This essential oil has proven antimicrobial [7 – 14] and antioxidant [7, 12 – 16] properties and other biological actions [16 – 20], which is why it is mainly used in medicine, perfumery and cosmetics [1 – 3, 20].

Essential oils are mainly obtained from plant materials by distillation with water or steam [1 – 3].

The present study aimed to perform a comparative analysis of the essential oil obtained from vegetatively propagated rosemary of Iranian origin, cultivated in Bulgaria, by two laboratory methods – hydrodistillatioan and steam distillation. The study involved the identification of aromatic substances using gas chromatography (GC/MS), determining the odor and antioxidant activity of the essential oils.

MATERIALS AND METHODS

Raw materials

Rosemary (*Rosmarinus officinalis* L.), from which the essential oil is extracted, is obtained by vegetative propagation originating in Iran. The plantation was established at the beginning of 2023 in the experimental field of the Institute of Roses, Essential and Medical Plants, Kazanlak, Bulgaria (42.61º94′418″ N 25.39º29′58″ E, altitude 407 m) (Figure 1).

***Figure 1**. Rosemary, cultivated in Institute of Roses, Essential and Medical Plants, Agricultural Academy, Kazanlak, Bulgaria (authors'images)*

The soils in the area are leached cinnamon-forest, develop on old diluvial deposits, structureless with good aeration and water permeability, with acidity *p*H 5.6 and poorly stocked with nitrogen 20.5 mg·1000 g^{-1}, phosphorus 4.25 mg·100 g^{-1}, but is well stocked with potassium 21.75 mg·100 g^{-1}, humus content 1.8 %.

The following planting scheme was implemented in this study: inter-row distance was 86 cm, and intra-row distance was between 35 cm and 3.3 plants·m^{-2}.

The raw material (twigs and flowering tops) was harvested at the beginning of August 2024.

Methods

The plants' moisture (60 - 65 %) was determined by drying to constant weight at 105 °C in a moisture analyzer Kern DLB (Kern & Sohn GmbH, Balingen-Frommern, Germany), and the chemical analysis results were given on a dry weight (dw) basis [21].

The essential oil was obtained in two ways - by steam distillation in a 10 dm^3 laboratory apparatus (Figure 2), with a duration of 2 h, a distillation rate of 10 % and a distillate temperature of 24 °C and by hydrodistillation in a British Pharmacopoeia laboratory glass apparatus modified by Balinova and Dyakov [22] (Figure 3). After preparation and dehydration with anhydrous sodium sulfate, the oils were stored in the dark at 4 °C throughout the analyses. Essential oil yield was calculated relative to absolute dry mass.

Figure 2. *Laboratory apparatus for steam distillation (authors' images)*

Figure 3. *Laboratory glass apparatus for hydrodistillation of the British Pharmacopoeia, modified by Balinova and Dyakov [22] (authors' images)*

Chemical composition of essential oils

The chemical composition of the essential oils was determined by GC/MS analysis. GC analysis was performed with an Agilent 5975C instrument (Agilent Technologies Inc., Santa Clara, CA, USA) under the following conditions: DB-5 ms column, 30 m × 0.32 mm × 0.25 µm; temperature 40 °C·min^{-1}, 5 °C·min^{-1} to 300 °C and finally retention 10 min; temperature of injector and detector 250 °C; temperature FID: 250 °C; helium as a carrier at a constant rate of 0.8 mL·min^{-1}; split 100:1. GC/MS analysis was performed on an Agilent 5975C under the same conditions as the GC analysis. The chemical compounds were identified by comparing their retention time to library data [23]. The identified constituents were arranged according to their retention time, and their quantity was given in percentages.

Antioxidant activity of essential oils

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated as the rosemary essential oils (0.15 mL) was added to 2.85 mL of freshly prepared 0.1 mM DPPH solution in methanol. The samples were incubated for 15 min at 37 °C in darkness. The reduction of the absorbance at 517 nm was measured by spectrophotometer (Camspec M107, Camspec Ltd., Cambridge, United Kingdom) compared to the blank containing methanol [24]. The radical scavenging activity of the rosemary essential oils was expressed as mM Trolox® equivalent (TE) per mL^{-1} rosemary essential oil.

ABTS assay

The assay is based on the reduction of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical. It was carried out according to the procedure described by Re *et al.* [25] with slight modifications. Briefly, ABTS radical cations were generated by reacting aqueous ABTS (Sigma-Aldrich Chemie, Merck) with potassium persulfate and maintaining the mixture in the dark at room temperature (20 °C ± 0.5) for at least 12 h before use. Afterwards, the absorbance of the ABTS solution was set to (0.70 ± 0.02) at 734 nm by adding phosphate buffer. The volume of 30 µL of appropriately diluted rosemary essential oil was mixed with 3 mL of ABTS solution. After reaction for 10 min, the absorbance was measured at 734 nm with a spectrophotometer. The radical scavenging activity of the thyme extracts was expressed as mM $TE \cdot mL^{-1}$ rosemary essential oil.

Statistical analysis

All experiments were performed in triplicate, with values in the tables averaged and represented by their mean and standard deviation (SD). The experimental data were subjected to statistical evaluation using analysis of variance (ANOVA) with Statgraphics Centurion XVI Version 16.2.04 software (StatPoint Technologies, Inc., USA). Duncan's multiple range tests determined the difference among the means, and the significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

The essential oils are light-yellow liquids with a specific odor, and these indicators are comparable with data from the literature [1 – 3].

The amount of essential oil obtained by hydrodistillation is 0.5 %, and by steam distillation - 0.37 %. It is nearly two to three times lower compared to literature data published by Bozin *et al.* [7] (1.18 %), by Tural and Turhan [12] (0.98 %), and by Conde-Hernández *et al.* [26] (by hydrodistillation between 0.35 and 2.53 %), but close to those of Zheljazkov *et al*. [6] (0.51 %). These differences in quantities can be explained by the different soil and climatic conditions and cultivation methods found in other essential oil plants [2, 3].

The chemical composition of the two essential oils is presented in Table 1.

Table 1. *Chemical composition of rosemary essential oils [% of TIC***]*

Peak	**RT****	**RI*****	**Name**	**Hydrodistillation**	**Steam distillation**
Oxygenated aliphatics					
1	11.03	980	3-Octanone	1.67 ± 0.01^{a}	1.94 ± 0.01^{a}
Monoterpene hydrocarbons					
2	8.89	919	Tricyclene	0.19 ± 0.0^{a}	0.22 ± 0.0^{a}
3	9.48	930	α-Pinene	41.09 ± 0.40^{a}	51.53 ± 0.50^{b}
4	9.88	945	Camphene	4.36 ± 0.04^{a}	6.13 ± 0.06^{b}
5	9.95	967	Sabinene	0.16 ± 0.0^{a}	0.28 ± 0.0^{b}
6	10.75	971	β-Pinene	1.55 ± 0.01^{a}	1.59 ± 0.01^{a}
7	11.17	989	β-Myrcene	1.89 ± 0.01^{a}	2.07 ± 0.0^{a}
8	11.64	1003	α-Phellandrene	0.10 ± 0.0^{a}	0.13 ± 0.0^{a}
9	11.99	1012	α-Terpinene	0.24 ± 0.0^{a}	0.30 ± 0.0^{b}
10	12.48	1023	Limonene	0.44 ± 0.0^{a}	0.37 ± 0.0^{b}
11	13.36	1057	γ-Terpinene	0.53 ± 0.0^{a}	0.60 ± 0.0^{a}
12	14.20	1085	Terpinolene	0.85 ± 0.0^{a}	0.97 ± 0.0^{a}
Oxygenated monoterpenes					
13	12.57	1026	1,8-cineole	10.22 ± 0.09^{a}	14.24 ± 0.13^{b}
14	13.74	1070	trans-Sabinene hydrate	0.18 ± 0.0^{a}	0.08 ± 0.0^{b}
15	14.82	1098	β-Linalool	1.86 ± 0.01^{a}	1.73 ± 0.01^{a}
16	15.31	1122	Chrysanthenone	0.34 ± 0.0^{a}	0.39 ± 0.0^{a}
17	15.48	1131	*cis*-2-p-Menthen-1-ol	0.12 ± 0.0^{a}	0.14 ± 0.0^{a}
18	16.16	1140	Camphor	9.27 ± 0.08^{a}	5.05 ± 0.05^{b}
19	16.54	1158	Pinocarvone	0.16 ± 0.0^{a}	0.22 ± 0.0^{a}
20	17.02	1166	Borneol	5.34 ± 0.05^{a}	2.63 ± 0.02^{b}
21	17.15	1170	Terpinen-4-ol	0.68 ± 0.0^{a}	0.41 ± 0.0^{a}
22	17.61	1177	2-Methyl isoborneol	0.74 ± 0.0^{a}	0.47 ± 0.0^{b}
23	17.81	1188	α-Terpineol	0.40 ± 0.0^{a}	0.52 ± 0.0^{a}
24	17.88	1193	Myrtenol	0.13 ± 0.0^{a}	0.29 ± 0.0^{b}
25	18.06	1202	Verbenone	9.40 ± 0.09^{a}	1.13 ± 0.01^{b}
26	20.17	1259	Bornyl acetate	5.67 ± 0.05^{a}	4.05 ± 0.04^{b}
Sesquiterpene hydrocarbons					
27	23.67	1413	β-Caryophyllene	1.30 ± 0.01^{a}	1.33 ± 0.01^{a}
28	24.57	1454	α-Caryophyllene	0.14 ± 0.0^{a}	0.19 ± 0.0^{a}
Oxygenated sesquiterpenes					
29	29.95	1650	α-Cadinol	0.16 ± 0.0^{a}	0.14 ± 0.0^{a}
Phenyl propanoic hydrocarbons					
30	12.32	1020	*p*-Cymene	0.15 ± 0.0^{a}	0.26 ± 0.0^{b}
Oxygenated phenyl propanoids					
31	23.05	1354	Eugenol	0.38 ± 0.0^{a}	0.14 ± 0.0^{b}
32	23.17	1399	Methyl eugenol	0.12 ± 0.0^{a}	0.07 ± 0.0^{b}

*total ion current; **retention time [min]; ***retention (Kovat's) index;
values with different letters in the row indicate significant differences ($p < 0.05$).

The data show that the same components were identified, but in different quantities, explainable by the type of distillation (hydrodistillation and steam distillation):

- 32 components (99.83 % of the total composition) have been identified in oil obtained by hydrodistillation. The main compounds (over 2 %) were α-pinene (41.09 %), 1,8-cineole (10.22 %), verbenone (9.40 %), camphor (9.27 %), bornyl acetate (5.67 %), borneol (5.34 %), and camphene (4.36 %).
- 32 components (99.61 % of the total composition) have been identified in oil obtained by steam distillation. The main compounds (over 2 %) were α-pinene (51.53 %), 1.8-cineole (14.24 %), camphene (6.13%), camphor (5.05 %), bornyl acetate (4.05 %), borneol (2.63 %), verbenone (2.48 %), and β-myrcene (2.07 %).

The comparative analysis of the data on the amounts of the main components identified in oils showed that in the hydrodistillation, the amounts of the main monoterpene hydrocarbons (pinene, camphene, and β-myrcene) and of the monoterpene oxide 1,8-cineole were small ($p < 0.05$), but the monoterpene alcohol borneol and the monoterpene ketones camphor and verbenone were in larger amounts ($p < 0.05$). These differences can be explained by the type of distillation, water or steam, and water vapor's effect on the individual functional groups, which have been found for other essential oils [2, 3].

There are many studies in the literature on the chemical composition of rosemary essential oil, and comparative analysis shows that the same components have been identified, but in different amounts, explainable by the soil and climatic conditions under which the plants are grown. It has been found that the main components in essential oils obtained from plants growing in different countries of the world is different, for example:

- Zheljazkov *et al*. [6] on plants from USA were α-pinene (30.4 %), 1,8-cineole (23.3 %), and camphor (18.9 %).
- Bozin *et al*. [7] on plants originating from Serbia were limonene (21.7 %), camphor (21.6 %), and Z-linalool oxide (10.8 %).
- Mahmoodi *et al*. [8] on plants from Iran were 1,8-cineole (78.6 %).
- Bajalan *et al*. [13] on plants from Iran were 1,8-cineole (5.63 - 26.89 %), camphor (1.66 - 24.82 %), and α-pinene (14.69 - 20.81 %).
- Wang *et al*. [15] on plants from China were 1,8-cineole (27.23 %), α-pinene (19.43 %), camphor (14.26 %), camphene (11.52 %), and β-pinene (6.71 %).
- Teneva *et al*. [14] (no plant origin given) are *p*-cymene (42.95 %).
- Khalil and Hassan [16] on plants from Iraq were 1,8-cineole (34.25 %), α-pinene (20.98 %), and camphor (13.75 %).

Eleven allergens [27] were identified in the two oils we studied: α- and β-pinene, limonene, γ-terpinene, terpinolene, β-linalool, α-terpineol, terpinene-4-ol, camphor, eugenol and β-caryophyllene. When essential oil is incorporated into perfumery or cosmetic products, their quantity should be considered and should not exceed the concentrations described.

The distribution of the identified components by groups of compounds (% of the composition) is presented in Figure 4. The data show that both oils are dominated by monoterpene hydrocarbons (51.49 % for hydrodistillation and 64.44 % for steam distillation), followed by oxygenated monoterpenes (44.59 % and 31.47 %, respectively), oxygenated aliphatics (1.67 % and 1.95 %), sesquiterpene hydrocarbons (1.44 % and 1.53 %) oxygenated phenyl propanoids (0.5 % and 0.21 %), phenyl propanoid hydrocarbons (0.15 % and 0.26 %), oxygenated sesquiterpenes (0.16 % and 0.14 %).

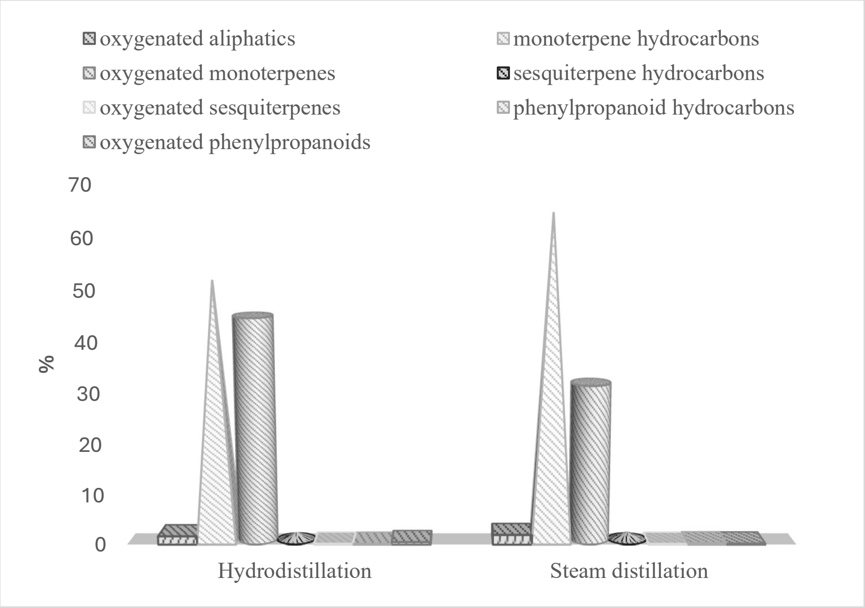

***Figure 4**. Groups of compounds in rosemary essential oils [%]*

The amount of identified groups of compounds differs from the data of Bozin *et al*. [7] oxygenated monoterpenes (46.9 %) and monoterpene hydrocarbons (46.7 %), explainable by the different origins of the plants.

Oxygen-containing compounds (% of all oxygenated derivatives) are known to determine the biological activity of essential oils, with phenols having the most prominent properties, followed by alcohols [28]. The distribution (by functional groups is presented in Figure 5.

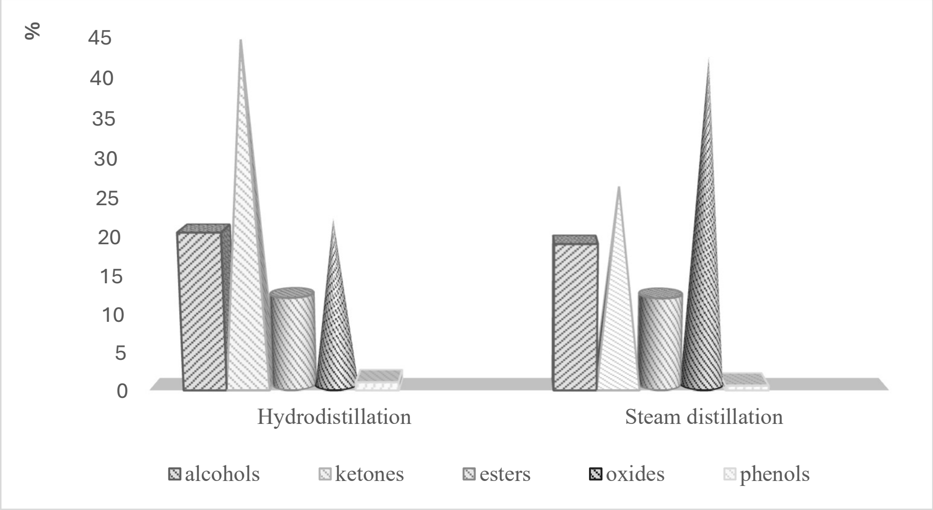

***Figure 5**. Groups of oxygen-containing compounds in rosemary essential oils [%]*

The data show that ketones predominate in the oil obtained by hydrodistillation, whereas oxides predominate in steam distillation. This difference in quantities may be due to the type of distillation, water and steam, explained in the case of essential oil extraction, also found for other essential oil plants [2, 3].

The results for antioxidant activity determined by two methods are presented in Table 2.

***Table 2**. Antioxidant activity of rosemary essential oils*

Method of obtaining the essential oils	**Hydrodistillation**	**Steam distillation**
DPPH [mM TE·mL^{-1}]	0.653 ± 0.004^{a}	0.616 ± 0.004^{a}
ABTS [mM TE·mL^{-1}]	0.630 ± 0.004^{a}	0.605 ± 0.004^{a}

values with letters in the same row no indicate significant differences ($p < 0.05$).

The data show that the values obtained are higher for the oil obtained by hydrodistillation ($p < 0.05$). This oil has a higher ketone content than that obtained by steam distillation. In the literature, there are data on the determination of antioxidant activity, but the values obtained cannot be compared because the chemical composition and the units of measurement are different, e.g. Bozin *et al*. [7] and Wang *et al*. [15] calculated IC_{50} against DPPH, with values obtained of 3.82 µL·mL^{-1} and 2.04 %, respectively); Tural and Turhan [12], Bajalan *et al*. [13] and Teneva *et al*. [14] determined values against DPPH, 21.31 %, 3.2 mg·mL^{-1} and from 81.7 to 82.8 %, respectively.

CONCLUSIONS

A comparative analysis of essential oils extracted by hydrodistillation and steam distillation from rosemary obtained by vegetative propagation originating from Iran, grown in the experimental field of the Institute of Roses, Essential and Medical Plants, Kazanlak, Bulgaria was carried out. The oils contain the same components but in different amounts, determining their different antioxidant activity. The use of these oils in perfumery and cosmetic products is limited (to 0.2 %) by the allergens identified in them. However, their use in food products is the subject of further study.

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