THE EFFECT OF DISTILLATION METHODS ON THE YIELD, COMPOSITION AND BIOLOGICAL ACTIVITY OF BASIL (*OCIMUM BASILICUM* L.) ESSENTIAL OIL

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(ORIGINAL SCIENTIFIC PAPER)
UDC 635.71:66.048:543.544.3
DOI 10.5937/savteh2202016D

Basil (*Ocimum basilicum* L.) is an aromatic plant, well known for its characteristic scent and healing properties, which has a wide application, from cooking to alternative medicine. The objective of this work was to evaluate the chemical composition, antimicrobial and antioxidant activity of basil essential oil obtained by hydrodistillation, microwave-assisted hydrodistillation and solvent-free microwave-assisted hydrodistillation. Gas chromatography analysis showed that the main components present in all three essential oils were linalool (30.3-58.2%) and epi-α-cadinol (5.6-7.3%). The distillation method mainly affected the content of terpenes, and aromatic compounds. The essential oils expressed good antimicrobial activity, most effective against *Escherichia coli* ATCC 25922, while the *Pseudomonas aeruginosa* ATCC 27853 was the most resistant strain. Good antioxidant activity was established after 120 min of incubation for all obtained essential oils with a significant difference regarding the applied distillation method. The results showed a great influence of the distillation method on the chemical composition, detected compounds, as well as antioxidant potential and antimicrobial activity of basil essential oils. The use of microwave assisted hydrodistillation has shown a significant difference in oil yield, energy consumption and environmental impact, which makes it a more suitable distillation process compared to conventional hydrodistillation.

Keywords: antimicrobial activity, antioxidant activity, essential oil, GC/MS, *Ocimum basilicum* L.

Introduction

Essential oils (EOs) represent a complex hydrophobic mixture of volatile compounds, which are formed as secondary metabolites of plants. Their characteristic odour enables the use of EOs in cosmetics, aromatherapy and gastronomy [1]. Due to their proven antibacterial, antifungal and antioxidant effects [2, 3, 4] and current trends in the food industry, their use in the process of food protection, as natural preservatives, is increasing [5, 6].

Various conventional and unconventional methods, such as steam distillation, supercritical fluid extraction and solvent extraction can be used for EOs isolation [1, 7]. The most common and traditional method of EOs extraction is hydrodistillation (HD) [8]. HD is a simple, safe and environmentally friendly EO extraction method with water as the solvent, which provides good-quality essential oils [9]. However, this method has several lacks as high energy consumption and exposure of the plant material to high temperatures which can cause the reduction of the extraction yield and oxidation of some compounds [10, 11]. In order to overcome these disadvantages, some innovative and novel “green” techniques should be considered.

A modification of the existing method of HD by additional microwave heating (microwave-assisted hydrodistillation (MAHD)) can represent a good alternative, since it increases the efficiency, reduces the processing time, accelerates the kinetics of the process, and provides better quality products [12, 13]. In MAHD, the limiting factor of the process is the ability of Eos` compounds to dissolve in water. Also, the efficiency depends on the dipolar moment of the extracted compounds, so aromatic compounds with a higher dipolar moment will react more energetically with the present microwaves, and their extraction will be faster compared to the compounds with a lower dipolar moment [14]. Good quality EOs can be obtained by solvent-free microwave distillation (SFMD) from the raw plant material without the presence of water or any organic solvent, and it is characterized as a “green method” successfully developed in laboratory conditions [13, 15].

The genus *Ocimum* L. belongs to the *Lamiaceae* family and includes over 150 different species, growing

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The manuscript received: January, 17, 2022.
Paper accepted: August, 22, 2022.

in the regions of Asia, Africa, Central and South America [16]. *Ocimum basilicum* L. or sweet basil represents an upright, branched plant with light green leaves, which can grow from 0.3 to 1.3 m [17]. It is widely used in gastronomy and food industry to improve the smell, taste and digestibility of food [18]. Its EO contains an abundance of active ingredients, whose presence and ratio depend on the growing conditions of the plant and the applied agrotechnical measures [16]. According to the literature data, basil EO mainly contains phenols (thymol, eugenol, methyl eugenol), alcohols (linalool), esters (methyl cinnamate), and oxide (1,8-cineole) [19, 20, 21]. The compounds responsible for the distinctive basil scent are 1,8-cineole, methyl cinnamate, methyl chavicol and linalool [22].

The aim of this research was to analyse the effect of different distillation methods on the chemical composition, antimicrobial and antioxidant activity of basil EO. For this purpose, hydrodistillation, microwave-assisted hydrodistillation and solvent-free microwave-assisted distillation were used for basil EO extraction.

Material and methods

Plant material

A raw *O. basilicum* L plant (moisture content 17.41±0.73%) was harvested in the area near Leskovac, Serbia. The harvest was carried out during the flowering period in August 2020. Drying of the plant material was performed for a period of 20 days at room temperature.

Solvent-free microwave distillation

Modified SFMD method [23] was performed to obtain basil EO (BEO1) from 150 g of the raw plant material subjected to the action of microwaves in a microwave oven (Beko, MWC 2000 MW, 800W) connected to a Clevenger apparatus, in a 500 ml flask bottle during 30 min.

Microwave hydrodistillation

Basil EO from 50 g of dried plant material mixed with 500 ml of distilled water (BEO2) was obtained by the modified MAHD method [23]. The distillation mixture was heated in a microwave oven (Beko, MWC 2000 MW, 800W) connected to a Clevenger apparatus for 30 min.

Conventional hydrodistillation

The basil EO obtained by conventional HD method (BEO3) on a Clevenger type apparatus was isolated from the mixture of 50 g of dry plant material and 500 ml of distilled water, where heating was performed on a heating mantle (HEAM-1K1-001, Labbox Labware S. L., Barcelona, Spain) during 120 min. The isolated EOs were stored at 4 °C until use.

Essential oil composition

Basil EOs composition was analysed by gas chromatography/mass spectroscopy (GC/MS) and gas chromatography/flame ionization detection (GC/FID) by the method already described by Stanojević et al., 2019 [25] and Vladimirov et al., 2019 [26]. GC/MS analysis was performed on Agilent Technologies 7890B gas chromatograph, on a nonpolar, silica capillary column, HP-5MS (5% diphenyl- and 95% dimethyl-polysiloxane, 30 m × 0.25 mm, 0.25 μm film thickness (Agilent Technologies, Santa Clara, CA, USA) connected to 5977A mass detector (Agilent Technologies, Santa Clara, CA, USA). Data were processed by MSD ChemStation, MassHunter Qualitative Analysis and AMDIS_32 software (Agilent Technologies, USA). The results are expressed as a percentage of the area of each peak compared to the total area of all the peaks in the run. It does not require prior calibration and does not depend upon the amount of sample injected within the limits of the detector. No response factors were used.

Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to determine the antioxidant activity of the obtained basil EOs, according to Akoto et al., 2020 [27], with some modifications. The extracted EOs were dissolved in ethanol (96%) and a series of dilutions of different concentrations (0.5 to 16 mg/ml) were prepared. An ethanol solution of DPPH radical ($3x10^{-4}$ mol/l) in the volume of 1 ml was added to 2.5 ml of EO solution of a certain concentration. Incubation of the samples was performed at 20, 60 and 120 min, and the absorbance (A_S) was measured at 517 nm on a spectrophotometer (2100 UV spectrophotometer, Cole-Parmer, Illinois, USA). Absorbance measurements at 517 nm were performed for both pure ethanol radical solution (control sample – A_C) and ethanol EOs solutions (blank sample - A_B). The degree of neutralization of free radical was calculated according to the following equation:

DPPH radical scavenging (%) =100 - $[(A_S-A_B) \times 100/A_C]$

The ability of EOs to neutralize free DPPH radicals was expressed as EC_{50} value (mg/ml), which represents the EOs concentration that neutralizes 50% of the radicals present during a certain incubation time.

Antimicrobial activity

Antimicrobial activity of obtained basil EOs was performed by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), according to Balouiri et al., 2016 [28]. Antimicrobial activity was determined by microdilution method against two Gram-positive strains: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 and four Gram-negative strains *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603. Dilutions of EOs ranged from 0.6 to 30 mg/ml. After incubation at 37 °C, results were recorded by EZ read 400 Elisa microplate reader (Biochrom, UK). EOs

concentrations that did not show growth of the tested microorganisms were transferred to the petri plates with Mueller-Hinton Agar ("Torlak", Belgrade, Serbia) and incubated for 48 h at 37 °C. The lowest concentration at which no growth was observed was recorded as the MBC value.

Energy consumption and CO_2 content

The energy consumption was recorded using the watt meter related to the input of the heating mantle and microwave oven [24]. The data used for the calculation of the carbon dioxide released into the atmosphere during distillations was found in the Electricity-Map database (2021) [29], which gives real-time data for CO_2 emissions of electricity consumption for almost a hundred countries around the world. According to this data, to generate 1 kWh of electricity from fossil fuels an average amount of 546 g CO_2 is released into the atmosphere.

Statistical analysis

All analyses were performed in triplicate and the results were shown as average value ± standard deviation. One-way ANOVA followed by Tukey's multiple comparison test was used to estimate statistically significant differences among the results in the software SPSS 21.0 (IBM, USA). Samples were considered significantly different when the *p*-value was lower than 0.05.

Results and discussion

Essential oils yield

EOs yields of the basil plant material were in the range of 0.37-1.07% (Table 1). The use of the MAHD method has proven to be more efficient than the conventional HD method, in a way of reducing the extraction time and resulting in a similar value of extraction yield. The lowest EO yield was recorded for BEO1 (0.37 %). Significantly higher ($p<0.05$) yield was obtained using MAHD from dry plant material (1.07%). The yield of BEO3 did not differ significantly ($p>0.05$) in relation to the yield of BEO2.

Table 1. Yields of essential oils extracted from basil plant material obtained by different extraction methods. Different letters indicate significant differences among the samples ($p<0.05$).

Sample	Yield (%)
BEO1	0.37 ± 0.05^{a}
BEO2	1.07 ± 0.11^{b}
BEO3	0.79 ± 0.08^{c}

BEO1 – Essential oil obtained from raw plant material by solvent-free microwave-assisted hydrodistillation; BEO2 – Essential oil obtained from dry plant material by microwave-assisted hydrodistillation; BEO3 – Essential oil obtained from dry plant material by Clevenger type hydrodistillation.

The obtained results are in accordance with the studies which indicated that the basil EO yield was in the range of 0.20-1.90% [16, 30]. Similar to this research, literature data showed that the use of microwaves, as a way of heating the system, reduced the extraction time achieving the same efficiency [7].

Some research showed that SFMD of basil EO gave higher yields of EOs, compared to MAHD [31, 32]. In addition, MAHD gave a low distillation yield of 0.38% [33]. A low yield of basil EO was also obtained by conventional HD (0.31%) [13]. The differences between the literature data and the results of this research can be explained by the fact that EO extraction efficiency may depend on the microwave power, extraction time and amount of water added [34, 35], as well as the type and age of plant material and its growing conditions [36].

Basil EOs composition

GC/MS analysis showed that the components of basil EOs can be classified into several groups: monoterpene hydrocarbons, oxygen-containing monoterpenes, sesquiterpene hydrocarbons, oxygen-containing sesquiterpenes and aromatic compounds (Table 2). The most abundant group of compounds in BEO1 were oxygen-containing monoterpenes contributing with 42.0%, followed by aromatic compounds (24.4%) and sesquiterpene hydrocarbons (23.8%) (Figure 1a, Table 2). The chemical composition of BEO2 included 62.5% oxygen-containing monoterpenes, 17.7% sesquiterpene hydrocarbons, 11.2% oxygen-containing sesquiterpenes and 6.4% aromatic compound (Figure 1b, Table 2). Finally, the main components identified in BEO3 were oxygen-containing monoterpenes (67.8%), followed by sesquiterpene hydrocarbons and oxygen-containing sesquiterpenes with 19.3% and 11.3% of contribution, respectively (Figure 1c, Table 2).

SFME method increased the extraction of monoterpene hydrocarbons (2.6% vs. 0.8% in HD) and sesquiterpene hydrocarbons (23.8% vs. 19.3% in HD). Their impact on the pleasant aroma of EOs is less important in comparison to the oxygen-containing compounds but they can exhibit antibacterial and antifungal properties [37]. On the other side, the efficiency of oxygen-containing monoterpenes and sesquiterpenes extraction via MAHD and HD were similar.

The most abundant component in all basil EOs analyzed in this study was linalool, contributing with 30.2-58.2%, regarding the method used for EOs isolation, which is in agreement with the study of Binello et al. (2014) [37]. The higher content of linalool was detected in the EOs extracted by MAHD from dry plant material (51.2%) compared to SFMD (30.3%). Linalool content decreased in the following order: BEO3 > BEO2 > BEO1. Linalool, in comparison to other essential oil components, has an appreciable solubility in water [39], which could be the reason for its high content in BEO3 and BEO2. However, the main disadvantage of HD is the degradation of unsaturated or ester compounds

through thermal or hydrolytic effects due to long extraction times. On the other side, microwave irradiation highly accelerates the extraction process, without causing considerable changes in the essential oil composition; due to easier degradation of plant cells caused by faster temperature arise [40]. According to the results obtained by Pavlić et al. (2018) [13], microwave irradiation could cause the decyclization of the phenol ring of carvacrol, then the migration of the hydroxylic group and finally lead to the formation of linalool [13]. This could also be the reason for higher linalool content in comparison to SFE. Additionally, higher content in BEO2 could be ascribed to the fact that drying of the plant material creates more space for the solvent to penetrate the cells and collect the secondary metabolites while the rate of biochemical reactions (enzymatic or metabolic alteration) is very poor [39].

On the other side, methyl chavicol content increased with the decrease of linalool content. It was not detected in BEO3 (Table 2), while its content in BEO2 was 2.4%. The highest methyl chavicol content (24.2%) was detected in BEO1. The presence and loss of some compounds in essential oils during distillation is not an uncommon situation. One of the most important variables is extraction time. Longer extraction times increase the chance of thermal and hydrolytic reactions of unsaturated and ester components present therein and the generation of their degradation products due to the local overheating. Water as a polar solvent can accelerate many reactions, especially those via carbocation as intermediates [41, 42, 43]. The conventional HD technique requires a large quantity of water and more time for EO isolation in comparison to MAHD and SFME. Thus, degradation could be the reason for the absence of methyl chavicol in BEO1. However, neither its possible hydrolytic products nor any kind of relationship with linalool could not be found in the literature available to the authors.

Regarding the linalool degradation, in the study of Binello et al. (2014) [37], the authors compared the results obtained with HD, in situ microwave-generated hydrodistillation and microwave hydrodiffusion and gravity techniques in the extraction of EO from dry plant material from *Lamiaceae* family (lavender, Greek oregano, sweet basil and sage). Linalool, eugenol and α-cadinol were the main components in the EO obtained from *Ocimum basilicum* L. dry leaves [37]. The highest content of terpinen-4-ol (1.0%) was detected in BEO3, containing linalool (58.2%) in the highest percentage.

Besides linalool, the main components present in BEO1 were methyl chavicol, germacrene D and epi-α-cadinol. Similar to this research, the main components of the EO obtained by SFMD from basil originating from Egypt were linalool and methyl chavicol [7]. On the other side, in the BEO3, besides linalool, there were present epi-α-cadinol and germacrene D i.e. epi-α-cadinol and 1,8-cineole in BEO2 (Table 2). On the other hand, the main components of basil EO obtained by HD originated from Turkey were methyl eugenol, α-cubebene, nerol and ε-muurolene [44]. Also, linalool was not detected in EOs obtained by HD from basil from Turkey [45] and Iran [46]. Different essential oils obtained from the same plant material support the hypothesis that the composition of essential oils may vary depending on the methods of extraction used. In addition, the basil EO obtained by supercritical fluid extraction contained a lower percentage of linalool (10.14%-16.60%), but also an increased content of eugenol and δ-cadinene, compared to EO obtained by HD [47].

Besides the extraction techniques discussed above, many other factors influence the chemical composition of the essential oils including climate, seasonal and geographic conditions as well as the harvesting period. These factors affect the exhibition of different biological activities between the EO obtained from the same species but from different geographic regions [48]. Variations in the chemical composition of EOs can be explained by the influence of different climatic and growing conditions [49, 50].

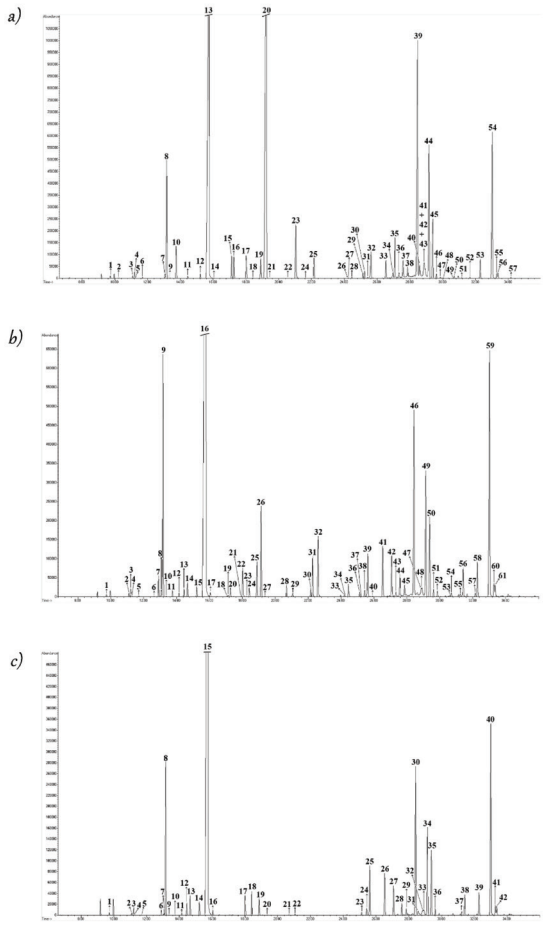

Figure 1. Chromatograms of basil essential oils: a) BEO1; b) BEO2; c) BEO3. BEO1 – Essential oil obtained from raw plant material by solvent-free microwave-assisted hydrodistillation; BEO2 – Essential oil obtained from dry plant material by microwave-assisted hydrodistillation; BEO3 – Essential oil obtained from dry plant material by Clevenger type hydrodistillation.

Table 2. The chemical composition of basil essential oils detected by GC/MS and GC/FID methods

No.	t_{ret}, min	Compound	RI^{exp}	RI^{lit}	Method of identification	Area% BEO1	BEO2	BEO3
1.	9.73	*α*-Pinene	935	923	RI, MS, Co-I	tr	tr	tr
2.	10.24	Camphene	949	946	RI, MS, Co-I	tr	-	-
3.	11.10	Sabinene	975	969	RI, MS	0.2	0.2	tr
4.	11.22	*β*-Pinene	978	974	RI, MS, Co-I	0.4	0.5	0.5
5.	11.27	1-Octen-3-ol	979	974	RI, MS	tr	tr	tr
6.	11.69	Mycrene	992	988	RI, MS	0.1	tr	tr
7.	12.63	*p*-Cymene	1018	1020	RI, MS	-	tr	0.3
8.	12.91	*o*-Cymene	1026	1022	RI, MS	-	tr	-
9.	13.07	Limonene	1030	1024	RI, MS, Co-I	0.3	0.3	0.3
10.	13.17	1,8-Cineole	1033	1026	RI, MS, Co-I	4.0	6.6	4.9
11.	13.37	(Z)-*β*-Ocimene	1039	1032	RI, MS	tr	tr	tr
12.	13.75	(E)-*β*-Ocimene	1049	1044	RI, MS	1.3	0.3	tr
13.	14.15	*γ*-Terpinene	1060	1054	RI, MS, Co-I	-	0.1	tr
14.	14.54	*cis*-Sabinene hydrate	1069	1065	RI, MS	0.1	0.3	0.6
15.	14.66	*cis*-Linalool oxide (furanoid)	1075	1061	RI, MS	-	0.5	0.8
16.	15.22	Terpinolene	1090	1086	RI, MS	0.3	-	-
17.	15.23	*trans*-Linalool oxide (furanoid)	1090	1084	RI, MS	-	0.5	0.7
18.	15.76	Linalool	1105	1095	RI, MS, Co-I	30.3	51.2	58.2
19.	16.05	1-Octen-3-yl acetate	1113	1110	RI, MS	tr	0.2	0.3
20.	17.06	trans-Sabinol	1142	1137	RI, MS	-	tr	-
21.	17.13	(E)-Epoxy-ocimene	1141	1137	RI, MS	1.0	tr	-
22.	17.27	Camphor	1148	1141	RI, MS, Co-I	0.8	0.3	-
23.	17.92	Pinocarvone	1166	1160	RI, MS	-	tr	-
24.	18.02	Borneol	1169	1165	RI, MS, Co-I	1.0	1.1	0.9
25.	18.26	*cis*-Linalool oxide (pyranoid)	1176	1173	RI, MS	-	tr	-
26.	18.42	Terpinen-4-ol	1180	1174	RI, MS	tr	0.3	1.0
27.	18.90	*α*-Terpineol	1194	1186	RI, MS, Co-I	0.9	1.0	0.7
28.	19.25	Methyl chavicol	1204	1195	RI, MS	24.2	2.4	-
29.	19.38	*trans*-Dihydro carvone	1208	1200	RI, MS	tr	tr	tr
30.	20.68	Carvacrol, methyl ether	1246	1241	RI, MS	tr	0.4	tr
31.	21.04	Geraniol	1257	1249	RI, MS	2.8	-	-
32.	21.07	Linalool acetate	1257	1254	RI, MS, Co-I	-	0.3	tr
33.	21.58	Geranial	1257	1249	RI, MS, Co-I	tr	-	-
34.	22.15	Bornyl acetate	1289	1287	RI, MS, Co-I	0.7	0.3	-
35.	22.27	Thymol	1293	1289	RI, MS, Co-I	-	1.2	-
36.	22.60	Carvacrol	1303	1298	RI, MS	-	1.8	-
37.	24.21	*α*-Terpinyl acetate	1353	1346	RI, MS	tr	0.1	-
38.	24.26	*α*-Cubebene	1354	1345	RI, MS	tr	tr	-
39.	24.44	Eugenol	1360	1356	RI, MS, Co-I	0.2	0.6	-
40.	25.13	*α*-Copaene	1381	1374	RI, MS	0.3	0.2	0.3
41.	25.24	Geranyl acetate	1385	1379	RI, MS	0.4	tr	-
42.	25.44	*β*-Bourbonene	1391	1389	RI, MS	tr	0.3	0.5
43.	25.63	*β*-Elemene	1397	1389	RI, MS	1.3	1.4	2.2
44.	25.91	Methyl eugenol	1406	1403	RI, MS	-	tr	-
45.	26.54	(E)-Caryophyllene	1426	1417	RI, MS	0.9	1.6	1.9
46.	26.96	*α*-trans-Bergamotene	1141	1132	RI, MS	0.3	-	-
47.	27.08	*α*-Guaiene	1444	1437	RI, MS	1.5	1.1	1.2
48.	27.33	trans-Muurola-3,5-diene	1453	1453	RI, MS	0.2	0.2	-
49.	27.58	*α*-Humulene	1461	1452	RI, MS	0.7	0.7	0.6
50.	27.86	*cis*-Muurola-4(14),5-diene	1470	1465	RI, MS	0.5	0.4	0.4
51.	28.46	Germacrene D	1489	1484	RI, MS	9.2	5.1	5.8
52.	28.59	*β*-Selinene	1494	1489	RI, MS	0.5	-	tr
53.	28.84	*α*-Selinene	1502	1498	RI, MS	tr	-	-
54.	28.88	Bycicloger macrene	1504	1500	RI, MS	1.0	0.3	tr
55.	28.94	Aciphyllene	1505	1501	RI, MS	0.3	0.2	tr
56.	29.16	*α*-Bulnesene	1514	1509	RI, MS	4.6	3.4	3.3
57.	29.39	*γ*-Cadinene	1522	1513	RI, MS	2.2	2.2	2.7
58.	29.62	*trans*-Calamenene	1530	1521	RI, MS	0.2	0.3	0.4
59.	29.86	*δ*-Cadinene	1538	1522	RI, MS	0.1	0.3	-
60.	30.05	*α*-Cadinene	1545	1537	RI, MS	tr	-	-
61.	30.62	*cis*-Muurol-5-en-4-α-ol	1566	1559	RI, MS	tr	tr	-
62.	30.71	(E)-Nerolidol	1569	1561	RI, MS	0.2	0.3	-
63.	31.16	Spathulenol	1587	1577	RI, MS	tr	0.2	0.4
64.	31.42	Caryophyllene oxide	1592	1582	RI, MS	tr	1.2	1.3
65.	32.16	Humulene oxide	1620	-	MS	-	0.3	.
66.	32.29	1, 10-di-epi-Cubenol	1625	1618	RI, MS	0.8	1.1	1.1
67.	33.03	epi-*α*-Cadinol	1648	1638	RI, MS	5.6	7.3	7.5
68.	33.30	*β*-Eudesmol	1659	1649	RI, MS	0.2	0.4	0.5
69.	33.37	*α*-Cadinol	1662	1652	RI, MS	0.3	0.4	0.5
70.	34.15	*α*-Bisabolol	1691	1685	RI, MS	tr	-	-
					Total identified (%)	99.9	99.4	99.8
					Grouped components (%)			
					Monoterpene hydrocarbons	2.6	1.4	0.8
					Oxygen-containing monoterpenes	42.0	62.5	67.8
					Sesquiterpene hydrocarbons	23.8	17.7	19.3
					Oxygen-containing sesquiterpenes	7.1	11.2	11.3
					Aromatic compounds	24.4	6.4	0.3
					Others	tr	0.2	0.3

t_{ret}: Retention time; RI^{lit}-Retention indices from literature [70]; RI^{exp}: Experimentally determined retention indices using a homologous series of n-alkanes (C8-C20) on the HP-5MS column; tr: trace. BEO1 – Essential oil obtained from raw plant material by solvent-free microwave-assisted hydrodistillation; BEO2 – Essential oil obtained from dry plant material by microwave-assisted hydrodistillation; BEO3 – Essential oil obtained from dry plant material by Clevenger type hydrodistillation.

Antioxidant activity

The antioxidant activity of obtained basil EOs was determined by the DPPH method, a simple method for rapid assessment of the ability to remove free DPPH radicals [7]. The results indicated that BEO1 had the highest EC_{50} value and therefore the lowest antioxidant activity, compared to the other two oil samples (Table 3). With the increase of incubation time, the EC_{50} values were significantly different (p<0.05) for all analysed EOs. BEO3 had the lowest EC_{50} value after 60 and 120 min of incubation, 0.63 and 0.44 mg/ml, respectively. Higher results of EC_{50} were stated for BEO2, 0.91 and 0.63 mg/ml, after 60 and 120 min, respectively. The obtained results for EC_{50} indicate stronger antioxidant activity than previously reported for basil EO obtained by HD after 60 min of incubation, 3.13 mg/ml, [51] and the values of 10-50 mg/ml for EOs extracted from different basil varieties [52]. On the other hand, other studies have shown a lower value for EC_{50} of basil EOs obtained by HD, 0.055 mg/ml [13], and 0.013 mg/ml [53]. Generally, the differences in antioxidant activity of EOs can be explained by different origins of the plant, extraction methods, experimental conditions, synergetic or antagonistic behaviour of EOs components and its interaction with the oxidizing material [54].

Table 3. Antioxidant activity of basil essential oils, obtained by DPPH assay

Sample	Time (min) 20	60	120
	EC_{50} (mg/ml)		
BEO1	6.69±0.09$^{a, A}$	3.44±0.03$^{b, A}$	2.68±0.02$^{c, A}$
BEO2	1.59±0.06$^{a, B}$	0.91±0.02$^{b, B}$	0.63±0.02$^{c, B}$
BEO3	2.72±0.05$^{a, C}$	0.63±0.03$^{b, C}$	0.44±0.02$^{c, C}$

BEO1 – Essential oil obtained from raw plant material by solvent-free microwave-assisted extraction; BEO2 – Essential oil obtained from dry plant material by microwave-assisted extraction; BEO3 – Essential oil obtained from dry plant material by Clavenger type of hydrodistillation. Different letters indicate statistically different (p<0.05) values in the same row (lowercase letters) and in the same column (uppercase letters).

Based on the results of antioxidant activity (Table 3) it can be observed that the distillation method significantly (p<0.05) affects the antioxidant potential of basil EOs. The lowest EC_{50} values were observed for EO extracted by HD (Table 3) in accordance with the study of Pavlić et al., 2018 [13] who reported that EO obtained by HD expressed stronger antioxidant activity compared to EO extracted by MAHD. On the other hand, other studies showed that the EO obtained by SFMD had a more pronounced antioxidant activity compared to EO obtained by HD [55].

The antioxidant activity of EOs is related to their chemical composition and can be roughly estimated through the content of phenols and unsaturated terpenes [54]. The results of antioxidant activity showed a good correlation with GC/MS results of EO's chemical composition. According to the GC/MS results, it can be stated that the antioxidant activity of basil EOs increased with the increase of the linalool concentration. The lowest antioxidant activity was observed in BEO1, probably as the result of the lower presence of linalool (Table 2) compared to BEO2 and BEO3. The use of linalool in pharmaceuticals and food has been widely studied due to its ability to prevent lipid oxidation and enhance the shelf life of the food [56-58].

Antimicrobial activity

The antimicrobial activity of obtained basil EOs was analysed against six bacterial strains, *B. subtilis, S. aureus, P. vulgaris, P. aeruginosa, E. coli* and *K. pneumoniae* (Table 4). Independently of the isolation method, all obtained EOs showed the highest antimicrobial activity against *E. coli.* In the research of Helal et al., 2019. [59] basil EO expressed significantly lower antimicrobial potential against *E. coli*, with a MIC value of 12.5 mg/ml, while the MBC value was 25 mg/ml. Similarly, the research of Mehdizadeh et al., 2016 [60] showed that the EO of basil cultivated in Iran had a lower effect on *E. coli*, with a MIC value of 10 mg/ml.

Based on the results (Table 4), *P. aeruginosa* and *K. pneumoniae* showed the greatest resistance to the action of these EOs, with a small difference between MIC and MBC values. According to the research of Rezzoug et al., 2019. [61] *P. aeruginosa* and *K. pneumoniae* also showed the highest resistance to the action of basil EO obtained by HD.

Table 4. Antimicrobial activity of basil essential oils.

Microorganism		BEO1	BEO2	BEO3
			mg/ml	
Bacillus subtilis	MIC	5.82	2.91	5.50
ATCC 6633	MBC	12.93	4.20	5.82
Staphylococcus aureus	MIC	10.35	7.76	11.64
ATCC 25923	MBC	13.93	7.76	14.23
Proteus vulgaris	MIC	9.05	5.82	5.82
ATCC 8427	MBC	10.35	7.11	5.82
Pseudomonas aeruginosa	MIC	12.93	12.93	14.23
ATCC 27853	MBC	14.23	15.52	15.52
Escherichia coli	MIC	4.53	3.23	3.23
ATCC 25922	MBC	5.82	3.88	4.53
Klebsiella pneumoniae	MIC	12.93	12.93	12.93
ATCC 700603	MBC	14.23	14.23	12.93

BEO1 – Essential oil obtained from raw plant material by solvent-free microwave-assisted hydrodistillation; BEO2 – Essential oil obtained from dry plant material by microwave-assisted hydrodistillation; BEO3 – Essential oil obtained from dry plant material by Clevenger type of hydrodistillation.

The mechanism of antimicrobial action of EOs can be explained by the fact that their lipophilic nature can stimulate cell membrane damage, disrupt pH homeostasis and cause an imbalance of inorganic cell ions [53]. The antimicrobial effects of different EOs depend on the Eos` composition, the functional groups within the main components, as well as their synergistic effects [62], such as the interaction of monoterpenoid alcohol and monoterpenoid phenol [63].

The antimicrobial effect of analysed EOs probably was the result of the high content of 1,8-cineole, methyl chavicol and epi-α-cadinol, but can also be attributed to other components of EOs. 1,8-cineole (eucalyptol) is a cyclic ether designed as the active agent of eucalyptus oil with antimicrobial activity [64]. Methyl chavicol (1-methoxy-4-prop-2-enylbenzene, estragole, or p-allylanisole) belongs to the class of phenylpropanoids and it has moderate antimicrobial activity [65]. Regarding the biological activity of epi-α-cadinol, the marked antimicrobial efficacy of white pepper EO is believed to, among other compounds, rely on the presence of a high percentage of sesquiterpenes like epi-α-cadinol [66]. Also, α-pinene and β-pinene are listed as important antimicrobial agents [66]. Some studies showed that the presence of linalool can cause a decrease in cell membrane permeability and facilitate the penetration of the active EOs components within the cytoplasm [67, 68]. A similar effect can be attributed to eugenol [69].

Energy consumption and CO_2 content

In addition to the advantages provided by MAHD compared to HD, regarding a shorter distillation time with higher extraction efficiency (Table 1), the use of MAHD is more favourable due to the reduction of costs but, also, possible environmental effects (Table 5).

Table 5. Energy consumption and released CO_2 for HD and MAHD

Parameter	HD	MAHD
Distillation time (min)	120	30
Energy consumption (kWh)	1	0.4
Released CO_2 (g)	546.0	218.4

HD – conventional hydrodistillation; MAHD – microwave-assisted hydrodistillation

The energy required for the distillation of basil EOs using HD was 1 kWh, while for MAHD was 0.4 kWh, which indicates a significantly lower cost. The use of electricity has a direct impact on the environment, through the release of CO_2 during the combustion process. Higher energy input also releases more CO_2, so during the MAHD more than half of the amount of CO_2 is released, compared to HD. For these reasons, MAHD represents a green technology, which successfully overcomes the disadvantages of HD.

Conclusion

The results of this study showed that the extraction method can significantly affect the chemical composition of basil essential oil. The distillation method resulted in a significant difference in the antioxidant activity and antimicrobial effect. Compared to solvent-free distillation and hydrodistillation, the largest number of compounds were identified in the basil essential oil obtained by microwave-assisted hydrodistillation. In addition to a significant reduction of process time, microwave-assisted

hydrodistillation also provided greater process efficiency, with reduced electricity consumption and significantly reduced release of CO_2 into the atmosphere. Therefore, this method seems to be a good alternative to the conventional hydrodistillation for obtaining basil essential oils. Additionally, the use of such obtained basil EO could be more focused on its ability to control microbial and lipid stability of some foods.

Acknowledgements

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant No. 451-03-68/ 2022-14/200133.

List of abbreviations

EO - essential oil
HD - hydrodistillation
MAHD – microwave-assisted hydrodistillation
SFMD – solvent-free microwave distillation
BEO1 – essential oil obtained from raw plant material by solvent-free microwave-assisted hydrodistillation
BEO2 – essential oil obtained from dry plant material by microwave-assisted hydrodistillation
BEO3 – essential oil obtained from dry plant material by Clevenger type hydrodistillation
GC/MS - gas chromatography/mass spectroscopy
DPPH - 2, 2-diphenyl-1-picrylhydrazyl
EC_{50} – the essential oil concentration that neutralize 50% of the radicals
MIC - minimal inhibitory concentration
MBC - minimal bactericidal concentration

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Izvod

UTICAJ METODE DESTILACIJE NA PRINOS, HEMIJSKI SASTAV I BIOLOŠKU AKTIVNOST ETARSKOG ULJA BOSILJKA (*OCIMUM BASILICUM* L.)

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(ORIGINALNI NAUČNI RAD)
UDK 635.71:66.048:543.544.3
DOI 10.5937/savteh2202016D

Bosiljak (*Ocimum basilicum* L.) predstavlja aromatičnu biljku, prepoznatljivu po karakterističnom mirisu i lekovitim svojstvima, koja ima široku primenu, od kulinarstva do alternativne medicine. Cilj ovog rada bio je proceniti hemijski sastav, antimikrobnu i antioksidativnu aktivnost etarskih ulja bosiljka dobijenih hidrodestilacijom, mikrotalasnom destilacijom i destilacijom bez rastvarača u prisustvu mikrotalasa. Metoda gasne hromatografije pokazala je da su glavne komponente prisutne u dobijenim uljima linalol (30.3-58.2%) i epi-α-kadinol (5.6-7.3%). Metoda ekstrakcije uticala je na sadržaj terpenskih, kao i aromatičnih jedinjenja. Etarska ulja su pokazala dobru antimikrobnu aktivnost, najviše izraženu prema *Escherichia coli* ATCC 25922, a najmanje prema *Pseudomonas aeruginosa* ATCC 27853. Dobra antioksidativna aktivnost zabeležena je posle 120 minuta inkubacije za sva tri dobijena ulja, sa značajnom razlikom u odnosu na primenjenu metodu destilacije. Rezultati su pokazali značajan uticaj metode destilacije na hemijski sastav, detektovana jedinjenja, kao i na antioksidativni potencijal i antimikrobnu aktivnost etarskih ulja bosiljka. Upotreba hidrodestilacije u prisustvu mikrotalasa pokazala je značajnu razliku u pogledu prinosa ulja, potrošnje energije i uticaja na životnu sredinu, što je čini pogodnijom metodom destilacije u odnosu na konvencionalnu hidrodestilaciju.

Ključne reči: antimikrobna aktivnost, antioksidativna aktivnost, etarsko ulje, GC/MS, *Ocimum basilicum* L.