COMPARATIVE ANALYSIS OF ANTIBACTERIAL ACTIVITY AND PHYSICOCHEMICAL PROPERTIES OF PEPPERMINT AND CORNMINT ESSENTIAL OILS AND THEIR MAIN COMPOUND MENTHOL

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ABSTRACT

The aim of the present paper was to study and present a comparative analysis of the antibacterial activity and physicochemical properties of commercial mint essential oils from two different species - peppermint (Mentha piperita Huds. (L.)) and cornmint (Mentha arvensis L.). Peppermint oils exhibited weak antibacterial activity, but were more pronounced against Gram-positive bacteria Staphylococcus aureus (1.3 - 2.0 mm) and Gram-negative bacteria Escherichia coli (1.2 - 1.9 mm) and Klebsiella sp. (2.6 mm). The essential oil of the species M. arvensis did not exhibit antimicrobial activity against the tested cultures. L-menthol, which is a commercial sample isolate, exhibited activity against all tested microorganisms, with the exception of Gram-positive bacteria Listeria monocytogenes and Bacillus cereus. The diameter of the inhibition zones was the largest against Gram-positive bacteria Bacillus subtilis (3.4 mm), and the smallest was against Gram-positive bacteria Staphylococcus aureus (1.6 mm) and Gram-negative bacteria Klebsiella sp. (1.4 mm). The surface tension, density and refractive index of different mint essential oils were determined experimentally. The surface energy and surface heat capacity were calculated based on the calculations of surface tension. All experiments and calculations were provided at a temperature range between 6°C and 30°C. A dependence between surface tension and temperature was not observed.

Keywords: peppermint, cornmint, essential oils, menthol, antibacterial activity, physicochemical properties.

INTRODUCTION

Peppermint oil is obtained from the flowering aerial parts of the genus *Mentha* of the family Lamiaceae. Four species are known to be cultivated for oil extraction: *Mentha piperita* (L.) Huds. - common or spicy mint, *M. arvensis* L. - cornmint (Chinese, Japanese, Brazilian), *M. spicata* Huds. = *M. viridis* L. - spearmint and *M. pulegium* L. - pennyroyal. The main component of their essential oils obtained from the first two Mentha species is the monoterpene alcohol menthol, in the third - the monoterpene ketone carvone, and in the fourth - the monoterpene ketone pulegone [1, 2].

Peppermint is grown in Central Europe, North America, South Asia. In our country, its cultivation dates back to the beginning of the 20th century. Bulgarian peppermint is an easy mobile transparent, yellow to yellow-greenish liquid with a characteristic minty smell and a cooling, non-bitter taste [2].

Various components have been identified in the oil, mainly *l*-menthol (about 40 – 45 %), l-menthone, menthyl acetate and menthofuran, which mainly determine its smell and taste. The amount of these components varies depending on the harvesting the raw material phase, the ratio between leaves, stems and inflorescences, climatic conditions and characteristics between varieties [3 - 6]. The essential oil has antimicrobial [4 - 9] and other biological properties [1, 10], which is why it is used in the food industry [1, 2, 11], medicine [1, 2, 10], cosmetics [12, 13], as well as for the isolation of menthol [2].

Cornmint is grown in countries of East Asia, North and South America, and Eastern Europe. The essential oil is a light yellow to yellow mobile liquid that freezes at a slight decrease in temperature due to the high menthol content. It has a strong minty smell and a cooling-bitter taste. Some of the menthols are separated, and the dementholated oil is more commonly found on the market. The dementholated oil is an almost colourless to pale yellow mobile liquid, with a solid and characteristic minty odour and a cooling moderately bitter taste. The composition of the crude oil includes the components mentioned in peppermint, but the total menthol is much more (up to 85 % - 90 %). Dementholized oils have less total menthol (about 40 % - 45 %), and the ratio of components varies depending on the origin and degree of dementholization. Dementholated oil is an additive to peppermint oil, and on its own, it is a cheap substitute in cosmetics, food industry, and pharmacy [2].

Menthol (*p*-menthan-3-ol, 1-methyl-4-isopropyl-cyclohexan-3-ol, hexahydrothymol) has four isomers: menthol, neo-, iso-, and neo-isomenthol, each of which has optically active forms (*d*) and (*l*) and a racemic mixture. L-menthol, which is a crystalline substance with a strong, fresh, cooling minty odour and a pungent, cooling taste, is used in cosmetics, the food industry, and medicine. For industrial purposes, it is isolated from the oils of peppermint and cornmint, but it could also be obtained synthetically [1, 2, 13].

In the literature, data on the physicochemical characteristics of essential oils are quite scarce, despite the fact that they are important in the processing of essential oil plants, as well as in the storage and transportation of essential oils.

Therefore, the aim of the present work was to compare the antibacterial activity and physicochemical parameters of commercial peppermint (*M. piperita*) and cornmint (*M. arvensis*) essential oils, which have different origin and menthol content than commercial sample of *l*-menthol.

EXPERIMENTAL

Materials

The materials used are:

- two commercial peppermint essential oils. One sample was provided by a company producing essential oils in Southern Bulgaria (*sample 1*), and the other - by a company in North-Eastern Bulgaria (*sample 2*).

- one commercial essential oil of cornmint, provided by a company in North-Eastern Bulgaria (*sample 3*).

- *l*-menthol - isolate from the company Symrise, Germany.

Methods

Determination of antibacterial activity

The microorganisms used in this study were most often found in food and cosmetic products, are also causative agents of various infections and diseases: Gram-positive bacteria: *Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* ATCC 25093, *Bacillus subtilis* ATCC 6633, and Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *Enterica serovar Abony* NCTC 6017. They were provided by National Bank for Industrial Microorganisms and Cell Cultures, Sofia. The Gram-positive bacteria *Bacillus cereus* and Gram-negative bacteria *Klebsiella* sp., were clinical isolates and were from the collection of the Department of Tourism and Culinary Management (University of Food Technologies, Plovdiv).

Antibacterial activity was determined by modifying the agar diffusion method by measuring the inhibition zones of pathogen growth ($1x10^4$ CFU mL^{-1}) around metal rings (Ø = 6 mm) in which a certain amount of test material was imported [14].

Determination of physicochemical properties

The density, refractive index and molar refraction were determined by Yankova [15]. Their temperature dependencies were represented by van der Waals and Guggenheim [16]. The two indicators surface energy and surface heat capacity were calculated from the obtained values for surface tension [16]. All physicochemical indicators were calculated at six different temperatures (6°C, 10°C, 15°C, 20°C, 25°C and 30°C). At the first temperature, the essential oils were stored and the rest they can be used in various food products.

Statistical analysis

The provided experiments were repeated three times. For data analysis ± standard error was used. The differences were considered statistically significant of $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition

The studied essential oils differ in chemical composition and content of the main component menthol [17]:

- Essential oil obtained from *M. piperita* (*sample 1*) contained menthol (40.84 %), *neo*-menthol (4.80 %), and iso-menthone (4.53 %), with a total content of oxygen derivatives of monoterpene compounds 92.86 %.

- Essential oil from *M. piperita* (*sample 2*) contained menthol (34.90 %) and neo-menthol (3.43 %), with a total content of oxygen derivatives of monoterpene compounds 85.20 %.

- Essential oil from *M. arvensis* (*sample 3*) contained menthol (30.35 %) and neo-menthol (4.36 %), with a total content of oxygen derivatives of monoterpene compounds 79.07 %. A low menthol content is an indication that it is a dementholized oil.

It is known that in essential oils, the compounds have different functional groups. The oxygen derivatives - phenols, followed by alcohols, aldehydes, ketones and esters are with the highest biological activity, and with the lowest are hydrocarbons [18]. The distribution of compounds by groups in the three studied essential oils of peppermint is presented in Fig. 1. The data shows a different ratio of oxygen-containing compounds, with alcohols being the highest, followed by ketones, esters and oxides.

Antibacterial activity

The antimicrobial activity of the mint essential oils and *l*-menthol against the studied pathogenic bacteria is presented in Table 1.

The data demonstrated that the essential oils of *M. piperita* showed very low antimicrobial activity. The essential oil of the first sample showed antibacterial activity only against two test cultures, the diameter of the inhibition zones being 2.0 mm against Gram-positive bacteria *S. aureus* and 1.2 mm against Gram-negative bacteria *E. coli.* The essential oil of *sample 2* exhibited antibacterial effect against three test microorganisms, the diameter of the zones of inhibition against Gram-positive bacteria *S. aureus* was 1.3 mm, against Gram-negative bacteria *E. coli* was 1.9 mm, and against *Klebsiella* sp. was 2.6 mm.

The essential oil of the species *M. arvensis* (*sample 3*) did not exhibit antimicrobial activity against the tested

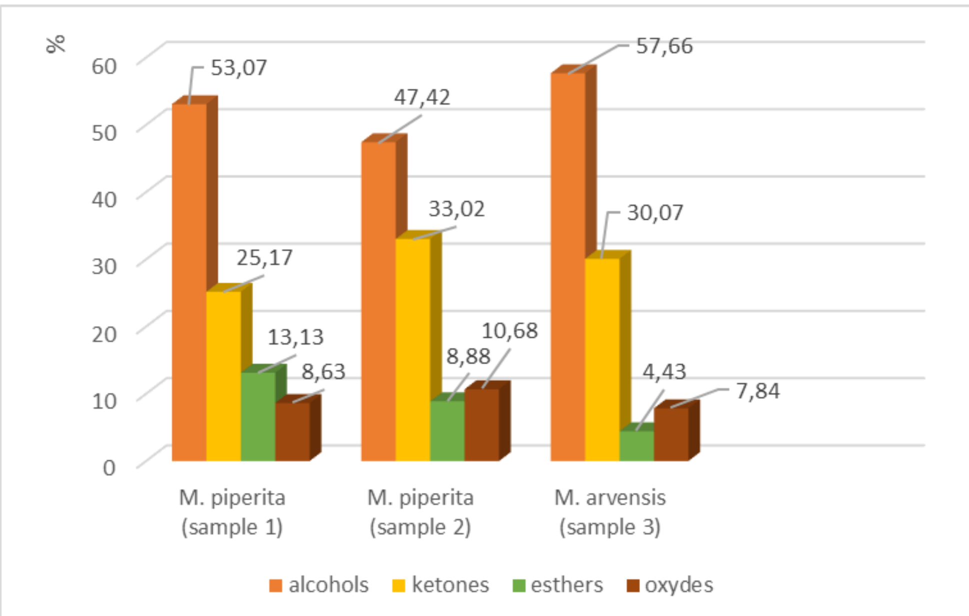

Fig. 1. Group of oxygenated components in peppermint and cornmint essential oils, %.

Table 1. Zones of inhibition of the growth of pathogenic bacteria of peppermint and cornmint essential oils and *l*-menthol.

Test-microorganisms	Diameter of zones of inhibition, mm			
	M. piperita (*sample 1*)	*M. piperita* (*sample 2*)	*M. arvensis* (*sample 3*)	*l*-menthol
Listeria monocytogenes	-*	-	-	-
Staphylococcus aureus	2.0 ± 0.01	1.3 ± 0.01	-	1.6 ± 0.01
Bacillus subtilis	-	-	-	3.4±0.03
Bacillus cereus (clinical isolate)	-	-	-	-
Escherichia coli	1.2 ± 0.01	1.9 ± 0.01	-	2.5 ± 0.02
Salmonella enterica subsp. *Enterica serovar Abony*	-	-	-	2.6 ± 0.02
Klebsiella (clinical isolate)	-	2.6 ± 0.02	-	1.4 ± 0.01

** no inhibitory activity was detected*

Table 2. Density and refractive index for peppermint and cornmint essential oils.

Temperature, °C	Density, kg m^{-3}			Refractive index, n_D		
	M. piperita		*M. arvensis*	*M. piperita*		*M. arvensis*
	sample 1	*sample 2*	*sample 3*	*sample 1*	*sample 2*	*sample 3*
6	0.901 ± 0.03	0.909 ± 0.18	0.921 ± 0.11	1.457 ± 0.07	1.458 ± 0.11	1.458 ± 0.08
10	0.897 ± 0.11	0.907 ± 0.11	0.906 ± 0.16	1.452 ± 0.04	1.453 ± 0.04	1.452 ± 0.12
15	0.895 ± 0.08	0.905 ± 0.15	0.892 ± 0.12	1.447 ± 0.16	1.448 ± 0.08	1.447 ± 0.06
20	0.892 ± 0.12	0.904 ± 0.07	0.881 ± 0.11	1.442 ± 0.12	1.444 ± 0.09	1.441 ± 0.11
25	0.889 ± 0.14	0.899 ± 0.09	0.866 ± 0.11	1.437 ± 0.09	1.439 ± 0.13	1.436 ± 0.06
30	0.886 ± 0.08	0.894 ± 0.04	0.851 ± 0.21	1.433 ± 0.13	1.434 ± 0.14	1.429 ± 0.09

test cultures.

L-menthol exhibited activity against all tested microorganisms, with the exception of Gram-positive bacteria *L. monocytogenes* and *B. cereus*. The diameter of the inhibition zones was the largest against Gram-positive bacteria *B. subtilis* (3.4 mm), and the lowest against Gram-positive bacteria *S. aureus* (1.6 mm) and Gram-negative bacteria *Klebsiella* (1.4 mm). Compared to the other two test microorganisms, the results were comparable.

The weak antibacterial activity of the investigated commercial samples of essential oils is probably due to the lower content of the main component menthol compared to the samples investigated from other authors obtained in the literature data. This can be explained by the origin of the plants in the type of peppermint - from Northern and Southern Bulgaria, as well as by the removal of part of the menthol in the cornmint, which is not cultivated in our country.

Differences in the antimicrobial activity of the peppermint and cornmint essential oils in this study and those reported in the literature could be explained by the differences obtained in the chemical composition of the oil, which depends on climatic and soil conditions, found in various essential oil plants [1].

Physicochemical properties

Surface tension, density and refractive index

Peppermint and cornmint essential oils were investigated by different methods. First, the surface tension was determined using maximum bubble pressure method. After that, the experimental density and refractive index of essential oil for two years were determined.

The experimental data of surface tension, density, and refractive index are presented in Table 2 and Figs. 2, 3 and 4. Surface tension decreases in a very small range. Density and refractive index presented values comparable in literature [19]. They decrease with an increase in temperature. Normally as the temperature

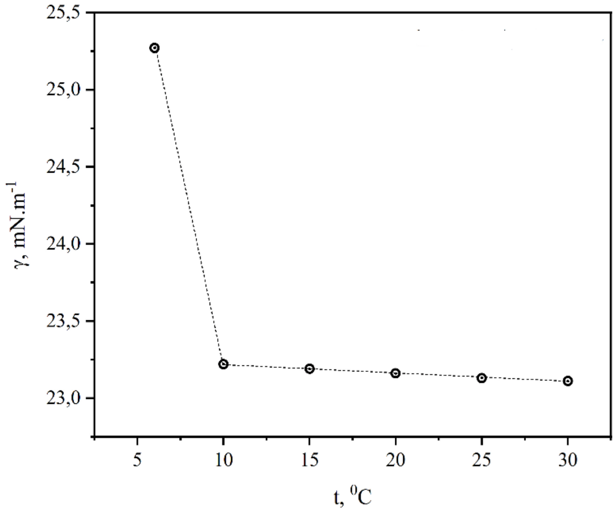

Fig. 2. Temperature dependence between surface tension and temperature for peppermint essential oil (*sample 1*).

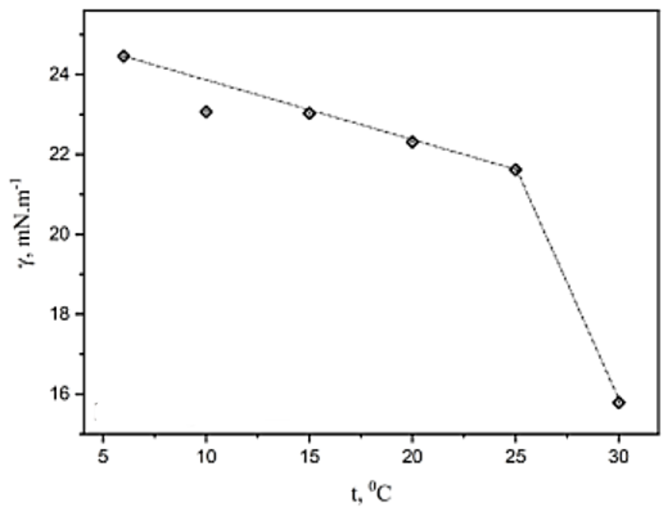

Fig. 3. Temperature dependence between surface tension and temperature for peppermint essential oil (*sample 2*).

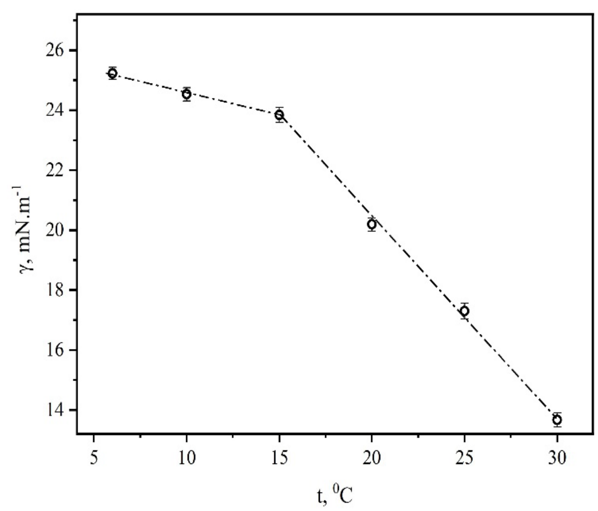

Fig. 4. Temperature dependence between surface tension and temperature for cornmint essential oil (*sample 3*).

Table 3. Surface energies, surface capacity and molar refraction for peppermint and cornmint essential oils.

Temperature, °C	Surface energies, mN m^{-1}			Surface capacity, N m^{-1}.K^{-1}			Molar refraction ($\times 10^6$), m^3 mol^{-1}		
	M. piperita		*M. arvensis*	*M. piperita*		*M. arvensis*	*M. piperita*		*M. arvensis*
	sample 1	*sample 2*	*sample 3*	*sample 1*	*sample 2*	*sample 3*	*sample 1*	*sample 2*	*sample 3*
6	78.11 ± 0.03	125.23 ± 0.15	159.49 ± 0.19	4679.69 ± 0.08	6974.13 ± 0.14	8979.95 ± 0.15	38.42 ± 0.11	38.49 ± 0.09	37.77 ± 0.17
10	77.02 ± 0.12	125.29 ± 0.09	160.73 ± 0.13	4746.75 ± 0.15	7074.04 ± 0.08	9108.62 ± 0.18	37.85 ± 0.05	37.92 ± 0.09	36.74 ± 0.26
15	77.94 ± 0.05	127.05 ± 0.15	162.44 ± 0.14	4830.57 ± 0.06	7198.96 ± 0.16	9269.47 ± 0.19	37.23 ± 0.13	37.32 ± 0.12	35.82 ± 0.12
20	78.86 ± 0.06	128.14 ± 0.18	161.20 ± 0.12	4914.39 ± 0.09	7323.87 ± 0.13	9430.31 ± 0.11	36.61 ± 0.11	36.75 ± 0.15	34.97 ± 0.14
25	79.78 ± 0.09	129.25 ± 0.09	160.71 ± 0.11	4998.21 ± 0.11	7448.79 ± 0.18	9591.16 ± 0.11	35.69 ± 0.08	35.84 ± 0.08	34.03 ± 0.14
30	80.71 ± 0.11	125.23 ± 0.13	159.48 ± 0.19	5082.03 ± 0.13	7573.70 ± 0.07	15025.82 ± 0.15	35.09 ± 0.11	35.17 ± 0.07	32.98 ± 0.12

increases, the surface tension decreases. For many substances, this dependence is linear to near-critical temperatures. At the critical temperature the surface tension becomes equal to zero, and the densities of the liquid and gas phases equalize [16].

Surface energy, surface heat capacity and molar refraction

Table 3 presents results for surface energies, surface capacity and molar refraction for peppermint and cornmint essential oils.

The energies presented very small differences in values at different temperatures because the temperature dependence was determined between surface tension and the temperature gradient of surface tension. The surface energy was calculated as a sum between them [16]. The data show that the values for *sample 1* were almost twice as low as those for *sample 3*, which can be explained by the different chemical compositions. Differences were also found between the values of *sample 1* and *sample 2*.

According to literature data when the heat capacities are positive the system is determined as stable [16]. In the present work for three samples, the heat capacities are positive too. Good temperature dependence between surface capacity and temperature was observed in *sample 3*. For *samples 1* and *2* heat capacity is as small as values connected with the bad connection between surface tension and temperature.

The data show that the values for molar refraction decrease with increasing the temperature. A similar trend was found with coriander oil, although water-alcohol solutions of the oil were studied ($11.21.10^{-6}$ - $14.99.10^{-6}$ m^3 mol^{-1}) [19]. It is known that molar refraction is related to polarization effects in molecules that can be caused in the electromagnetic spectrum. Relationships have been found between molar refraction and surface tension at temperatures sufficiently distant from the critical temperature [20]. A number of authors have studied the temperature dependence of density and refractive indices in both various aromatic substances [21, 22] and vegetable oils [23, 24]. However, a comparison of the data is not possible due to their different chemical composition.

The established differences in the values of the studied samples of commercial peppermint essential oils can be explained both by their different chemical composition and by their origin and year of processing of the plants, also found in other essential oil plants [1, 2].

CONCLUSIONS

The comparative study conducted between three samples of commercial mint essential oils shows that those from the type of peppermint (*M. piperita*) had weak antibacterial activity compared to the tested test cultures, and those from cornmint (*M. arvensis*) did not exhibit antibacterial activity. L-menthol had a more pronounced antibacterial activity compared to the tested essential oils. On the investigated peppermint and cornmint essential oils some physicochemical parameters were determined for the first time at six different temperatures from 6 to 30°C with a step of 5°C.

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