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ESSENTIAL OIL FROM LAURACEAE AND ROSACEAE / β-CYCLODEXTRIN COMPLEXES

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ABSTRACT:
The paper presents the synthesis and characterization of some essential oil from Lauraceae and Rosaceae botanical families / β-cyclodextrin complexes. Essential oils from *Cinnamomum cassia* L. from Lauraceae family and *Rosa damascena* L. from Rosaceae family were used for cyclodextrin complexation by using the crystallization from ethanol-water solution. The complexes were crystallized by a program temperature and the complexes were filtered, washed with ethanol and dried at room temperature. The yields of complex recovering were 67% and 72%, respectively. Cyclodextrin complexes were characterized by gas chromatography-mass spectrometry in order to identify and quantify the main volatile compounds from raw essential oil and from the essential oil recovered from the complex. The main compound from *Cinnamomum* essential oil was cinnamaldehyde, which was in a relative concentration of 62.3% in the raw oil and the ratio between the relative concentrations in recovered and raw essential oils was 1.1. The most important compound for the rose flavor was β-phenylethanol, which was identified in a concentration of 27.8% in the raw essential oil; the corresponding concentration ratio was 0.6. The formation of complexes was also demonstrated by thermogravimetric analysis; the mass loss in the *Cinnamomum cassia* and *Rosa damascena* essential oils/β-cyclodextrin complexes was 9.7% and 7.7%, respectively, while the commercial β-cyclodextrin reveals a mass loss of 14.1%.

KEYWORDS:
β-cyclodextrin, essential oils, *Cinnamomum cassia* L., *Rosa damascena* L., nanoencapsulation, thermogravimetry, gas chromatography-mass spectrometry

1. INTRODUCTION

Cyclodextrins are cyclic oligosaccharides consist of several glucopyranose units (6, 7, and 8 for the natural α-, β-, and γ-cyclodextrin, respectively) and having a structural architecture such as a truncated cone [1,2]. The hydroxyl groups from the glucopyranose moieties are located in the exterior, the primary ones being located on one side of the structure (the primary face), while the secondary groups forms the secondary face of cyclodextrin. These outer hydroxyl groups furnish the water solubility of cyclodextrins, while the inner cavity remain hydrophobic and can encapsulate (molecular encapsulation, molecular inclusion) hydrophobic geometrically compatible compounds [2-5]. The host-guest complex have many advantages: hydrosolubilizing of hydrophobic bioactive molecules, protection

against degradation by action of oxygen/air, light, humidity, controlled release of biocompounds, obtaining solid powders from liquid biological systems [4].

Essential oils are valuable bioactive materials, having pharmaceutical and odorant properties. They are volatile compounds and are susceptible to degradation in the presence of above mentioned factors. From the huge number of essential oils, those obtained from Lauraceae and Rosaceae have valuable properties. *Cinnamomum* species have antiviral, antidiabetic, and anticarcinogenic effects [6-12]; some studies reveal that *C. cassia* have important anti-HIV properties. The *Cinnamomum* bark is used as spice all over the world, the essential oil being 0.5-1% from the bark [7]. The main compounds are cinnamaldehyde, ethyl cinnamate, eugenol (mostly in leaves), and sesquiterpenes. On the other hand, *Rosa damascena* essential oil has antibacterial activity [13]. Among the volatile compounds [14] from *R. damascena* (*i.e.* β-phenylethanol), this plant contains also flavonoids (kaempferol and quercetin), with important antioxidant activity [15].

In the present study the synthesis and characterization of *Cinnamomum cassia* L. and *Rosa damascena* L. essential oils/β-cyclodextrin complexes were performed. The main volatile compounds with pharmaceutical properties from the essential oils were evaluated by means of the competitivity to the encapsulation in β-cyclodextrin.

2. MATERIALS AND METHOD

Materials. Essential oils from *Cinnamomum cassia* L. (Lauraceae family) and *Rosa damascena* L. (Rosaceae family) were achieved from SC Fares SA Orăştie and β-cyclodextrin used for nanoencapsulation was obtained from Merck&Co., Inc., New Jersey. Alkane standard solution C_8-C_{20}, used for the calculation of the Kovats index, was obtained from Fluka Chemie AG. Ethanol 96% (v/v, Chimopar, Bucharest) and hexane (GC grade, Fluka) were also used for synthesis and analysis of essential oils and their β-cyclodextrin complexes.

Synthesis of essential oils/β-cyclodextrin complexes. The essential oil / β-cyclodextrin complexes were obtained by crystallization from ethanol-water solution. Thus, the β-cyclodextrin sample was dissolved (suspended) in distilled water at 50°C and the ethanolic solution of essential oil (corresponding to a cyclodextrin : main essential oil compound (cinnamaldehyde or β-phenylethanol) ratio of 1:1) was dropped into the complexation reactor for 15 minutes under continuous stirring. The mixture was stirred at the same temperature for another 15 minutes and after that the slow crystallization process was started (controlled cooling from 50°C to 20°C with cooling rate of 0.125°C/min. The crystallization process was continued by maintaining the complex suspension at 4°C over night. The complex crystals were filtered in vacuum, washed with ethanol, and dried at room temperature. The complex recovering yields were obtained as the ratio between the final complex mass and the sum of the starting cyclodextrin and essential oil masses (Table 1).

Table 1. Quantities of essential oils and β-cyclodextrin, as well as the yields of complexation process for *C. cassia* and *R. damascena* essential oil/β-cyclodextrin complexes.

N°	*Complex*	$m_{ess.\ oil}$ *(mg)*	$m_{\beta CD}$ *(mg)*	$m_{compl.}$ *(mg)*	*Yield (%)*
1	*C. cassia L. ess. oil / β-cyclodextrin*	*69*	*673*	*500*	*67.4*
2	*R. damascena L. ess. oil / β-cyclodextrin*	*116*	*671*	*569*	*72.3*

Recovering of the essential oil from complex. The recovering of the essential oil from complexes was performed by multiple extraction in hexane. Thus, the essential oil / β-cyclodextrin complex (0.1 g) was dissolved in 4 mL of water in a thersomstated extractor. Two mL of hexane was added to the cyclodextrin complex solution and the heterogeneous mixture was stirred for 20 minutes at 60°C; the extraction mass was then cooled to room temperature and the organic layer was separated; the aqueous residue was extracted with the same hexane volume for another three times in the same manner. All hexane extracts were dried over anhydrous calcium chloride and analyzed by GC-MS.

Gas chromatography – mass spectrometry (GC-MS) analysis. The GC-MS analysis of the raw essential oils and those recovered from the cyclodextrin complexes was performed by using a Hewlett Packard HP 6890 Series gas chromatograph device coupled with a Hewlett Packard 5973 Mass Selective Detector with a calibration factor of 1.0. The following GC parameters were used: column HP-5 MS (length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm), temperature program of 50°C to 250°C with a heating rate of 4°C/min, both injector and detector temperatures of 280°C, injection volume of 2 μL; He was used as flow gas. An energy of 70 eV was used for the MS analysis, with a source temperature of 150°C, a scanning range of 50-300 amu, and a scanning rate of 1 s^{-1}. The experimental MS spectra were compared with those from the NIST/EPA/NIH Mass Spectral Library 2.0 (2002). The acquisition and handling of the data was performed by using the Hewlett Packard Enhanced ChemStation G1701BA ver. B.01.00/1998 soft and Hewlett Packard Enhanced Data Analysis program, respectively. Further, the Kovats indices (KI) for all separated compounds were calculated by using the GC data of a C_8-C_{20} alkane standard mixture in the same conditions.

Thermogravimetric (TG) analysis. The behavior of essential oil / cyclodextrin complexes among the heating can reveals the nanoencapsulation process. The TG analysis was performed by using a TG 209 Netzsch device in the following conditions: temperature program of 20-200°C with a heating rate of 4°C/min, followed by a higher heating rate of 10°C/min for the range of 200-900°C. All samples were analyzed under nitrogen atmosphere. The acquisition and data handling were performed by using the Netzsch Proteus-Thermal Analysis ver. 4.0 / 2000 program.

3. RESULTS AND DISCUSSION

Essential oils/β-cyclodextrin complexes were obtained with good yields (evaluated as the recovered complex in relation with the starting essential oil and cyclodextrin). Thus, *C. cassia* essential oil/β-cyclodextrin complex was obtained with a yield of 67.4%; in the case of *R. damascena* essential oil/β-cyclodextrin complex this yield was little bit higher (72.3%) (Table 1).

The complexes were analyzed by thermogravimetry in order to evaluate the content of encapsulated volatile compounds (including water) and establish quality of complexation process. Thus, the *C. cassia* essential oil / β-cyclodextrin complex reveals a mass loss of 8.5% up to 100°C and 3.4% up to 220°C; the first mass loss is corresponding to the loss of water as well as the loss of some volatile compounds from encapsulated essential oil, while the second mass loss is due to the dissociation of the main volatile compounds from the complex (Fig. 1). In the case of *R. damascena* essential oil / β-cyclodextrin complex these TG values are 5.95% for the first interval (corresponding to water dissociation) and 2.4% for the second one, which is corresponding to the volatile compounds release from the complex (Fig. 2). The β-cyclodextrin release water up to 100°C with a mass loss of 14.1% and no

significant mass loss was observed for higher temperatures. The reducing of water mass loss (5.5-8% lowered values for the water content in complexes in comparison with the water concentration in β-cyclodextrin) in β-cyclodextrin complexes and the release of volatile compounds at higher temperatures demonstrates the formation of essential oil / β-cyclodextrin complexes.

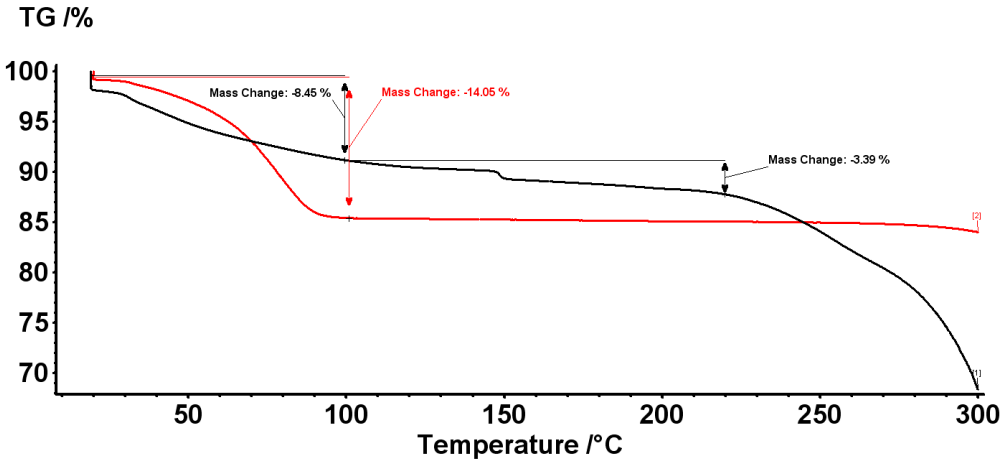

Fig. 1. Thermogravimetric analysis of the *C. cassia* essential oil / β-cyclodextrin complex (up-black) and the commercial β-cyclodextrin (down-red)

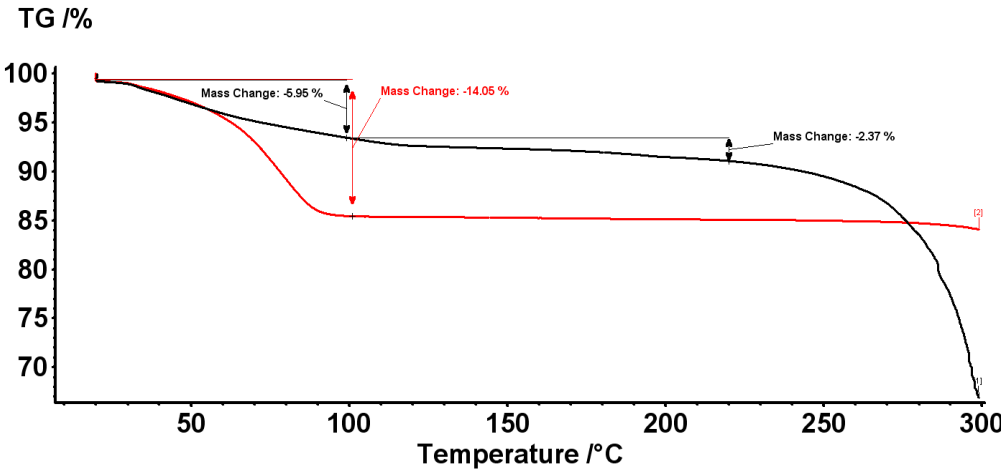

Fig. 2. Thermogravimetric analysis of the *R. damascena* essential oil / β-cyclodextrin complex (up-black) and the commercial β-cyclodextrin (down-red)

The main compounds identified in the *C. cassia* essential oil were cinnamaldehyde (62.3%), ethyl cinnamate (10%), and o-methoxycinnamaldehyde (12.1%) (Table 2). Other compounds with significant concentrations were benzaldehyde (2.4%), *o*-anisaldehyde (1%), and coumarin (1.8%). The total number of compounds separated from *C. cassia* essential oil was more than 100 (Figs. 3 and 4). In the case of *R. damascena* essential oil more than 150 compounds were separated by GC and the main compounds were β-phenylethanol (27.8%) and dimethylphtalate (29.9%), which probably was used as additive. Other compounds were benzyl acetate (5.2%), β-phenylethyl formate (1.2%), citronellol (3.9%), guaniol (1.9%), a,a-dimethylphenethyl acetate (1.3%), a-terpinyl acetate (3.7%), and cupraene (1.1%) (Table 3 and Figs. 5 and 6).

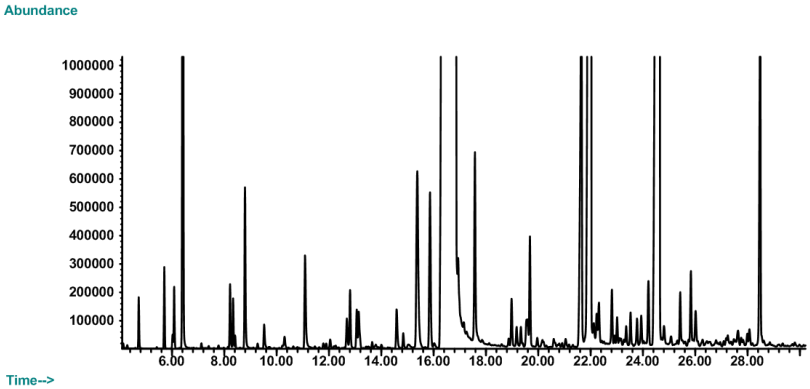

Fig. 3. The GC chromatogram from the GC-MS analysis of *C. cassia* essential oil

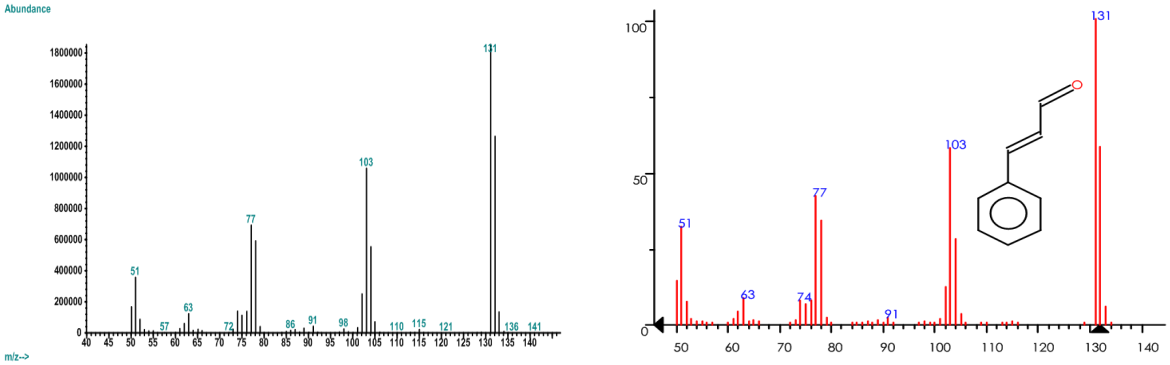

Fig. 4. Experimental (left) and NIST database (right) MS spectra for cinnamaldehyde

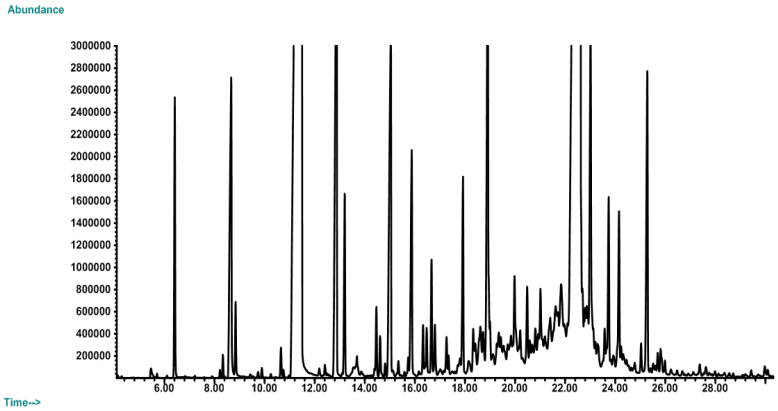

Fig. 5. The GC chromatogram from the GC-MS analysis of *R. damascena* essential oil

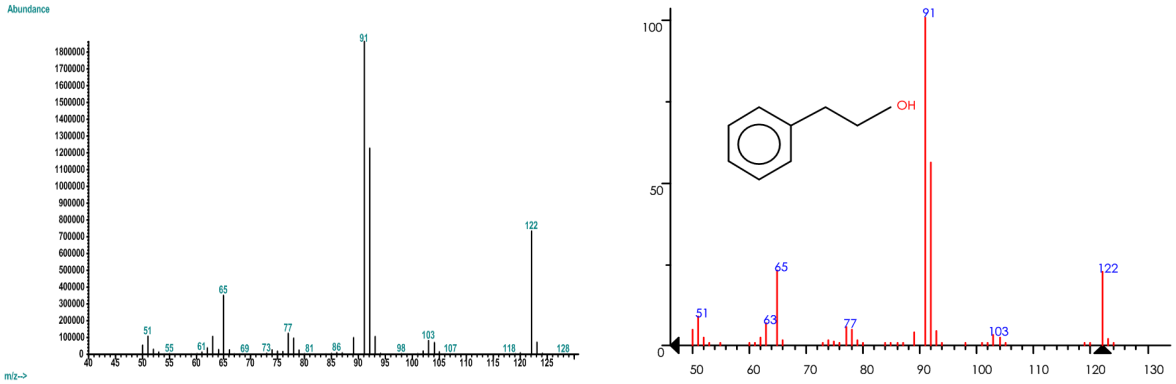

Fig. 6. Experimental (left) and NIST database (right) MS spectra for β-phenylethanol

Tabel 2. The relative concentrations and the corresponding ratio of the main compounds identified by MS in *C. cassia* essential oils.

N°	*MS Identification*	*Kovats index (KI)*	*Relative concentration in raw essential oil (%)*	*Relative concentration in recovered essential oil (%)*	*The ratio between concentrations in recovered and raw essential oils*
1	*α-Pinene*	*936*	*0.17*	*0.18*	*1.06*
2	*Benzaldehyde*	*963*	*2.41*	*0.84*	*0.35*
3	*p-Cymene*	*1026*	*0.15*	*0.07*	*0.45*
4	*Limonene*	*1030*	*0.12*	*0.13*	*1.10*
5	*Salicylaldehyde*	*1045*	*0.45*	*0.04*	*0.09*
6	*Borneol*	*1168*	*0.17*	*0.04*	*0.22*
7	*o-Anisaldehyde*	*1244*	*0.92*	*0.72*	*0.79*
8	*β-Phenethyl acetate*	*1259*	*0.59*	*0.40*	*0.67*
9	*Cinnamaldehyde*	*1289*	*62.31*	*69.67*	*1.12*
10	*α-Copaene*	*1377*	*0.32*	*0.14*	*0.44*
11	*Coumarin*	*1440*	*1.75*	*0.65*	*0.37*
12	*Cinnamyl alcohol, acetate*	*1452*	*9.96*	*6.54*	*0.66*
13	*γ-Cadinene*	*1478*	*0.16*	*0.06*	*0.40*
14	*δ-Cadinene*	*1525*	*0.21*	*0.04*	*0.18*
15	*Cinnamaldehyde, o-methoxy-*	*1539*	*12.09*	*10.19*	*0.84*
	Other compounds		*8.22*	*10.29*	

Tabel 3. The relative concentrations and the corresponding ratio of the main compounds identified by MS in *R. damascena* essential oils.

N°	*MS Identification*	*Kovats index (KI)*	*Relative concentration in raw essential oil (%)*	*Relative concentration in recovered essential oil (%)*	*The ratio between concentrations in recovered and raw essential oils*
1	*Benzeneacetaldehyde*	*1047*	*0.45*	*0.44*	*0.98*
2	*Linalool*	*1103*	*0.20*	*0.47*	*2.34*
3	*Phenylethyl Alcohol*	*1128*	*27.82*	*17.00*	*0.61*
4	*Benzyl acetate*	*1170*	*5.17*	*7.81*	*1.51*
5	*Phenethyl alcohol, formate*	*1179*	*1.18*	*2.50*	*2.12*
6	*Citronelol*	*1234*	*3.89*	*8.28*	*2.13*
7	*Guaniol*	*1259*	*1.89*	*3.32*	*1.76*
8	*(E)-Citral*	*1273*	*0.30*	*0.56*	*1.84*
9	*p-Menth-8-en-1-ol, acetate*	*1283*	*0.70*	*2.08*	*2.95*
10	*α,α-Dimethylphenethyl acetate*	*1322*	*1.32*	*4.23*	*3.20*
11	*α-Terpinyl acetate*	*1353*	*3.72*	*11.34*	*3.05*
12	*β-Citronellal*	*1419*	*0.31*	*0.53*	*1.72*
13	*Dimethyl phthalate*	*1472*	*29.94*	*11.31*	*0.38*
14	*Cuparene*	*1509*	*1.09*	*3.74*	*3.44*
15	*α-Methyl ionone*	*1523*	*1.11*	*2.79*	*2.50*
16	*Elemol*	*1553*	*0.20*	*0.14*	*0.66*
	Other compounds		*20.71*	*23.46*	

The efficiency of encapsulation process for the main volatile compounds from essential oils was evaluated by quantification of the concentrations of these compounds in the recovered essential oil from the complex. In the case of *C. cassia* essential oil / β-cyclodextrin complex the most concentrated compound, cinnamaldehyde, was encapsulated in higher relative concentration (69.7%) in comparison with the raw essential oil (62.3%), the ratio between these concentrations in the recovered and raw essential oils being 1.12. Other important compounds were encapsulated in lower relative concentrations, such as for benzaldehyde, coumarin, cinnamyl alcohol acetate, and *o*-methoxycinnamaldehyde (the corresponding ratios of 0.35, 0.37, 0.66, and 0.84, respectively) (Table 2).

The main compounds from *R. damascena* essential oil, β-phenylethanol and dimethyl phtalate were encapsulated in lower relative concentration than in the raw essential oil (ratios of 0.61 and 0.38, respectively). Other minor compounds were encapsulated in higher relative concentrations than in the raw essential oils, such as benzyl acetate (ratio of 1.51), phenethyl alcohol formate (2.12), citronellol (2.13), guaniol (1.76), a,a-dimethylphenethyl acetate (3.2), a-terpinyl acetate (3.05), cupraene (3.44), and a-methylionone (2.5) (Table 4).

4. CONCLUSION

The nanoencapsulation of essential oils from Lauraceae and Rosaceae botanical families was performed with good yields by using the crystallization from alcohol-water solution method. The formation of the complexes was proved by thermogravimetry, where a reducing of the water content in the complexes in comparison with β-cyclodextrin, as well as a concentration of 2.4-3.4% of essential oil in the complexes were observed. Some volatile compounds are also release from the complexes in the same temperature ranges such as the water of hydration; therefore the concentrations of encapsulated essential oils are higher.

The competitivity of encapsulation of the main volatile compounds from essential oils varies according to the hydrophobicity and geometry of structures. The relatively higher hydrophobicity of cinnamaldehyde (log*P* 1.8) conduct to a higher relative concentration in the recovered *C. cassia* essential oil in comparison with other compounds (*e.g.* benzaldehyde and *o*-methoxycinnamaldehyde with log*P* of 1.7 and 1.5, respectively). The same conclusion can be drawn in the case of the main volatile compounds from *R. damascena* essential oil (a lower log*P* value of 1.7 for β-phenylethanol conduct to a lower relative concentration in the recovered essential oil, in comparison with the more hydrophobic compounds, such as citronellol and a,a-dimethylphenethyl acetate, with log*P* of 2.8 and 2.7, respectively).

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