

## III.2. COMPARISON OF CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SOME ESSENTIAL OILS FROM ROMANIAN MARKET

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### Abstract

Essential oils have been used in aromatherapy for centuries because they are easily found in plants and they are usually extracted through a popular method named water distillation. The present study investigates the chemical composition and antioxidant activity of some essential oils. The investigated oils are Basil oil (*Ocimum basilicum*), Rosemary oil (*Rosmarinus officinalis*) and Peppermint oil (*Mentha piperita* and *Mentha arvensis*). They were purchased from Romanian market and they are produced by four different manufacturers. The main volatile constituents of these essential oils are analyzed by Gas Chromatography coupled with Mass Spectrometry Detector (GC-MSD) and Gas Chromatography coupled with a Flame Ionized Detector (GC-FID). The main compounds detected in Basil essential oil were Estragol and  $\beta$ -Linalool, in Rosemary oil were Eucalyptol (1.8-Cineole), d-Camphor and  $\alpha$ -Pinene and in Peppermint oil were Menthan-1-ol and l-Menthone. The antioxidant activity of these oils is evaluated using DPPH method. The highest value for antioxidant activities of Basil essential oil is  $IC_{50} = 49.17$  mg/ml, for Rosemary essential oil is  $IC_{50} = 14$  mg/ml and for Peppermint essential oil is  $IC_{50} = 69.65$  mg/ml.

**Key words:** essential oils, aromatherapy, chemical compounds, GC-MS, DPPH.

### INTRODUCTION

Essential oils have been used in aromatherapy for centuries. The geographic locations and environmental conditions influence the composition of essential oils, which can vary. Essential oils are obtained from the plant most active parts, such as flowers, seeds, leaves, herbs and roots, and it can be obtained by various extraction methods.

Nowadays, water distillation is the most used extraction method for commercial production. This extraction method is applied to the plant material using selective solvents and standard procedures (Badal & Delgoda, 2016).

*Lamiaceae* family is one of the most distinguished and largest families of aromatic plants. It includes about 235 genera and 7 000 species worldwide (Andrade et al., 2018). Basil (*Ocimum basilicum*), Peppermint (*Mentha piperita* and *Mentha arvensis*) and Rosemary (*Rosmarinus officinalis*) belong to this family. These flowered plants are cultivated for their aromatic properties and their commercial use. For example, these herbs are used in gastronomy as seasonings; as flavours in juices; essential oils are used in aromatherapy and in relaxing

massage; sanitary articles like tooth paste; in food supplements like sore throat tablets and intestinal tympanites (Dunning, 2013).

Essential oils are consisting of a mixture of phenols, iridoids esters, unsaturated hydrocarbons, saturated hydrocarbons, ethers, ketones, alcohols, aldehydes and terpenes. Essential oils are colorless and they have a pleasant characteristic odour (Ziosi et al., 2013). The present study investigates the chemical composition and antioxidant activity of some commercially available essential oils: Basil oil, Rosemary oil and Peppermint oil.

### MATERIALS AND METHODS

P<sub>1</sub>-Basil oil (*Ocimum basilicum*), P<sub>2</sub>-Rosemary oil (*Rosmarinus officinalis*) and P<sub>3</sub>-Peppermint oil (*Mentha piperita* and *Mentha arvensis*) were purchased from Romanian market and they are produced by four different manufacturers.

Essential oil composition depends on the plant genotype and the environmental factory like soil properties, altitude, wind, sun, humidity, plant age and harvesting methods and on the used extraction method.

The tested oils were produced by four manufacturers named in this article as A, B, C and D and the essential oils are named as: P<sub>1</sub> for Basil essential oil, P<sub>2</sub> for Rosemary essential oil and P<sub>3</sub> for Peppermint essential oil.

The main volatile constituents of these essential oils are analyzed by Gas Chromatography coupled with Mass Spectrometry Detector (GC-MSD) and Gas Chromatography coupled with a Flame Ionized Detector (GC-FID).

Gas Chromatography coupled with a Flame Ionized Detector (GC-FID) is a quantitative method. Gas Chromatography coupled with Mass Spectrometry Detector (GC-MSD) is a qualitative method.

The Gas Chromatography (GC) method was performed on Agilent GC 7890A chromatograph using PHENOMENEX capillary column named ZEBRON (ZB)-5ms, an inlet temperature at 250°C, a 50:1 split ratio and a 0.8 mL/min Helium flow rate (Deleanu et al., 2018). Temperature of the GC column was kept at 50°C for 1 min. Then, the temperature gradually increased with the gradients of 8°C/min up to 100°C and was kept constant for 2 minute, 2°C/min up to 110°C was kept constant for 2 minute, 5°C/min up to 185°C and 30°C/min up to 280°C was kept constant for 10 minute. Mass Spectrometry Detector (GC-MSD) is a qualitative method. The Mass Spectrometry (MS) detection was performed on Triple Quad MS Agilent 7000A detector and ZEBRON (ZB)-5 ms plus (30 m × 0.25 i.d. mm, 0.25 film thickness μm) from Phenomenex, capillary column (Deleanu et al., 2018).

The Electron Ionization (EI) is a direct process where the energy is transferred, by different methods like collision, from electrons to the sample molecules (Miroslaw et al., 2015).

The Electron Ionization used was 70 eV (electron Volt). Transfer line temperature was 280°C, the source temperature was 230°C and the quadrupole temperature was 150°C. Chromatographic peaks were identified according to National Institute of Standards and Technology (NIST) database.

The antioxidant activity of these oils is evaluated using DPPH (2,2-diphenyl-1-picryl-

hydrazyl radical) method. We use a 0.3 mM DPPH ethanol solution mixed with 125 μL of the diluted oils in a 96-well clear microplate (Deleanu et al., 2018).

The control used at this analysis is a 50 μL DPPH solution mixed with 125 μL ethanol. The microplate with the oil samples and the control sample were incubated in dark, at room temperature, for 30 minutes. The plates were read at 518 nm at TECAN M200 INFINITE PRO MICROPLATE READER. The antiradical activity (AA) of the oil samples were determined using the formula:

$$AA\% = 100 - \left( \frac{(A_{SD} - A_{SWD}) \times 100}{A_C} \right)$$

where:

*AA* = Antiradical Activity;

*A<sub>SD</sub>* = Absorbance of the oil sample with DPPH;

*A<sub>SWD</sub>* = Absorbance of the oil sample without DPPH;

*A<sub>C</sub>* = Absorbance of the control sample.

## RESULTS AND DISCUSSIONS

The chemical composition of these essential oils was analyzed by Gas Chromatography coupled with Mass Spectrometry Detector (GC-MSD) and Gas Chromatography coupled with a Flame Ionized Detector (GC-FID).

The compounds concentrations of the studied essentials oils and the Retention Time (RT) are presented in Tables 1-3. The GC-MSD and GC-FID chromatograms of these essential oils are presented in Figures 1-12.

Twenty chemical compounds, representing 99.85% of P<sub>1</sub>A, were identified as major like Estragole (75.54%), β-Linalool (17.86%), 1.5.9.9-Tetramethyl-1.4.7-cycloundecatriene (1.87%), and minor compounds like citral (0.7%), α-Bergamotene(0.59%), Caryophyllene (0.51%), β-Citral (0.46%), β-Cubebene (0.37%), Isomenthol (0.36%), cis-β-Farnesene (0.25%), Humulene (0.24%), 3-Carene (0.16%), β-Pinene (0.15%), D-Limonene (0.14%), Eucalyptol (0.14%), l-Menthone (0.12%), 3-Methoxycinnamaldehyde (0.11%), 2.7-Dimethyl-2.6-octadien-1-ol (0.1%), α-Pinene (0.09%) and β-Bisabolene (0.09%).

Table 1. The chemical composition of Basil (*Ocimum basilicum*) essential oils

No	Compound	RT min	P <sub>1</sub> A%	P <sub>1</sub> B %	P <sub>1</sub> C %	P <sub>1</sub> D%	
1	$\alpha$ -Pinene	7.87	0.09	0.07	0.10	0.08	
2	$\beta$ -Pinene	8.86	0.15	0.25	0.15	0.16	
3	2-Ethyl-1-hexanol	9.87	-	0.12	0.60	-	
4	Cymene	9.92	-	0.06	-	-	
5	D-Limonene	10.05	0.14	0.06	0.28	0.22	
6	Eucalyptol	10.17	0.14	0.18	0.10	0.10	
7	3-Carene	10.40	0.16	0.15	0.13	0.11	
8	$\beta$ -Linalool	12.0	17.8	18.0	17.6	18.7	
9	l-Menthone	14.19	0.12	-	-	0.09	
10	Isomenthol	15.05	0.36	0.19	0.13	0.48	
11	Estragole	16.1	75.5	76.2	76.4	75.4	
12	$\beta$ -Citral	17.31	0.46	0.39	0.33	0.37	
13	2,7-Dimethyl-2,6-octadien-1-ol	17.69	0.1	0.07	-	-	
14	Citral	18.37	0.7	0.61	-	0.55	
15	Caryo-phyllene	23.4	0.51	0.41	0.35	0.40	
16	$\alpha$ -Bergamotene	23.7	0.59	0.49	0.49	0.47	
17	cis- $\beta$ -Farnesene	24.2	0.25	-	0.25	0.19	
18	Humulene	24.5	0.24	0.19	0.18	0.19	
19	$\beta$ -Cubebene	25.1	0.37	0.35	0.24	0.28	
20	$\beta$ -Bisabolene	25.8	0.09	0.08	-	-	
21	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	26.6	1.87	1.60	1.43	1.56	
22	3-Methoxycinnamaldehyde	27.4	0.11	0.21	0.38	0.19	
23	Caryo-phyllene oxide	27.8	-	0.06	0.11	0.07	
<b>Total</b>			<b>-</b>	<b>99.85</b>	<b>99.88</b>	<b>99.32</b>	<b>99.77</b>

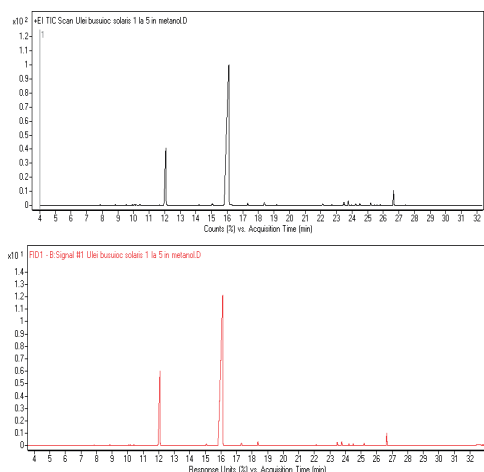


Figure 1. GC-MS and GC-FID chromatogram of P<sub>1</sub>A

Twenty-one chemical compounds, representing 99.88% of P<sub>1</sub>B, were identified as major like Estragole (76.26%),  $\beta$ -Linalool (18.08%), 1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene (1.6%) and minor compounds like Citral (0.61%),  $\alpha$ -Bergamotene (0.49%), Caryophyllene (0.41%),  $\beta$ -Citral (0.39%),  $\beta$ -Cubebene (0.35%),  $\beta$ -Pinene (0.25%), 3-Methoxycinnamaldehyde (0.21%), Isomenthol (0.19%), Humulene (0.19%), Eucalyptol (0.18%), 3-Carene (0.15%), 2-Ethyl-1-hexanol (0.12%),  $\beta$ -Bisabolene (0.08%),  $\alpha$ -Pinene (0.07%), 2,7-Dimethyl-2,6-octadien-1-ol (0.07%), D-Limonene (0.06%), Cymene (0.06%) and Caryophyllene oxide (0.06%).

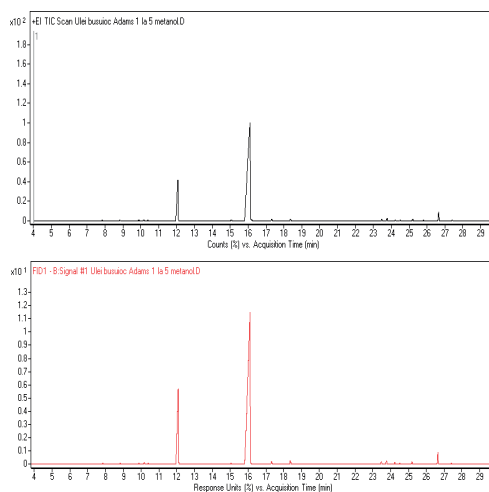


Figure 2. GC-MS and GC-FID chromatogram of P<sub>1</sub>B

Seventeen chemical compounds, representing 99.32% of P<sub>1</sub>C, were identified as major like Estragole (76.41%),  $\beta$ -Linalool (17.66%), 1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene (1.43%) and minor compounds like 2-Ethyl-1-hexanol (0.6%),  $\alpha$ -Bergamotene (0.49%), 3-Methoxycinnamaldehyde (0.38%), Caryophyllene (0.35%),  $\beta$ -Citral (0.33%), D-Limonene (0.28%), cis- $\beta$ -Farnesene (0.25%),  $\beta$ -Cubebene (0.35%), Humulene (0.18%),  $\beta$ -Pinene (0.15%), 3-Carene (0.13%), Isomenthol (0.13%), Caryophyllene oxide (0.11%) and Eucalyptol (0.1%).

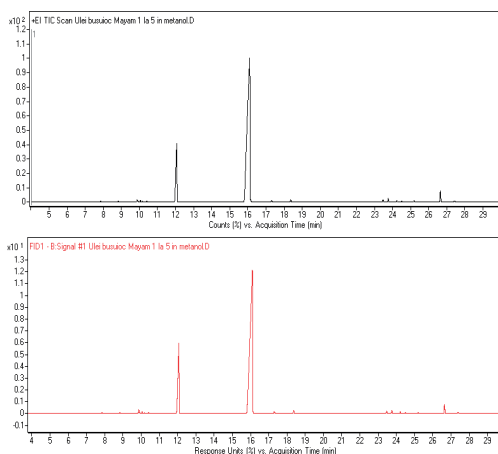


Figure 3. GC-MS and GC-FID chromatogram of P<sub>1</sub>C

Nineteen chemical compounds, representing 99.77% of P<sub>1</sub>D, were identified as major like Estragole (75.48%),  $\beta$ -Linalool (18.78%) and minor compounds like Citral (0.55%), Isomenthol (0.48%),  $\alpha$ -Bergamotene (0.47%), Caryophyllene (0.4%),  $\beta$ -Citral (0.37%),  $\beta$ -Cubebene (0.28%), D-Limonene (0.22%), cis- $\beta$ -Farnesene (0.19%), Humulene (0.19%), 3-Methoxycinnamaldehyde (0.19%),  $\beta$ -Pinene (0.16%), 3-Carene (0.11%), Eucalyptol (0.1%), l-Menthone (0.09%),  $\alpha$ -Pinene (0.08%) and Caryophyllene oxide (0.07%).

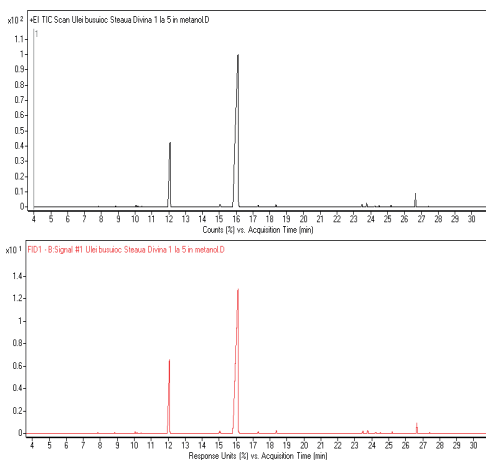


Figure 4. GC-MS and GC-FID chromatogram of P<sub>1</sub>D

According to the GC-MS and GC-FID results obtained for the Basil essential oil, the P<sub>1</sub>-Basil (*Ocimum basilicum*) essential oil compounds are representing approximately 99% of total composition. P<sub>1</sub>B has twenty-one chemical

compounds P<sub>1</sub>A has twenty, P<sub>1</sub>D has nineteen and P<sub>1</sub>C has seventeen chemical compounds. The main compounds detected in P<sub>1</sub>-Basil essential oil were: Estragol and  $\beta$ -Linalool. P<sub>1</sub>C (76.41%) has the most Estragole, then P<sub>1</sub>B (76.26), P<sub>1</sub>A (75.54%) and P<sub>1</sub>D (75.48%), followed by the second largest component Linalool with more than 17%.

Avetisyan et al. (2017) has reported that the main chemical constituents of Basil essential oil of aromatic plant origin include monoterpenes like Estragole/Methyl chavicol (more than 57.3%),  $\beta$ -Linalool (more than 18%), sesquiterpenes like  $\alpha$ -Bergamotene, Humulene and their oxygenated derivatives.

Stanojevic et al. (2019) found in his study that the most abundant component in Basil essential oil was  $\beta$ -Linalool (39.9 %).

According to Abbasy et al. (2015) a total of thirty six chemical constituents were identified in Basil essential oil and Linalool (69.87%) was found to be the major constituent. Other main identified constituents included Geraniol (9.75%), 1,8-Cineole (4.90%) and  $\alpha$ -Bergamotene (2.36%).

Table 2. The chemical composition of Rosemary (*Rosmarinus officinalis*) essential oils

No	Compound	RT min	P <sub>2</sub> A %	P <sub>2</sub> B %	P <sub>2</sub> C %	P <sub>2</sub> D %
1	Tricyclene	7.64	0.58	1.68	0.32	-
2	$\alpha$ -Pinene	7.87	7.54	24.7	14.5	2.45
3	d-Camphene	8.19	-	1.88	-	0.32
4	Camphene	8.24	1.69	6.61	4.80	1.82
	$\beta$ -Phellandrene	8.68	4.78	-	-	-
5	Isocamphane	8.72	-	0.2	0.21	-
6	L- $\beta$ -Pinene	8.86	2.41	4.79	5.28	0.63
7	$\beta$ -Pinene	8.95	1.98	2.36	1.25	0.20
8	$\alpha$ -Phellandrene	9.01	0.53	0.13	-	0.22
9	3-Carene	9.55	-	4.57	0.14	-
10	Isocineole	9.66	-	0.11	-	2.19
11	$\alpha$ -Terpinene	9.73	0.84	0.11	0.43	5.13
12	Cymene	9.92	0.89	2.90	1.26	10.02
13	D-Limonene	10.0	3.98	4.75	2.05	21.68
14	Eucalyptol	10.17	36.07	19.55	41.67	23.09
15	$\gamma$ -Terpinene	10.8	0.62	0.19	0.63	0.20
16	Terpinolene	11.6	-	0.78	0.29	-
17	Fenchone	11.8	-	0.52	-	-
18	$\beta$ -Linalool	12.0	1.10	2.60	0.87	1.38
19	d-Camphor	13.9	22.4	12.5	15.4	19.03
20	Isoborneol	14.5	0.74	3.57	-	1.83
21	endo-Borneol	14.8	0.50	0.48	2.91	3.06
22	Terpinen-4-ol	15.2	1.76	-	0.63	-
23	$\alpha$ -Terpinol	15.7	5.01	2.55	2.07	2.79
24	$\gamma$ -Terpinol	15.8	-	0.73	-	1.03
25	Borneol acetate	19.1	-	0.95	0.44	0.96
26	safole	19.2	5.62	-	-	-
27	Caryophyllene	23.2	-	0.24	3.88	1.69
28	( $\pm$ )-trans-Nerolidol	27.1	0.95	-	-	-
	<b>Total</b>	-	<b>99.99</b>	<b>99.57</b>	<b>99.06</b>	<b>99.72</b>

Twenty chemical compounds, representing 99.99% of P<sub>2</sub>A, were identified as major like Eucalyptol (36.07%), d-Camphor (22.4%),  $\alpha$ -Pinene (7.54%), safrole (5.62%),  $\alpha$ -Terpineol (5.01%),  $\beta$ -Phellandrene (4.78%), D-Limonene (3.98%), L- $\beta$ -Pinene (2.41%),  $\beta$ -Pinene (1.98%), Terpinen-4-ol (1.76%), Camphene (1.69%),  $\beta$ -Linalool (1.1%) and minor compounds like ( $\pm$ )-trans-Nerolidol (0.95%), Cymene (0.89%),  $\alpha$ -Terpinene (0.84%), Isoborneol (0.74%),  $\gamma$ -Terpinene (0.62%), Tricyclene (0.58%),  $\alpha$ -Phellandrene (0.53%) and endo-Borneol (0.5%).

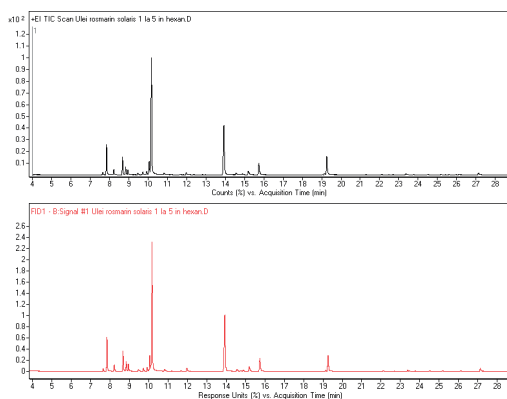


Figure 5. GC-MS and GC-FID chromatogram of P<sub>2</sub>A

Twenty-five chemical compounds, representing 99.57% of P<sub>2</sub>B, were identified as major like d-Camphene (24.76%),  $\alpha$ -Terpineol (19.55%), Terpinen-4-ol (12.56%), 3-Carene (6.61%), Fenchone (4.79%), Safrole (4.75%), Caryophyllene (4.57%), Camphene (3.57%),  $\alpha$ -Pinene (2.9%),  $\beta$ -Pinene (2.6%), Cymene (2.55%),  $\gamma$ -Terpineol (2.36%), Isocamphane (1.88%), Tricyclene (1.68%) and minor compounds like Isoborneol (0.95%), D-Limonene (0.78%),  $\alpha$ -Terpinene (0.73%), L- $\beta$ -Pinene (0.52%),  $\beta$ -Linalool (0.48%),  $\alpha$ -Phellandrene (0.24%), Terpinolene (0.2%),  $\beta$ -Phellandrene (0.19%), Borneol acetate (0.13%), Eucalyptol (0.11%) and d-Camphor (0.11%).

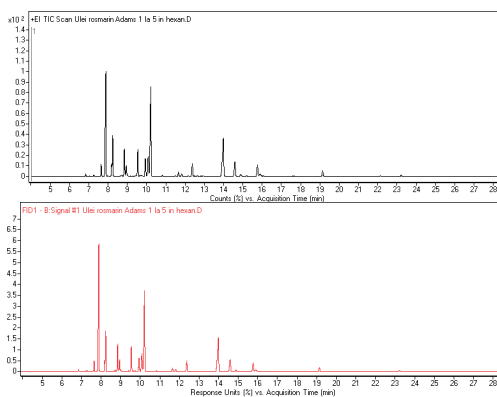


Figure 6. GC-MS and GC-FID chromatogram of P<sub>2</sub>B

Twenty chemical compounds, representing 99.06% of P<sub>2</sub>C, were identified as major like Eucalyptol (41.67%), d-Camphor (15.4%),  $\alpha$ -Pinene (14.53%), L- $\beta$ -Pinene (5.28%), Camphene (4.8%), Caryophyllene (3.88%), endo-Borneol (2.91%),  $\alpha$ -Terpineol (2.07%), D-Limonene (2.05%), Cymene (1.26%),  $\beta$ -Pinene (1.25%) and minor compounds like  $\beta$ -Linalool (0.87%),  $\gamma$ -Terpinene (0.63%), Terpinen-4-ol (0.63%), Borneol acetate (0.44%),  $\alpha$ -Terpinene (0.43%), Tricyclene (0.32%), Terpinolene (0.29%), Isocamphane (0.21%) and 3-Carene (0.14%).

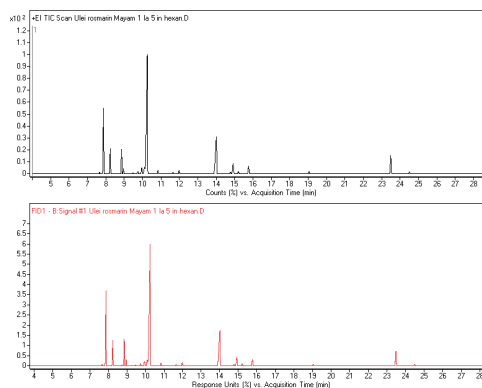


Figure 7. GC-MS and GC-FID chromatogram of P<sub>2</sub>C

Twenty chemical compounds, representing 99.72% of P<sub>2</sub>D, were identified as major like Caryophyllene (23.09%), Camphene (21.68%),  $\beta$ -Pinene (19.03%), L- $\beta$ -Pinene (10.02%),  $\alpha$ -Pinene (5.13%),  $\gamma$ -Terpinene (3.06%), Borneol acetate (2.79%), d-Camphene (2.45%), d-Camphor (2.19%),  $\beta$ -Linalool (1.83%),  $\alpha$ -Phellandrene (1.82%), 3-Carene (1.69%), Cymene (1.38%),  $\alpha$ -Terpinene (1.03%) and minor compounds like Terpinolene (0.96%), Isoborneol (0.63%),  $\beta$ -Phellandrene (0.32%), ( $\pm$ )-trans-Nerolidol (0.22%),  $\gamma$ -Terpineol (0.2%) and endo-Borneol (0.2%).

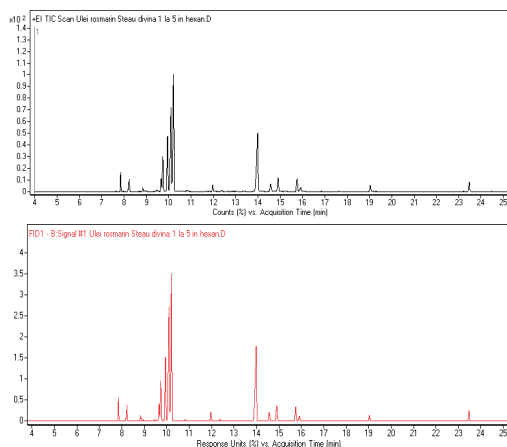


Figure 8. GC-MS and GC-FID chromatogram of P<sub>2</sub>D

P<sub>2</sub>-Rosemary (*Rosmarinus officinalis*) essential oil compounds are representing approximately 99% of total composition. P<sub>2</sub>B has twenty-five chemical compounds, P<sub>2</sub>A, P<sub>2</sub>C and P<sub>2</sub>D has twenty chemical compounds. The main compounds detected in P<sub>2</sub>-Rosemary essential oil were: Eucalyptol (1.8-Cineole), d-Camphor and  $\alpha$ -Pinene. P<sub>2</sub>C (41.67%) has the most Eucalyptol, then P<sub>2</sub>A (36.07%), P<sub>2</sub>D (23.09%) and P<sub>2</sub>B (19.55%).

Jiang et al. (2011) reported that Rosemary essential oil has twenty-two chemical constituents like 1.8-Cineole (26.54%) and  $\alpha$ -Pinene (20.14%). Since 2007, Djeddi et al. reported that it can be distinguished two major types of Rosemary oil: the one with over 40% of 1.8-cineole, which is characteristic of oils from Morocco, Tunisia, Turkey, Greece, Serbia, Italy, and France, and oils with approximately equal ratios (20-30%) of 1.8-Cineole,  $\alpha$ -Pinene, and Camphor (oils from France, Spain, Italy, Greece, Bulgaria). This difference could be due

to the different climates between south Europe and North Africa Mediterranean areas: higher percentage mean monthly temperatures and longer sunshine duration.

Silveira et al. (2012) found that the essential oils of Basil and Rosemary have a great potential for utilization as natural antimicrobial agents in foods.

Table 3. The chemical composition of Peppermint (*Mentha piperita* and *Mentha arvensis*) essential oils

No	Compound	RT min	P <sub>3</sub> A %	P <sub>3</sub> B %	P <sub>3</sub> C %	P <sub>3</sub> D %
1	$\alpha$ -Pinene	7.87	3.77	-	0.61	2.46
2	Camphene	8.24	0.55	-	-	0.26
3	$\beta$ -Phellandrene	8.68	1.11	-	0.24	0.37
4	L- $\beta$ -Pinene	8.86	5.46	-	0.83	1.99
5	$\beta$ -Pinene	8.95	2.19	-	0.20	0.54
6	3-octanol	9.11	1.32	-	0.22	1.25
7	2-Menthene	9.35	0.75	-	-	-
8	3-Carene	9.55	2.53	-	-	-
9	$\alpha$ -Terpinene	9.73	0.91	-	-	-
10	Cymene	9.92	9.91	0.04	0.19	0.50
11	D-Limonene	10.05	17.53	2.15	2.61	4.23
12	Eucalyptol	10.17	5.67	0.13	4.77	0.23
13	$\gamma$ -Terpinene	10.82	6.73	-	-	-
14	Terpinolene	11.65	3.01	-	-	-
15	Fenchone	11.81	0.36	-	-	-
16	Isopulegol	13.93	0.38	0.05	-	1.45
17	I-Menthone	14.23	4.12	28.5	24.98	21.05
18	O-Menthone	14.45	-	0.10	1.05	0.29
19	D-Menthone	14.59	1.67	10.3	3.81	9.93
20	Neo-menthol	14.75	1.13	2.09	3.69	4.56
21	p-Menthan-1-ol	15.2	28.2	50.1	44.50	41.79
22	Isomenthol	15.68	-	0.96	1.35	0.63
23	( $\pm$ )-Pulegone	17.43	0.35	0.04	0.45	1.06
24	Isomenthol acetate	19.19	0.90	3.49	5.94	3.83
25	Caryophyllene	23.22	-	1.08	3.61	0.86
26	Germa-crene D	25.19	-	-	0.18	0.30
27	Caryo-phyllene oxide	27.85	-	0.10	-	-
<b>Total</b>			<b>98.62</b>	<b>99.31</b>	<b>99.23</b>	<b>97.58</b>

Twenty-two chemical compounds, representing 98.62% of P<sub>3</sub>A, were identified as major like p-Menthan-1-ol (28.27%), D-Limonene (17.53%), Cymene (9.91%),  $\gamma$ -Terpinene (6.73%), Eucalyptol (5.67%), L- $\beta$ -Pinene (5.46%), I-Menthone (4.12%),  $\alpha$ -Pinene (3.77%), Terpinolene (3.01%), 3-Carene (2.53%),  $\beta$ -Pinene (2.19%), D-Menthone (1.67%), 3-octanol (1.32%), Neo-menthol (1.13%),  $\beta$ -Phellandrene (1.11%) and minor compounds like  $\alpha$ -Terpinene (0.91%), Isomenthol acetate (0.90%), 2-Menthene (0.75%), Camphene (0.55%), Isopulegol (0.38%), Fenchone (0.36%) and ( $\pm$ )-Pulegone (0.35%).



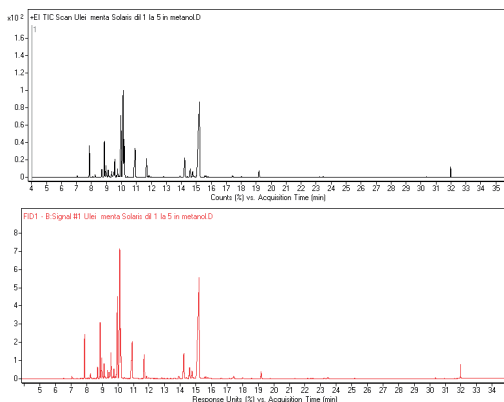


Figure 9. GC-MS and GC-FID chromatogram of P<sub>3</sub>A

Fourteen chemical compounds, representing 99.31% of P<sub>3</sub>B, were identified as major like p-Menthan-1-ol (50.19%), I-Menthone (28.57%), D-Menthone (10.32%), Isomenthol acetate (3.49%), D-Limonene (2.15%), Neo-menthol (2.09%), Caryophyllene (1.08%) and minor compounds like Isomenthol (0.96%), Eucalyptol (0.13%), O-Menthone (0.10%), Caryophyllene oxide (0.10%), Isopulegol (0.05%), (±)-Pulegone (0.04%) and Cymene (0.04%).

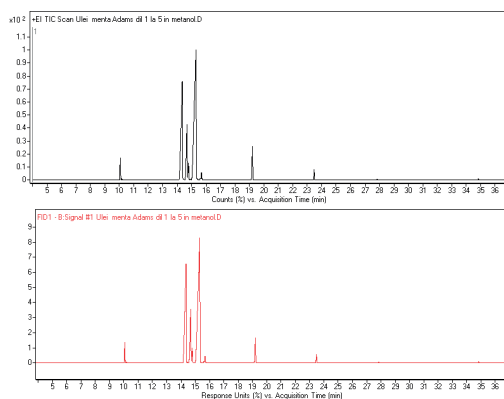


Figure 10. GC-MS and GC-FID chromatogram of P<sub>3</sub>B

Eighteen chemical compounds, representing 99.23% of P<sub>3</sub>C, were identified as major like p-Menthan-1-ol (44.50%), I-Menthone (24.98%), Isomenthol acetate (5.94%), Eucalyptol (4.77%), D-Menthone (3.81%), Caryophyllene (3.61%), Neo-menthol (3.69%), D-Limonene (2.61%), Isomenthol (1.35%), O-Menthone (1.05%) and minor compounds like L-β-Pinene (0.83%), α-Pinene (0.61%), (±)-Pulegone (0.45%), β-Phellandrene (0.24%), 3-octanol

(0.22%), β-Pinene (0.20%), Cymene (0.19%), Germa-crene D (0.18%).

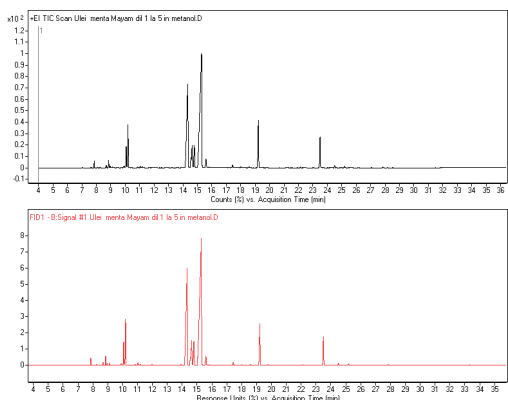


Figure 11. GC-MS and GC-FID chromatogram of P<sub>3</sub>C

Twenty chemical compounds, representing 97.58% of P<sub>3</sub>D, were identified as major like p-Menthan-1-ol (41.79%), I-Menthone (21.05%), D-Menthone (9.93%), Neo-menthol (4.56%), D-Limonene (4.23%), Isomenthol acetate (3.83%), α-Pinene (2.46%), L-β-Pinene (1.99%), Isopulegol (1.45%), 3-octanol (1.25%), (±)-Pulegone (1.06%) and minor compounds like Caryophyllene (0.86%), Isomenthol (0.63%), β-Pinene (0.54%), Cymene (0.50%), β-Phellandrene (0.37%), Germa-crene D (0.30%), O-Menthone (0.29%), Camphene (0.26%), Eucalyptol (0.23%).

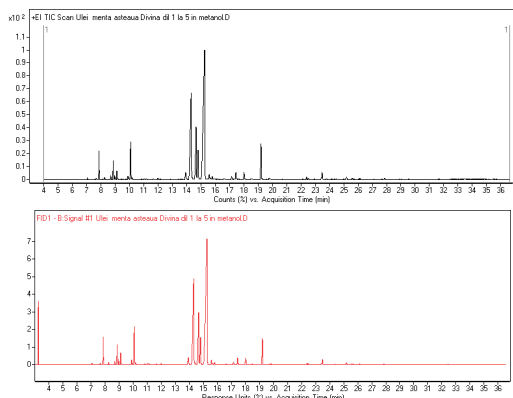


Figure 12. GC-MS and GC-FID chromatogram of P<sub>3</sub>D

P<sub>3</sub>-Peppermint (*Mentha piperita* and *Mentha arvensis*) essential oil compounds are representing 97-99% of total composition. P<sub>3</sub>A has twenty-two chemical compounds, P<sub>3</sub>D has

twenty, P<sub>3</sub>C has eighteen and P<sub>3</sub>B has fourteen chemical compounds. The main compounds detected in P<sub>3</sub>-Peppermint essential oil were: Menthan-1-ol and I-Menthone. *Mentha arvensis* P<sub>3</sub>B (50.19%) has the most Menthan-1-ol, then P<sub>3</sub>C (44.50%), P<sub>3</sub>D (41.79%) and P<sub>3</sub>A (28.27%). *Mentha arvensis* from P<sub>3</sub>B (28.57%) has the most I-Menthone, then P<sub>3</sub>C (24.98%), P<sub>3</sub>D (21.05%) and P<sub>3</sub>A (4.12%). *Mentha arvensis* has more Menthan-1-ol and I-Menthone than *Mentha piperita*.

Beigi et al. (2018), reported that the main volatile compounds detected in the Peppermint essential oil were Menthol (44.39%), Menthone (15.36%), Menthofuran (10.27%), 1,8-Cineole (5.81%), Menthyl acetate (4.78%), Neoisomenthol (2.37%), and Limonene (1.87%). According to Pino et al. (2018) the major constituents in Peppermint grown in Jalisco (Italy) were identified to be Menthol (35.4%), Menthofuran (18.2%), Menthone (15.4%), and Menthyl acetate (12.4%).

Stanojevic et al. (2019) found in his study that the most abundant component in Peppermint oil was Menthol (45.4 %).

Dorin et al. (2017) identified in Peppermint oil high concentrations of Menthol (33%), Menthone (17%), Limonene (2%), Pulegone (1.8%) and Izomentil acetate (5%). Among sesquiterpene was determined  $\beta$ -Caryophyllene at a concentration of 1.2%

The antioxidant activity of essential oils is expressed as the Inhibitory Concentration (IC<sub>50</sub>). This IC<sub>50</sub> is defined as the concentration expressed in mg/ml, of the studied essential oils, required to inhibit the formation of DPPH radicals by 50%.

The antioxidant activities of P<sub>1</sub>-Basil essential oils are presented in Figures 13 and 14. P<sub>1</sub>A (IC<sub>50</sub> = 49.17 mg/ml) exhibited a higher scavenging ability for DPPH radicals than the other three essential oils: P<sub>1</sub>B (IC<sub>50</sub> = 52.11 mg/ml), P<sub>1</sub>C (IC<sub>50</sub> = 61.15 mg/ml) and P<sub>1</sub>D (IC<sub>50</sub> = 75.17 mg/ml).

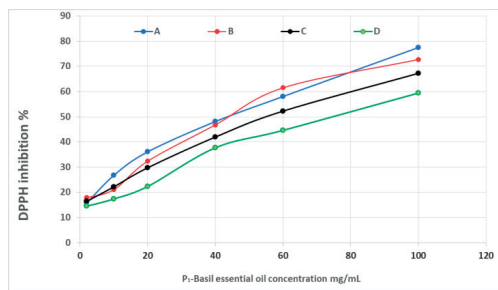


Figure 13. DPPH activity of P<sub>1</sub> in increasing concentrations

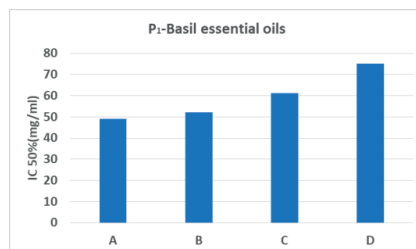


Figure 14. Inhibitory Concentration of P<sub>1</sub>

The antioxidant activities of P<sub>2</sub>-Rosemary essential oils are presented in Figures 15 and 16. P<sub>2</sub>A (IC<sub>50</sub> = 14 mg/ml) exhibited a higher scavenging ability for DPPH radicals than the other three essential oils: P<sub>2</sub>B (IC<sub>50</sub> = 224.93 mg/ml), P<sub>2</sub>C (IC<sub>50</sub> = 247.55 mg/ml) and P<sub>2</sub>D (IC<sub>50</sub> = 306.4 mg/ml).

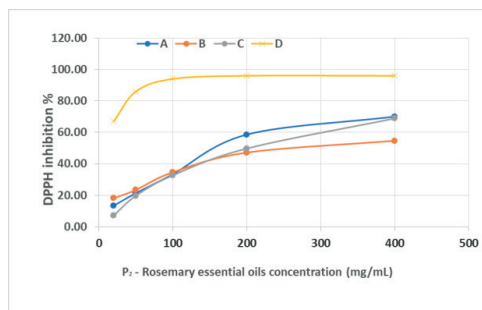


Figure 15. DPPH activity of P<sub>2</sub> in increasing concentrations



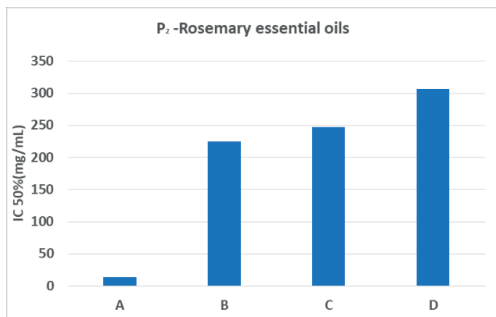


Figure 16. Inhibitory Concentration of P<sub>2</sub>

The antioxidant activities of P<sub>3</sub>-Peppermint essential oils are presented in Figures 17 and 18. P<sub>3</sub>C (IC<sub>50</sub> = 69.65 mg/ml) exhibited a higher scavenging ability for DPPH radicals than the other three essential oils: P<sub>3</sub>D (IC<sub>50</sub> = 82.62 mg/ml), P<sub>3</sub>A (IC<sub>50</sub> = 134.97 mg/ml) and P<sub>3</sub>B (IC<sub>50</sub> = 413.91 mg/ml).

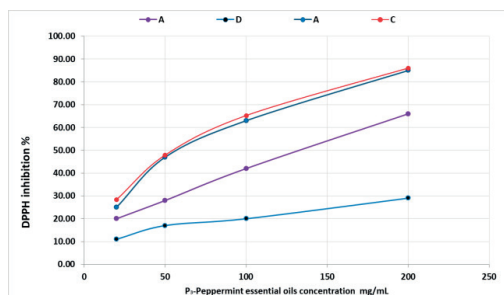


Figure 17. DPPH activity of P<sub>3</sub> in increasing concentrations

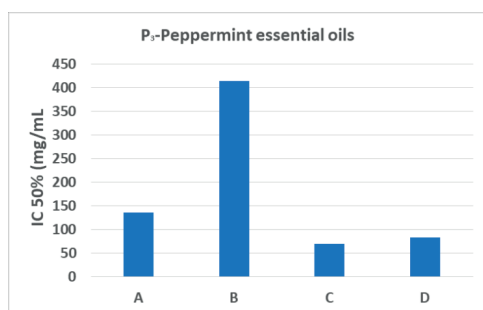


Figure 18. Inhibitory Concentration of P<sub>3</sub>

The antioxidant activities of P<sub>1</sub>-Basil essential oils is higher for P<sub>1</sub>A (IC<sub>50</sub> = 49.17 mg/ml) than for P<sub>1</sub>B (IC<sub>50</sub> = 52.11 mg/ml), P<sub>1</sub>C (IC<sub>50</sub> = 61.15 mg/ml) and P<sub>1</sub>D (IC<sub>50</sub> = 75.17 mg/ml).

The antioxidant activities of P<sub>2</sub>-Rosemary essential oils is higher for P<sub>2</sub>A (IC<sub>50</sub> = 14 mg/ml)

than for P<sub>2</sub>B (IC<sub>50</sub> = 224.93 mg/ml), P<sub>2</sub>C (IC<sub>50</sub> = 247.55 mg/ml) and P<sub>2</sub>D (IC<sub>50</sub> = 306.4 mg/ml).

The antioxidant activities of P<sub>3</sub>-Peppermint essential oils is higher for P<sub>3</sub>C (IC<sub>50</sub> = 69.65 mg/ml) than for P<sub>3</sub>D (IC<sub>50</sub> = 82.62 mg/ml), P<sub>3</sub>A (IC<sub>50</sub> = 134.97 mg/ml) and P<sub>3</sub>B (IC<sub>50</sub> = 413.91 mg/ml).

## CONCLUSIONS

In conclusion, the Basil essential oil with the most Estragole, the Rosemary essential oil with the most Eucalyptol and the Peppermint oil with the most Menthan-1-ol are produced by “C manufacturer”. *Mentha arvensis* from “B manufacturer” has more Menthan-1-ol and I-Menthone than *Mentha piperita* from “C manufacturer”. The antioxidant activities of Basil and Rosemary essential oils are higher for “A manufacturer”. The antioxidant activities of Peppermint essential oils is higher for “C manufacturer”. So, it is obviously that many extrinsic factors are influencing bioactivity of various essential oils available on market.

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