Multidisciplinary Approach in Determination of Optimal Conditions for Essential Oil Extraction from Selected Lamiaceae and Apiaceae Species

#### Tutor: Prof. Rino Ragno





Faculty of Pharmacy and Medicine Department of Drug Chemistry and Technology



### **MIJAT BOŽOVIĆ**





- EO is present at low concentrations and it requires high performance extraction techniques in order for high yields to be achieved;
- EOs are produced by different methods: solvent extraction, supercritical fluid extraction, hydro- and steam distillation, ultrasound and microwave-assisted processes;
- The extraction method is important in that the composition of EOs is somewhat dependent on the applied practice;
- Inappropriate extraction procedure can damage or alter the chemical signature, resulting in the loss of bioactivity and natural characteristics.





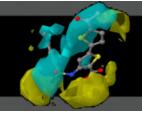
 A separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point;

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- It enables a compound/mixture of compounds to be distilled at a temperature significantly below the corresponding individual constituent(s) boiling point(s).
- Isolated EOs are different in composition from those naturally occurring in plants, since the process conditions cause chemical reactions to occur, which result in the formation of certain artifacts.
- Different factors determine the composition and quality of EO,
   such as cultivation, soil and climatic conditions, harvesting time.









### 24-hour Steam Distillation Method: Background

#### A comprehensive study on essential oil extraction from wild Mentha suaveolens Ehrh. in terms of different harvesting and extraction time

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Article

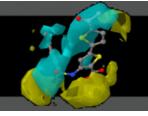
Multidisciplinary Approach to Determine the Optimal Time and Period for Extracting the Essential Oil from *Mentha suaveolens* Ehrh<sup>†</sup>

Stefania Garzoli<sup>1</sup>, Adele Pirolli<sup>2</sup>, Elisabetta Vavala<sup>3</sup>, Antonella Di Sotto<sup>4</sup>, Gianni Sartorelli<sup>2</sup>, Mijat Božović<sup>2</sup>, Letizia Angiolella<sup>3</sup>, Gabriela Mazzanti<sup>4</sup>, Federico Pepi<sup>1</sup> and Rino Ragno<sup>2,\*</sup>





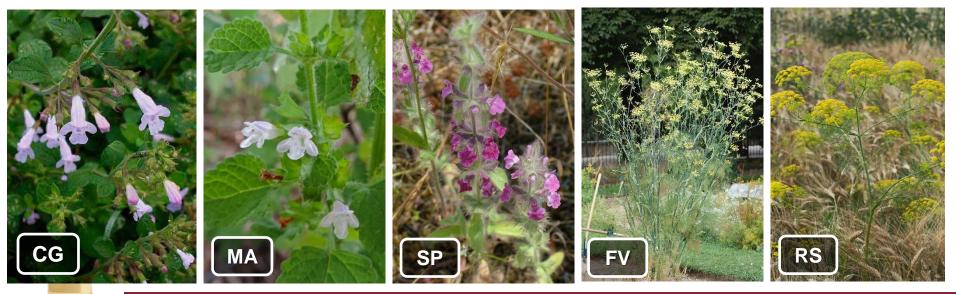
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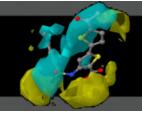
### **Investigated Plant Species**

- Calamintha nepeta (L.) Savi subsp. glandulosa (Req.) Ball (CG),
- Melissa officinalis L. subsp. altissima (Sibth. & Sm.) Arcangel (MA),
- Sideritis romana L. subsp. purpurea (Tal. ex Benth.) Heywood (SP),
- Foeniculum vulgare Miller (FV) and
- Ridolfia segetum Moris (RS).



Mijat Božović, PhD Dissertation







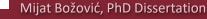
### **Plant Material Collecting**



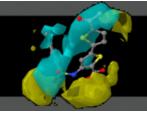


Tarquinia city, Province of Viterbo (Italy)

Kuće Rakića, Podgorica city (Montenegro)







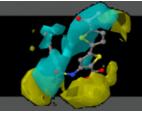


### **EO Extraction: Aims and Objectives**

- The aim was to develop a systematic extraction system using steam distillation technique, in terms of different harvesting and extraction times;
- Having in mind that EO is made up of many distinct molecules which come together to form its aroma and therapeutic properties, it should be emphasized that some of these molecules are delicate structures that can be altered or destroyed by adverse environmental conditions;
- Longer distillation may give more complete oil, but on the other hand, it may lead to the accumulation of more artifacts; all of that may have a curious effect on the physical characteristics of EO (odor, density, color), as well as on its biological activities.



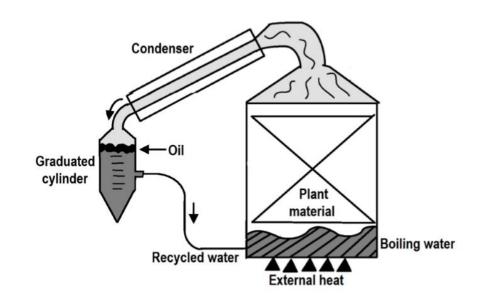






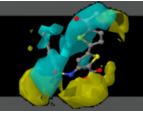
### **EO Extraction: In General**





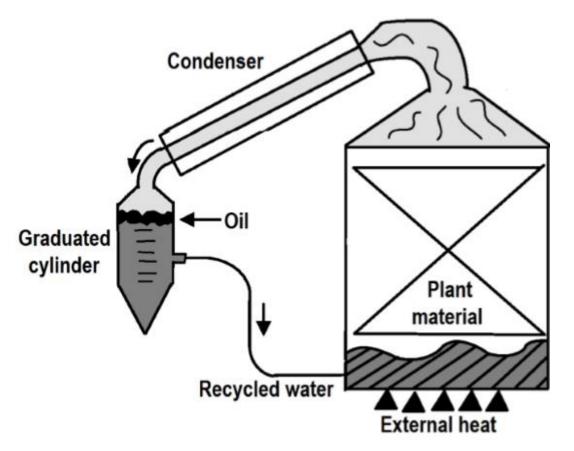
A direct steam distillation process using a 62 L steel distillator apparatus (Albrigi Luigi E0131, Verona, Italy)







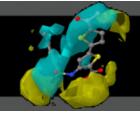
- EO fractions were separated at different time intervals;
- The accumulated EO/water double phase was extracted 3 times with diethyl ether;
- The organic layers were dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and deprived of the solvent *in vacuo* to furnish oils.



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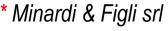
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### **EO Extraction: The Method Validation and Application**

- Rosmarinus officinalis L. \*
- Thymus serphyllum L. \*
- Origanum vulgare L. \*
- Origanum dictamnus L.\*
- Mentha spicata L. \*
- Melissa officinalis L. \*
- Salvia officinalis L. \*
- Salvia sclarea L. \*
- Hyssopus officinalis L. \*
- Stachys officinalis L. \*
- Aloysia citridora Paláu \*
- Matricaria chamomilla L. \*
- Calendula officinalis L. \*

- Heterotheca inuloides Cass. \*
- Helichrysum italicum (Roth.) Don fil. \*
- Eucalyptus globulus Labill. \*
- Jasminum officinale L. \*
- Citrus x aurantium L. \*
- Rosa gallica L. \*
- Agrimonia eupatoria L. \*
- Alchemilla vulgaris L. \*
- Ocimum basilicum L. \*\*
- Mentha x villosa Hunds. \*\*
- Origanum vulgare L.
- Mentha longifolia Huds.
- Mentha suaveolens Ehrh. \*\*\*

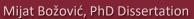
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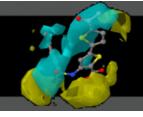






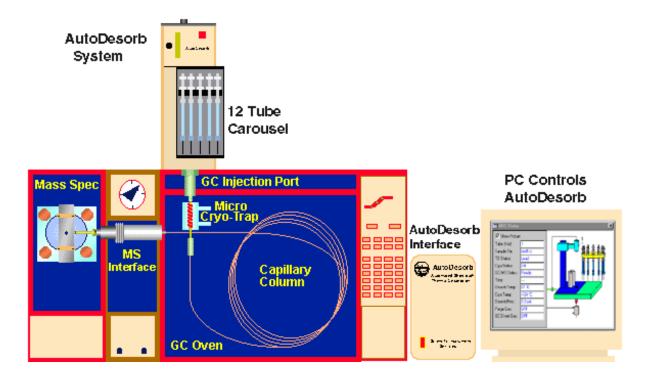
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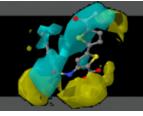
### **Chemical Analysis of EOs**





GC-MS analysis using a Perkin Elmer GS-MS equipped with a Stabilwax fused silica capillary column







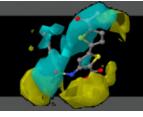
### Microbiology: Antifungal and Anti-biofilm Activities



- The *in vitr*o antifungal activity of EOs was evaluated against the reference strain *Candida albicans* ATCC 10231;
- The MIC was determined by *microbroth dilution method* described in the CLSI M27-A3 Standard following the international recommendations given by the National Committee for Clinical Laboratory Standards;
- The anti-biofilm activity was tested on bacterial cultures of 5 different strains belonging to genera *Pseudomonas* and *Staphylococcus*;
- Biofilm formation was assessed in static conditions;
- To measure the formation of biofilm, crystal violet staining was used;
- The biofilm-forming inhibition of less than 35% has been considered as not effective.







### **EO Extraction: Yields Numbers**

	<b>h</b> <sup>1</sup>	1	2	3	6	12	24
Plant species	<i>m</i> <sup>2</sup>						
SP	Jun.	0.011	0.014	0.016	0.020	0.027	0.038
MA	Jul.	0.010	0.014	0.016	0.019	0.024	0.030
	Aug.	0.020	0.026	0.029	0.033	0.036	0.039
	Sep.	0.020	0.026	0.028	0.031	0.034	0.037
MS	Jul.	0.030	0.040	0.050	0.050	0.060	0.070
	Aug.	0.070	0.090	0.100	0.120	0.150	0.190
	Sep.	0.050	0.090	0.090	0.100	0.110	0.180
CG	Jul.	0.300	0.350	0.360	0.366	0.370	0.373
	Aug.	0.300	0.360	0.400	0.420	0.426	0.432
	Sep.	0.190	0.250	0.300	0.360	0.376	0.381
	Oct.	0.180	0.260	0.290	0.320	0.328	0.328
FV	Aug.	0.070	0.110	0.140	0.180	0.196	0.213
	Sep.	0.090	0.140	0.170	0.200	0.218	0.240
	Oct.	0.360	0.640	0.830	1.090	1.210	1.250
RS	Jul.	0.200	0.300	0.440	0.640	0.740	0.800

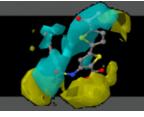
#### Relative yield % of essential oils over time

by www.

<sup>1</sup> Extraction hour, <sup>2</sup> Month of harvesting.







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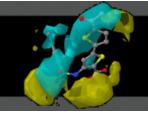
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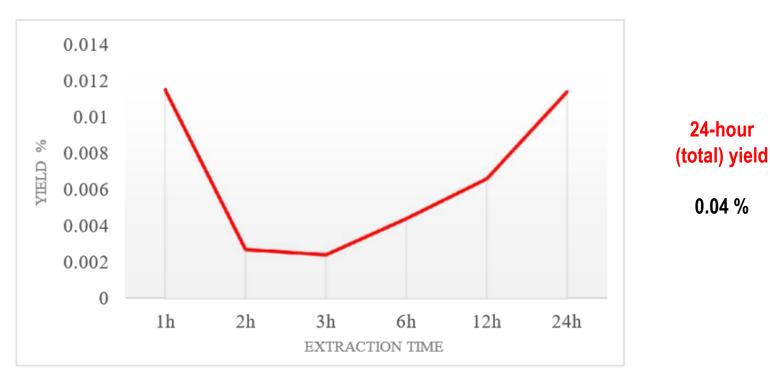
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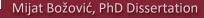




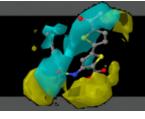
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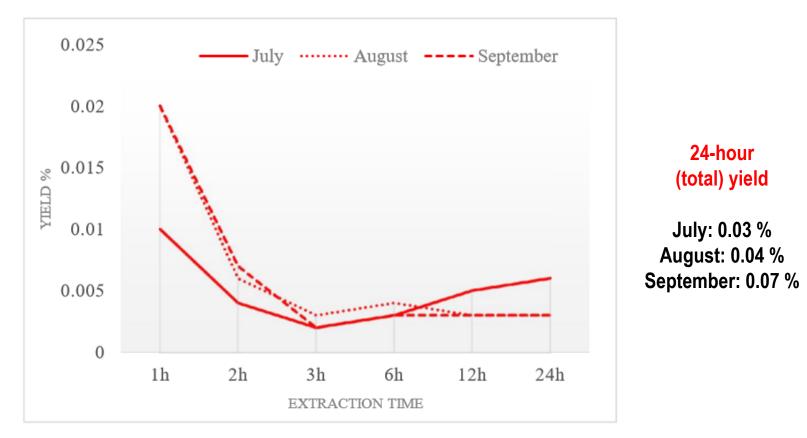




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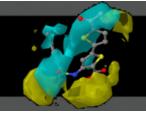


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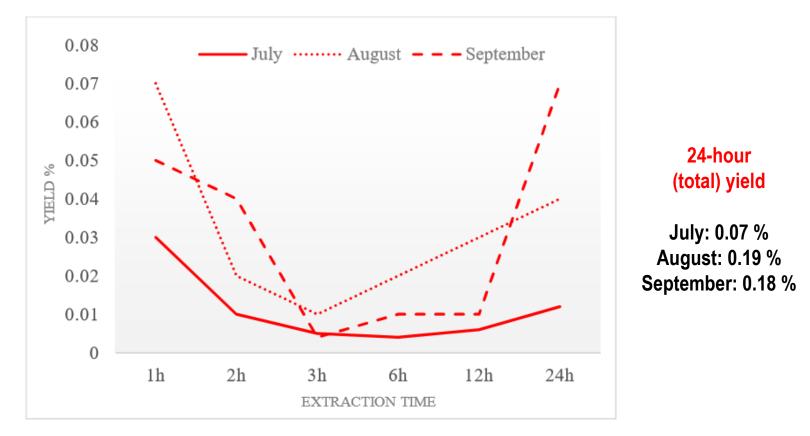


#### Yields curves for Melissa altissima monitored for 3 months







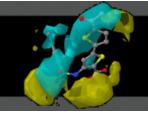


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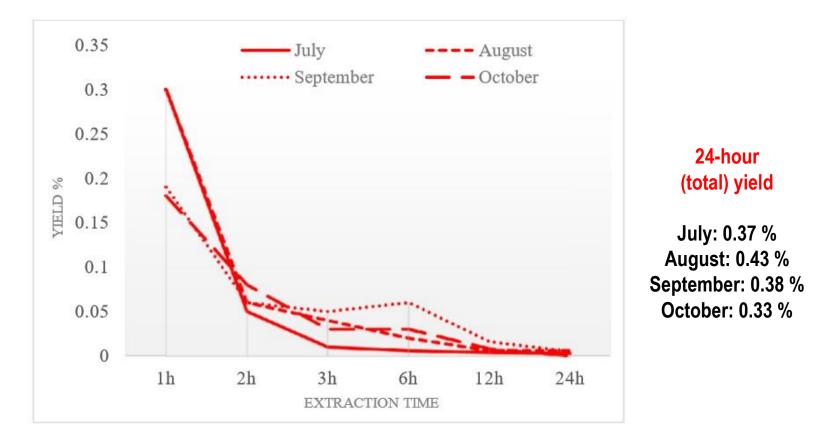


#### Yields curves for Mentha suaveolens monitored for 3 months





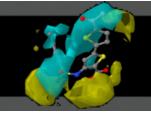




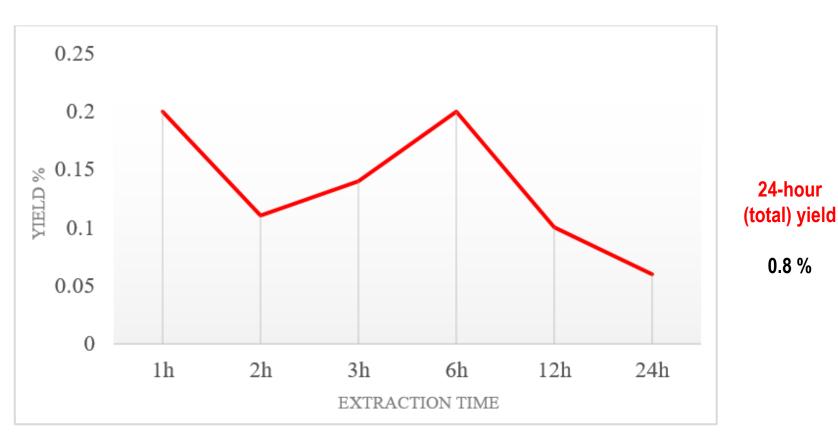
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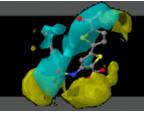


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#### Yield curve for **Ridolfia segetum** harvested in July

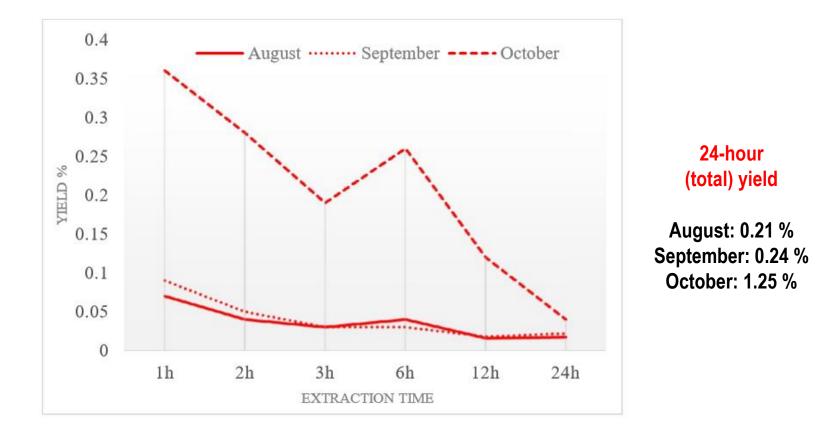


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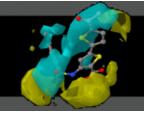
### **EO Extraction: Yields Plots**





#### Yields curves for Foeniculum vulgare monitored for 3 months







### **Chemical Analyses of EOs**

[		July <sup>2</sup>			August		September			
$h^{1}$	PO	PHA	CIN	PO	PHA	CIN	PO	PHA	CIN	
1	87.2	-	0.2	65.0	5.1	-	38.7	1.6	18.8	
2	70.6	-	0.1	77.5	8.0	-	35.6	10.1	-	
3	65.6	-	-	50.0	16.5	-	69.5	0.1	-	
6	26.0	-	-	16.9	9.1	23.1	13.2	12.1	2.7	
12	14.0	-	-	2.4	2.6	34.5	5.5	6.4	13.0	
24	-	-	7.7	-	0.4	38.8	5.6	6.3	19.0	

by www.

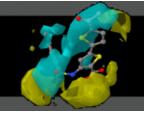
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PO: *piperitenone oxide*; PHA: *α-pharnesene*; CIN: *cinerolone*

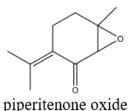
#### Chemical composition of Mentha suaveolens EOs







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								piperi	tenone oxide
		July <sup>2</sup>			August		S	Septembe	r
h 1	PO	PHA	CIN	PO	PHA	CIN	PO	PHA	CIN
1	87.2	-	0.2	65.0	5.1	-	38.7	1.6	18.8
2	70.6	-	0.1	77.5	8.0	-	35.6	10.1	-
3	65.6	-	-	50.0	16.5	-	69.5	0.1	-
6	26.0	-	-	16.9	9.1	23.1	13.2	12.1	2.7
12	14.0	-	-	2.4	2.6	34.5	5.5	6.4	13.0
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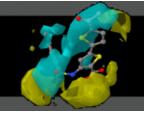
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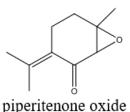




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2	70.6	-	0.1	77.5	8.0	-	35.6	10.1	-		
3	65.6	-	-	50.0	16.5	-	69.5	0.1	-		
6	26.0	-	-	16.9	9.1	23.1	13.2	12.1	2.7		
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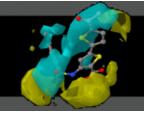
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Chemical composition of Mentha suaveolens EOs









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2	70.6	-	0.1	77.5	8.0	-	35.6	10.1	-	
3	65.6	-	-	50.0	16.5	-	69.5	0.1	-	
6	26.0	-	-	16.9	9.1	23.1	13.2	12.1	2.7	
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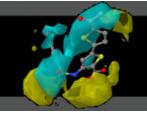
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3	65.6	-	-	50.0	16.5		69.5	0.1	
6	26.0	-	-	16.9	9.1	23.1	13.2	12.1	2.7
12	14.0	-	-	2.4	2.6	34.5	5.5	6.4	13.0
24	-	-	7.7	-	0.4	38.8	5.6	6.3	19.0

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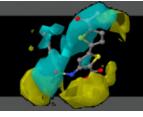
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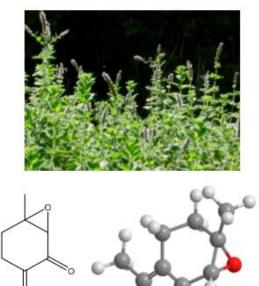








### **Chemical Analyses of EOs**



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Review

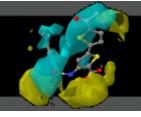
#### *Mentha suaveolens* Ehrh. (Lamiaceae) Essential Oil and Its Main Constituent Piperitenone Oxide: Biological Activities and Chemistry <sup>†</sup>

#### Mijat Božović, Adele Pirolli and Rino Ragno \*

Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University, P.le Aldo Moro 5, 00185 Rome, Italy; E-Mails: mijatboz@gmail.com (M.B.); adele.pirolli@uniroma1.it (A.P.)



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[		July <sup>2</sup>			August	t	S	eptemb	er	October		
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

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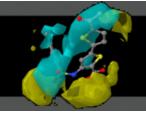
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*

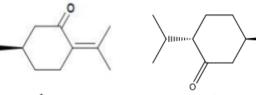








### Chemical Analyses of EOs



pulegone

by www.

menthone

		July <sup>2</sup>			August	t	S	eptemb	er	October		
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

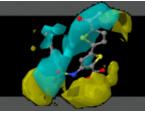
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*

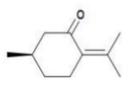








### **Chemical Analyses of EOs**



by www.

											pul	egone
[		July <sup>2</sup>			August	t	S	eptemb	er		October	•
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

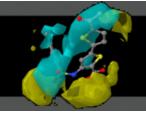
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*

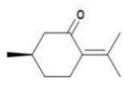








### **Chemical Analyses of EOs**



by www.

											pul	egone
		July <sup>2</sup>			August	t	S	eptemb	er		October	•
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

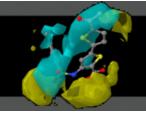
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*

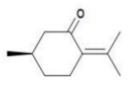








### **Chemical Analyses of EOs**



by www.

											pule	egone
		July <sup>2</sup>			August	t	S	eptemb	er		October	
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

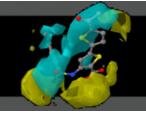
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*

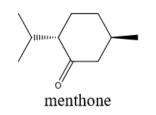












be www.

_												
	July <sup>2</sup>				August			ept <mark>o 1</mark> b	er	Oc		
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

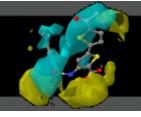
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*











[	July <sup>2</sup>				August	t	September			October		
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

EN WARD

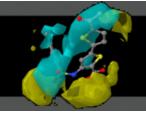
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*









### **Chemical Analyses of EOs**

by www.

chrysanthenone

	July <sup>2</sup>			August			September			October		
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

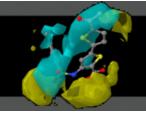
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*









### **Chemical Analyses of EOs**

by www.

chrysanthenone

	July <sup>2</sup>			August			September			October		
h 1	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY	PUL	MEN	CRY
1	76.8	3.1	4.4	80.8	3.9	2.6	48.8	20.3	1.3	42.5	35.4	1.3
2	77.7	0.8	10.5	84.7	2.1	5.2	62.5	20.0	2.0	57.5	27.8	2.3
3	64.3	0.6	20.3	80.0	1.0	9.0	72.9	11.2	3.4	53.3	23.6	3.3
6	53.2	0.6	22.7	66.0	0.8	18.4	74.9	5.9	6.8	68.2	10.9	5.3
12	41.1	0.6	33.9	55.4	0.7	24.0	64.8	4.1	13.6	68.8	7.0	5.4
24	37.7	-	27.3	49.9	0.7	29.5	43.2	3.7	18.6	51.8	6.8	13.4

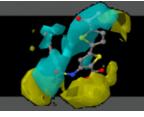
<sup>1</sup> Extraction hours, <sup>2</sup> Month of narvesting.

#### PUL: *pulegone*; MEN: *menthone*; CRY: *chrysanthenone*





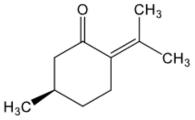






### **Chemical Analyses of EOs**









Review

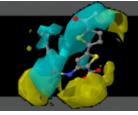
#### *Calamintha nepeta* (L.) Savi and Its Main Essential Oil Constituent Pulegone: Biological Activities and Chemistry

Mijat Božović<sup>§</sup> and Rino Ragno<sup>§,c,\*</sup>

<sup>§</sup>Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University, P.le Aldo Moro 5, 00185 Rome, Italy; E-mail: mijat.bozovic@uniroma1.it (M.B.)
<sup>§</sup>Alchemical Dynamics s.r.l., 00125 Rome, Italy

\* Corresponding author: rino.ragno@uniroma1.it (R.R.); Tel.: +39-06-4991-3937; Fax: +39-06-4991-3627

30/01/2017 36





[		August <sup>2</sup>	2	S	eptemb	er	October			
$h^{1}$	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH	
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7	
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6	
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0	
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6	
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4	
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8	

by www.

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

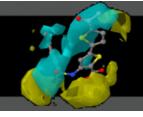
#### OCI: *o-cymene*; EST: *estragole*; APH: *α-phellandrene*





Mijat Božović, PhD Dissertation





## **Chemical Analyses of EOs**



by www.

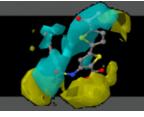
					o-cymene							
[		August <sup>2</sup>	2	S	eptemb	er	October					
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH			
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7			
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6			
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0			
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6			
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4			
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8			

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

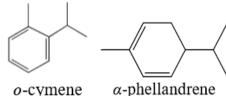








### **Chemical Analyses of EOs**



EN WARD

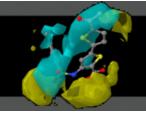
							o-cymen	e a-phena	indrene	
[		August <sup>2</sup>		S	eptemb	er	October			
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH	
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7	
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6	
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0	
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6	
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4	
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8	

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

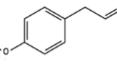








### **Chemical Analyses of EOs**



by www.

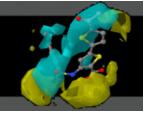
estragole

[		August <sup>2</sup>	2	S	eptemb	er	Ocuber		
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.









by www.

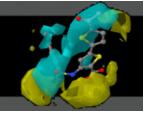
									o-cymene
		August <sup>2</sup>	2	S	eptemb	er		October	
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.











by www.

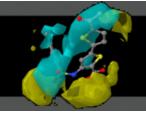
									o-cymene	
[		August <sup>2</sup>	2	S	eptemb	er		October		
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH	
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7	
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6	
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0	
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6	
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4	
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8	

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

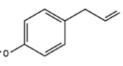








### **Chemical Analyses of EOs**



by www.

estragole

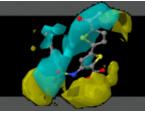
[		August <sup>2</sup>	2	S	eptemb	er	October		
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

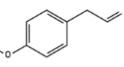








## **Chemical Analyses of EOs**



by www.

estragole

[		Aus st <sup>2</sup>	2	S	eptemb	er	October		
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8

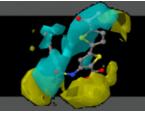
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### OCI: o-cymene; EST: estragole; APH: *α-phellandrene*

Chemical composition of Foeniculum vulgare EOs







### **Chemical Analyses of EOs**

					П		estragole		
[		Aus st <sup>2</sup>	2	S	eptensb	er	Oct ber		
h 1	OCI	EST	APH	OCI	EST	APH	OCI	EST	APH
1	22.9	12.0	11.8	52.2	-	12.9	3.9	57.6	5.7
2	21.5	12.5	12.3	35.9	-	15.7	2.7	54.8	6.6
3	24.4	14.0	5.9	25.7	-	1.3	3.5	39.1	8.0
6	18.7	5.9	20.0	18.8	-	6.0	1.6	30.1	8.6
12	13.6	1.7	3.8	12.7	-	0.9	0.6	9.4	3.4
24	2.4	0.9	-	18.1	-	0.4	6.0	8.6	2.8

by www.

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

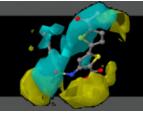
#### OCI: o-cymene; EST: estragole; APH: *α-phellandrene*





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[		July <sup>2</sup>			August		September			
h 1	CAR	CAO	PUL	CAR	CAO	PUL	CAR	CAO	PUL	
1	22.4	0.5	10.7	-	18.8	2.4	6.2	61.1	1.5	
2	24.0	16.6	14.1	-	23.7	-	25.6	19.3	8.6	
3	17.6	18.0	17.7	-	18.6	3.1	21.4	20.8	10.8	
6	10.9	12.2	9.0	9.3	20.4	11.7	17.1	7.5	11.0	
12	18.9	5.8	-	-	16.1	18.6	4.3	8.5	63.1	
24	5.0	-	29.6	6.9	17.3	22.9	6.6	14.0	19.2	

by www.

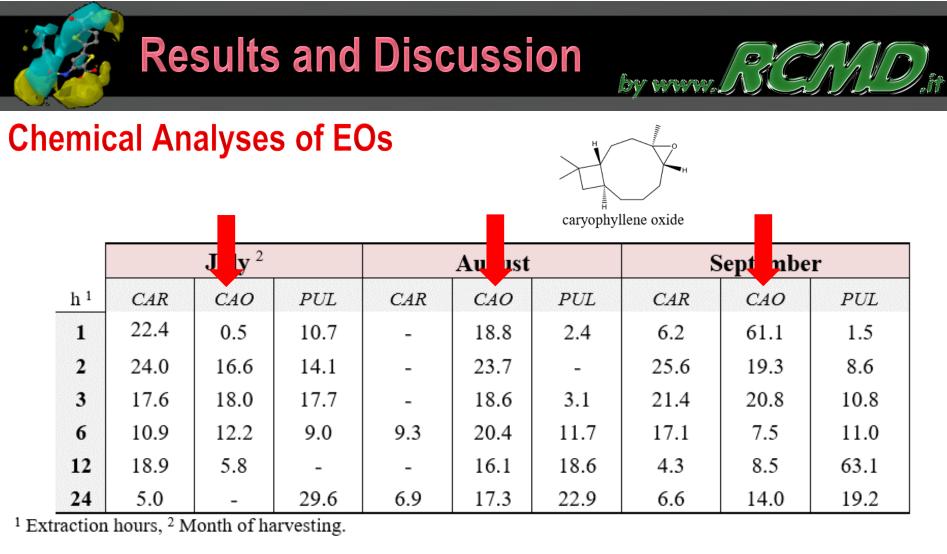
<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

#### CAR: caryophyllene; CAO: caryophyllene oxide; PUL: pulegone

### Chemical composition of Melissa altissima EOs



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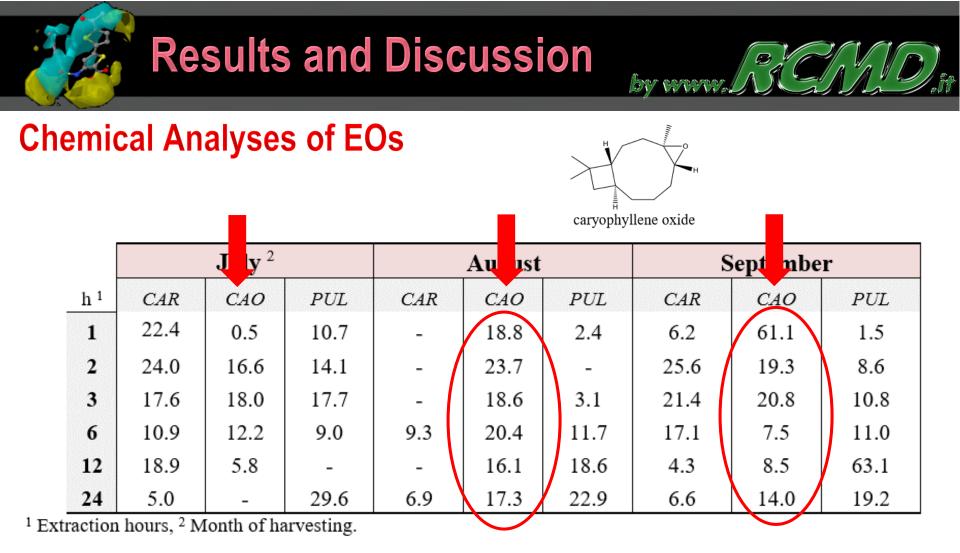
#### CAR: caryophyllene; CAO: caryophyllene oxide; PUL: pulegone

Chemical composition of Melissa altissima EOs



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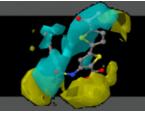


CAR: caryophyllene; CAO: caryophyllene oxide; PUL: pulegone

Chemical composition of Melissa altissima EOs



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### **Chemical Analyses of EOs**

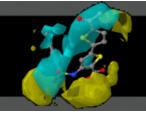
[		July <sup>2</sup>			August		September			
h 1	CAR	CAO	PUL	CAR	CAO	PUL	CAR	CAO	PUL	
1	22.4	0.5	10.7	-	18.8	2.4	6.2	61.1	1.5	
2	24.0	16.6	14.1	-	23.7	-	25.6	19.3	8.6	
3	17.6	18.0	17.7	-	18.6	3.1	21.4	20.8	10.8	
6	10.9	12.2	9.0	9.3	20.4	11.7	17.1	7.5	11.0	
12	18.9	5.8	-	-	16.1	18.6	4.3	8.5	63.1	
24	5.0	-	29.6	6.9	17.3	22.9	6.6	14.0	19.2	

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

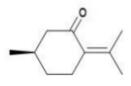
#### CAR: caryophyllene; CAO: caryophyllene oxide; PUL: pulegone

### Chemical composition of Melissa altissima EOs





### **Chemical Analyses of EOs**



by www.

							pule	gone	
		July <sup>2</sup>			August		S	Septembe	er
h 1	CAR	CAO	PUL	CAR	CAO	PUL	CAR	CAO	PUL
1	22.4	0.5	10.7	-	18.8	2.4	6.2	61.1	1.5
2	24.0	16.6	14.1	-	23.7	-	25.6	19.3	8.6
3	17.6	18.0	17.7	-	18.6	3.1	21.4	20.8	10.8
6	10.9	12.2	9.0	9.3	20.4	11.7	17.1	7.5	11.0
12	18.9	5.8	-	-	16.1	18.6	4.3	8.5	63.1
24	5.0	-	29.6	6.9	17.3	22.9	6.6	14.0	19.2

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

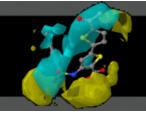
CAR: caryophyllene; CAO: caryophyllene oxide; PUL: pulegone

Chemical composition of Melissa altissima EOs

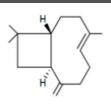








### **Chemical Analyses of EOs**



by www.

caryophyllene July<sup>2</sup> September August h 1 CAR CAO PUL CAR CAO PUL CAR CAO PUL 22.46.2 1 0.5 10.718.8 2.4 61.1 1.5 2 24.0 23.7 25.6 8.6 16.6 14.119.3 3 17.6 18.0 17.718.6 3.1 21.4 20.8 10.86 12.2 9.0 9.3 20.411.717.17.5 11.010.9 12 18.9 5.8 16.1 18.6 4.3 8.5 63.1 24 5.0 29.6 6.9 17.322.9 6.6 14.0 19.2

<sup>1</sup> Extraction hours, <sup>2</sup> Month of harvesting.

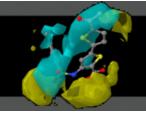
CAR: caryophyllene; CAO: caryophyllene oxide; PUL: pulegone

Chemical composition of Melissa altissima EOs



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	July					
h 1	OCI	API	LIM			
1	40.1	6.5	7.4			
2	23.6	11.7	4.5			
3	3.3	18.2	5.8			
6	12.1	22.3	3.3			
12	6.5	52.2	1.1			
24	3.5	60.3	0.2			

 $h^{1}$ GEL. SPA VER 22.21 18.12 25.227.7 3 14.426.76.4 9.2 15.48.5 6 12 6.3 11.5 10.46.7 24 8.9 13.9

June

<sup>1</sup> Extraction hours.

<sup>1</sup> Extraction hours.

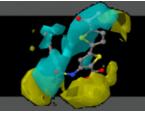
by www.

OCI: o-cymene; API: apiol; LIM: limonene; GEL: γ-elemene; SPA: spathulenol; VER: verbenone

5

Chemical composition of Ridolfia sefetum and Sideritis purpurea EOs

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### **Chemical Analyses of EOs**

		Ju					June	
h 1	OCI	API	LIM		h 1	GEL	SPA	VER
1	40.1	6.5	7.4	o-cymene	1	22.2	18.1	-
2	23.6	11.7	4.5	0	2	25.2	27.7	-
3	3.3	18.2	5.8		3	14.4	26.7	6.4
6	12.1	22.3	3.3		6	9.2	15.4	8.5
12	6.5	52.2	1.1	م apiol	12	6.3	10.4	11.5
24	3.5	60.3	0.2	apioi	24	6.7	8.9	13.9

<sup>1</sup> Extraction hours.

<sup>1</sup> Extraction hours.

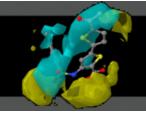
by www.

OCI: o-cymene; API: apiol; LIM: limonene; GEL: γ-elemene; SPA: spathulenol; VER: verbenone

6

Chemical composition of Ridolfia sefetum and Sideritis purpurea EOs

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	July					
h <sup>1</sup>	OCI	API	LIM			
1	40.1	6.5	7.4			
2	23.6	11.7	4.5			
3	3.3	18.2	5.8			
6	12.1	22.3	3.3			
12	6.5	52.2	1.1			
24	3.5	60.3	0.2			

22.21 18.125.22 27.7 3 14.426.76.4 9.2 15.48.5 6 12 6.3 11.5 10.46.7 24 8.9 13.9

June

SPA

VER

<sup>1</sup> Extraction hours.

<sup>1</sup> Extraction hours.

by www.

GEL.

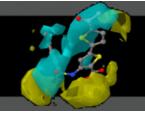
 $h^{1}$ 

OCI: o-cymene; API: apiol; LIM: limonene; GEL: γ-elemene; SPA: spathulenol; VER: verbenone

5

Chemical composition of Ridolfia sefetum and Sideritis purpurea EOs

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		July					Ju e	
h 1	OCI	API	LIM		h 1	GEL	SPA	VER
1	40.1	6.5	7.4		1	22.2	18.1	-
2	23.6	11.7	4.5	γ-elemene	2	25.2	27.7	-
3	3.3	18.2	5.8		3	14.4	26.7	6.4
6	12.1	22.3	3.3	HIMIN	6	9.2	15.4	8.5
12	6.5	52.2	1.1	он	12	6.3	10.4	11.5
24	3.5	60.3	0.2	spathulenol	24	6.7	8.9	13.9

<sup>1</sup> Extraction hours.

<sup>1</sup> Extraction hours.

by www.

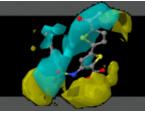
OCI: o-cymene; API: apiol; LIM: limonene; GEL: γ-elemene; SPA: spathulenol; VER: verbenone

5

Chemical composition of Ridolfia sefetum and Sideritis purpurea EOs

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	July		July			June		
h1	OCI	API	LIM	_	h <sup>1</sup>	GEL	SPA	VER
1	40.1	6.5	7.4		1	22.2	18.1	-
2	23.6	11.7	4.5		2	25.2	27.7	-
3	3.3	18.2	5.8		3	14.4	26.7	6.4
6	12.1	22.3	3.3		6	9.2	15.4	8.5
12	6.5	52.2	1.1	verbenone	12	6.3	10.4	11.5
24	3.5	60.3	0.2		24	6.7	8.9	13.9

<sup>1</sup> Extraction hours.

<sup>1</sup> Extraction hours.

by ward.

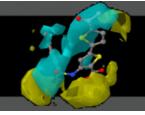
OCI: o-cymene; API: apiol; LIM: limonene; GEL: γ-elemene; SPA: spathulenol; VER: verbenone

5

Chemical composition of Ridolfia segetum and Sideritis purpurea EOs

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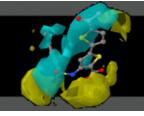
Sample <sup>1</sup>	MIC mg/mL	PO %
J1h	0.10	87.2
J2h	0.10	70.6
J3h	0.10	65.6
J6h	6.25	26.0
J12h	6.25	14.8
J24h	12.50	-
A1h	0.10	65.0
A2h	0.02	77.5
A3h	0.10	50.0
A6h	0.78	16.9
A12h	3.12	2.4
A24h	6.25	-
S1h	0.20	38.7
S2h	0.20	35.6
S3h	0.10	69.5
S6h	6.25	13.2
S12h	6.25	5.5
S24h	0.20	5.6
Miconazole #	0.016	-

by www.l







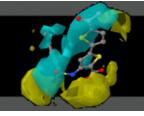




Sample <sup>1</sup>	MIC mg/mL	PO %
J1h	0.10	87.2
J2h	0.10	70.6
J3h	0.10	65.6
J6h	6.25	26.0
J12h	6.25	14.8
J24h	12.50	-
A1h	0.10	65.0
A2h	0.02	77.5
A3h	0.10	50.0
A6h	0.78	16.9
A12h	3.12	2.4
A24h	6.25	-
S1h	0.20	38.7
S2h	0.20	35.6
S3h	0.10	69.5
S6h	6.25	13.2
S12h	6.25	5.5
S24h	0.20	5.6
Miconazole #	0.016	-

by www.l







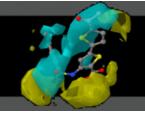
Sample <sup>1</sup>	MIC mg/mL	PO %	
J1h	0.10	87.2	
J2h	0.10	70.6	
J3h	0.10	65.6	
J6h	6.25	26.0	
J12h	6.25	14.8	
J24h	12.50	÷	$\sim$
A1h	0.10	65.0	
A2h	0.02	77.5	
A3h	0.10	50.0	
A6h	0.78	16.9	piperitenone oxide
A12h	3.12	2.4	
A24h	6.25	÷	
S1h	0.20	38.7	
S2h	0.20	35.6	
S3h	0.10	69.5	
S6h	6.25	13.2	
S12h	6.25	5.5	
S24h	0.20	5.6	
Miconazole #	0.016	-	

by www.l











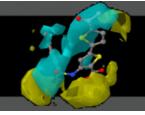
Sample <sup>1</sup>	MIC mg/mL	PO %
J1h	0.10	87.2
J2h	0.10	70.6
J3h	0.10	65.6
J6h	6.25	26.0
J12h	6.25	14.8
J24h	12.50	-
A1h	0.10	65.0
A2h	0.02	77.5
A3h	0.10	50.0
A6h	0.78	16.9
A12h	3.12	2.4
A24h	6.25	-
S1h	0.20	38.7
S2h	0.20	35.6
S3h	0.10	69.5
S6h	6.25	13.2
S12h	6.25	5.5
S24h	0.20	5.6
Miconazole #	0.016	

by www.l







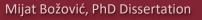




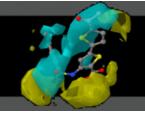
Sample <sup>1</sup>	MIC mg/mL	PO %
J1h	0.10	87.2
J2h	0.10	70.6
J3h	0.10	65.6
J6h	6.25	26.0
J12h	6.25	14.8
J24h	12.50	-
A1h	0.10	65.0
A2h	0.02	77.5
A3h	0.10	50.0
A6h	0.78	16.9
A12h	3.12	2.4
A24h	6.25	-
S1h	0.20	38.7
S2h	0.20	35.6
S3h	0.10	69.5
S6h	6.25	13.2
S12h	6.25	5.5
S24h	0.20	5.6
/liconazole #	0.016	-

by www.l







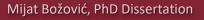




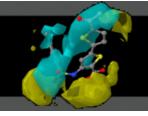
Sample <sup>1</sup>	MIC mg/mL	PO %
J1h	0.10	87.2
J2h	0.10	70.6
J3h	0.10	65.6
J6h	6.25	26.0
J12h	6.25	14.8
J24h	12.50	-
A1h	0.10	65.0
A2h	0.02	77.5
A3h	0.10	50.0
A6h	0.78	16.9
A12h	3.12	2.4
A24h	6.25	
S1h	0.20	38.7
S2h	0.20	35.6
S3h	0.10	69.5
S6h	6.25	13.2
S12h	6.25	5.5
S24h	0.20	5.6
Miconazole #	0.016	-

by www.











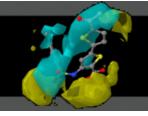
### **Antifungal Activity of EOs**

Sample 1	MIC mg/mL PUL %		DIT 04	Sample <sup>1</sup>	MIC mg/mL		PUL %
Sampic	24h	48h	ICL /0	Sample -	24h	48h	FUL 70
J1h	6.24	6.24	76.8	S1h	6.24	12.48	48.8
J2h	6.24	12.48	77.7	S2h	6.24	12.48	62.5
J3h	0.78	6.24	64.3	S3h	3.12	12.48	72.9
J6h	na	na	53.2	S6h	1.56	6.24	74.9
J12h	12.48	12.48	41.1	S12h	3.12	12.48	64.8
J24h	na	na	37.7	S24h	12.48	na	43.2
A1h	3.12	12.48	80.8	O1h	6.24	12.48	42.5
A2h	3.12	6.24	84.7	O2h	6.24	12.48	57.5
A3h	1.56	3.12	80.0	O3h	6.24	12.48	53.3
A6h	3.12	6.24	66.0	O6h	12.48	12.48	68.2
A12h	6.24	na	55.4	O12h	12.48	12.48	68.8
A24h	6.24	12.48	49.9	O24h	na	na	51.8



Anti-Candida albicans activities of Calamintha glandulosa EOs







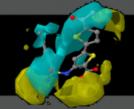
### **Antifungal Activity of EOs**

MIC mg/mL		DIT 0/-	Sampla	MIC mg/mL		PUL %
24h	48h	FUL 70	Sample -	24h	48h	FUL 70
6.24	6.24	76.8	S1h	6.24	12.48	48.8
6.24	12.48	77.7	S2h	6.24	12.48	62.5
0.78	6.24	64.3	S3h	3.12	12.48	72.9
na	na	53.2	S6h	1.56	6.24	74.9
12.48	12.48	41.1	S12h	3.12	12.48	64.8
na	na	37.7	S24h	12.48	na	43.2
3.12	12.48	80.8	O1h	6.24	12.48	42.5
3.12	6.24	84.7	O2h	6.24	12.48	57.5
1.56	3.12	80.0	O3h	6.24	12.48	53.3
3.12	6.24	66.0	O6h	12.48	12.48	68.2
6.24	na	55.4	O12h	12.48	12.48	68.8
6.24	12.48	49.9	O24h	na	na	51.8
	24h 6.24 6.24 0.78 na 12.48 na 3.12 3.12 1.56 3.12 6.24	24h     48h       6.24     6.24       6.24     12.48       0.78     6.24       na     na       12.48     12.48       na     na       12.48     12.48       na     na       3.12     6.24       1.56     3.12       3.12     6.24       1.56     3.12       3.12     6.24       1.56     3.12       3.12     6.24       1.56     3.12       3.12     6.24       1.56     3.12       3.12     6.24       1.56     3.12	24h48h6.246.2476.86.2412.4877.70.786.2464.3nana53.212.4812.4841.1nana37.73.1212.4880.83.126.2484.71.563.1280.03.126.2466.06.24na55.4	24h         48h         PUL %         Sample 1           6.24         6.24         76.8         S1h           6.24         12.48         77.7         S2h           0.78         6.24         64.3         S3h           na         na         53.2         S6h           12.48         12.48         41.1         S12h           na         na         37.7         S24h           3.12         12.48         80.8         O1h           3.12         6.24         84.7         O2h           1.56         3.12         80.0         O3h           3.12         6.24         66.0         O6h           6.24         76.8         10.0         10.0	24h48h24h6.246.2476.8S1h6.246.2412.4877.7S2h6.240.786.2464.3S3h3.12nana53.2S6h1.5612.4812.4841.1S12h3.12nana37.7S24h12.483.1212.4880.8O1h6.243.126.2484.7O2h6.241.563.1280.0O3h6.243.126.2466.0O6h12.483.126.2466.0O6h12.486.24na55.4O12h12.48	24h48h24h24h48h $6.24$ $6.24$ $76.8$ S1h $6.24$ $12.48$ $6.24$ $12.48$ $77.7$ S2h $6.24$ $12.48$ $0.78$ $6.24$ $64.3$ S3h $3.12$ $12.48$ $na$ $na$ $53.2$ S6h $1.56$ $6.24$ $12.48$ $12.48$ $41.1$ S12h $3.12$ $12.48$ $na$ $na$ $37.7$ S24h $12.48$ $na$ $3.12$ $12.48$ $80.8$ O1h $6.24$ $12.48$ $3.12$ $12.48$ $80.8$ O1h $6.24$ $12.48$ $3.12$ $6.24$ $84.7$ O2h $6.24$ $12.48$ $3.12$ $6.24$ $84.7$ O2h $6.24$ $12.48$ $3.12$ $6.24$ $66.0$ O6h $12.48$ $12.48$ $6.24$ $na$ $55.4$ O12h $12.48$ $12.48$



Anti-Candida albicans activities of Calamintha glandulosa EOs







81-1	MIC n	ng/mL	PUL % Sample <sup>1</sup>	G1. 1	MIC n	ng/mL	DIT 04
Sample <sup>1</sup>	24h	48h		Sample -	24h	48h	PUL %
J1h	6.24	6.24	76.8	S1h	6.24	12.48	48.8
J2h	6.24	12.48	77.7	S2h	6.24	12.48	62.5
J3h	0.78	6.24	64.3	S3h	3.12	12.48	72.9
J6h	na	na	53.2	S6h	1.56	6.24	74.9
J12h	12.48	12.48	41.1	S12h	3.12	12.48	64.8
J24h	na	na	37.7	S24h	12.48	na	43.2
A1h	3.12	12.48	80.8	O1h	6.24	12.48	42.5
A2h	3.12	6.24	84.7	O2h	6.24	12.48	57.5
A3h	1.56	3.12	80.0	O3h	6.24	12.48	53.3
A6h	3.12	6.24	66.0	O6h	12.48	12.48	68.2
A12h	6.24	na	55.4	O12h	12.48	12.48	68.8
A24h	6.24	12.48	49.9	O24h	na	na	51.8

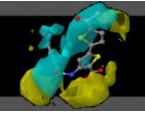
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Anti-Candida albicans activities of Calamintha glandulosa EOs

Mijat Božović, PhD Dissertation









#### Article



#### Essential Oil Extraction, Chemical Analysis and Anti-*Candida* Activity of *Calamintha nepeta* (L.) Savi subsp. *glandulosa* (Req.) Ball—New Approaches

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Mijat Božović <sup>1,2,†</sup>, Stefania Garzoli <sup>2,†</sup>, Manuela Sabatino <sup>1,2</sup>, Federico Pepi <sup>2</sup>, Anna Baldisserotto <sup>3</sup>, Elisa Andreotti <sup>4</sup>, Carlo Romagnoli <sup>4</sup>, Antonello Mai <sup>2</sup>, Stefano Manfredini <sup>3,\*</sup> and Rino Ragno <sup>1,2,5,\*</sup>

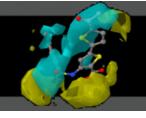
- <sup>1</sup> Rome Center for Molecular Design, Sapienza University, P.le Aldo Moro 5, 00185 Rome, Italy; mijat.bozovic@uniroma1.it (M.B.); manuela.sabatino@uniroma1.it (M.S.)
- <sup>2</sup> Department of Drug Chemistry and Technology, Sapienza University, P.le Aldo Moro 5, 00185 Rome, Italy; stefania.garzoli@uniroma1.it (S.G.); federico.pepi@uniroma1.it (F.P.); antonello.mai@uniroma1.it (A.M.)
- <sup>3</sup> Department of Life Sciences and Biotechnology, School of Pharmacy and Heath Products, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy; anna.baldisserotto@unife.it
- <sup>4</sup> Department of Life Sciences, University of Modena and Reggio Emilia, Viale Caduti in Guerra 127, 41121 Modena, Italy; elisa.andreotti@unimo.it (E.A.); carlo.romagnoli@unimo.it (C.R.)
- <sup>5</sup> Alchemical Dynamics s.r.l., 00125 Rome, Italy
- \* Correspondence: stefano.manfredini@unife.it (S.M.); rino.ragno@uniroma1.it (R.R.), Tel.: +39-532-974-635 (S.M.); +39-6-4991-3937 (R.R.); Fax: +39-532-455-953 (S.M.); +39-6-4991-3627 (R.R.)
- † M.B. and S.G. contributed equally to the paper.





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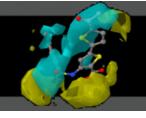
### **Antifungal Activity of EOs**

Sample 1	MIC mg/mL		C 1 1	MIC n	MIC mg/mL			MIC mg/mL	
Sample <sup>1</sup>	24h	48h	Sample <sup>1</sup>	24h	48h	Sample <sup>1</sup>	24h	48h	
A1h	na	na	S1h	na	na	O1h	1.56	6.24	
A2h	12.48	na	S2h	6.24	12.48	O2h	3.12	12.48	
A3h	na	na	S3h	6.24	12.48	O3h	1.56	12.48	
A6h	na	na	S6h	6.24	na	O6h	3.12	6.24	
A12h	na	na	S12h	6.24	na	O12h	6.24	12.48	
A24h	na	na	S24h	12.48	12.48	O24h	na	na	



#### Anti-Candida albicans activities of Foeniculum vulgare EOs

Mijat Božović, PhD Dissertation





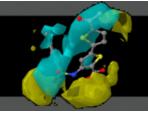
### **Antifungal Activity of EOs**

Comple 1	MIC mg/mL		Commits 1	MIC n	MIC mg/mL			MIC mg/mL	
Sample <sup>1</sup>	24h	48h	Sample <sup>1</sup>	24h	48h	Sample <sup>1</sup>	24h	48h	
A1h	na	na	S1h	na	na	O1h	1.56	6.24	
A2h	12.48	na	S2h	6.24	12.48	O2h	3.12	12.48	
A3h	na	na	S3h	6.24	12.48	O3h	1.56	12.48	
A6h	na	na	S6h	6.24	na	O6h	3.12	6.24	
A12h	na	na	S12h	6.24	na	O12h	6.24	12.48	
A24h	na	na	S24h	12.48	12.48	O24h	na	na	



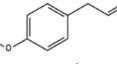
#### Anti-Candida albicans activities of Foeniculum vulgare EOs

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### **Antifungal Activity of EOs**

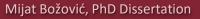


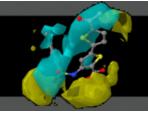
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Cample 1	MIC mg/mL 24h 48h		C 1 - 1	MIC n	MIC mg/mL			MIC mg/mL	
Sample <sup>1</sup>			Sample <sup>1</sup>	24h	48h	Sample <sup>1</sup>	24h	48h	
A1h	na	na	S1h	na	na	O1h	1.56	6.24	
A2h	12.48	na	S2h	6.24	12.48	O2h	3.12	12.48	
A3h	na	na	S3h	6.24	12.48	O3h	1.56	12.48	
A6h	na	na	S6h	6.24	na	O6h	3.12	6.24	
A12h	na	na	S12h	6.24	na	O12h	6.24	12.48	
A24h	na	na	S24h	12.48	12.48	O24h	na	na	



### Anti-Candida albicans activities of Foeniculum vulgare EOs





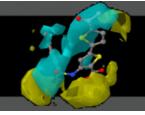


 The activity was evaluated against 5 bacterial pathogens: 2 strains belonging to Staphylococcus epidermidis, 2 belonging to S. aureus species and Pseudomonas aeruginosa PaO1;

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- The effect is reported as % of residual biofilm after treatment in comparison to untreated bacteria;
- In general, both strains of S. aureus were found to be the most resistant, whereas the strains of S. epidermidis species showed highest susceptibility.
- However, none of the concentrations caused the complete inhibition of biofilm;





### **Anti-biofilm Activity of EOs**

Calamintha glandulosa

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.

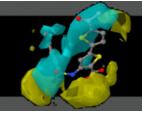


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Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
J1h	0.0003814	0.0003814	na	na	na
J2h	0.0003814	0.0003814	na	na	na
J3h	12.5	12.5	na	na	na
J6h	12.5	25	1.55	na	na
J12h	25	25	na	na	na
J24h	25	25	3.125	na	na
A1h	0.0003814	3.125	na	na	25
A2h	0.0003814	0.18	na	na	na
A3h	0.0003814	0.18	na	na	25
A6h	0.0003814	0.39	na	na	25
A12h	25	na	25	na	na
A24	12.5	na	6.25	12.5	na
S1h	0.0003814	na	25	na	na
S2h	0.0003814	0.18	25	na	na
S3h	0.0003814	0.18	na	na	na
S6h	0.0003814	0.18	na	na	na
S12h	na	na	12.5	na	na
S24h	na	na	0.39	na	na
O1h	0.0003814	0.39	na	6.25	na
O2h	0.0003814	0.39	na	na	na
O3h	12.5	12.5	25	na	na
O6h	0.0003814	0.39	na	na	na
O12h	na	na	na	na	na
O24h	25	na	na	na	na

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Calamintha glandulosa

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.

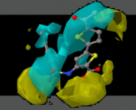


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Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
J1h	0.0003814	0.0003814	na	na	na
J2h	0.0003814	0.0003814	na	na	na
J3h	12.5	12.5	na	na	na
J6h	12.5	25	1.55	na	na
J12h	25	25	na	na	na
J24h	25	25	3.125	na	na
A1h	0.0003814	3.125	na	na	25
A2h	0.0003814	0.18	na	na	na
A3h	0.0003814	0.18	na	na	25
A6h	0.0003814	0.39	na	na	25
A12h	25	na	25	na	na
A24	12.5	na	6.25	12.5	na
S1h	0.0003814	na	25	na	na
S2h	0.0003814	0.18	25	na	na
S3h	0.0003814	0.18	na	na	na
S6h	0.0003814	0.18	na	na	na
S12h	na	na	12.5	na	na
S24h	na	na	0.39	na	na
O1h	0.0003814	0.39	na	6.25	na
O2h	0.0003814	0.39	na	na	na
O3h	12.5	12.5	25	na	na
O6h	0.0003814	0.39	na	na	na
O12h	na	na	na	na	na
O24h	25	na	na	na	na

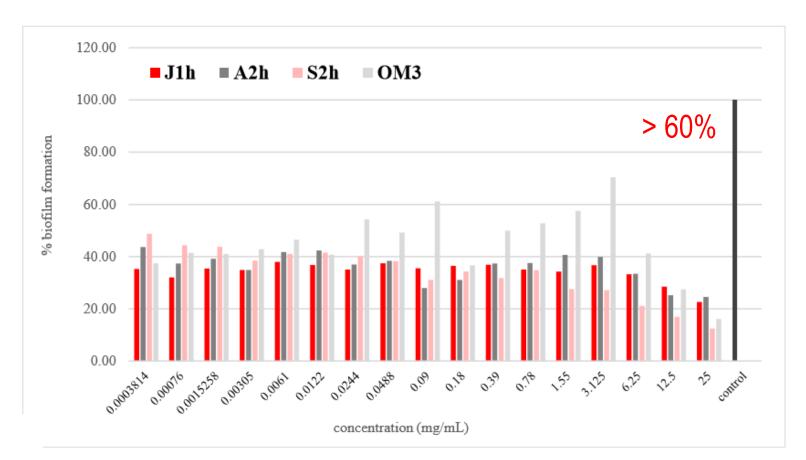
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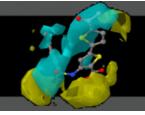


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Effects of selected Calamintha glandulosa samples treatment on biofilm formation for S. epidermidis O47







### **Anti-biofilm Activity of EOs**

<u>Calamintha glandulosa</u>

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.

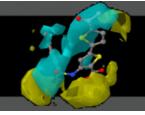
Sample <sup>1</sup>	S. epidermidis	S. epidermidis RT02A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
J1h	0.0003814	0.0003814	na	na	na
J2h	0.0003814	0.0003814	na	na	na
J3h	12.5	12.5	na	na	na
J6h	12.5	25	1.55	na	na
J12h	25	25	na	na	na
J24h	25	25	3.125	na	na
A1h	0.0003814	3.125	na	na	25
A2h	0.0003814	0.18	na	na	na
A3h	0.0003814	0.18	na	na	25
A6h	0.0003814	0.39	na	na	25
A12h	25	na	25	na	na
A24	12.5	na	6.25	12.5	na
S1h	0.0003814	na	25	na	na
S2h	0.0003814	0.18	25	na	na
S3h	0.0003814	0.18	na	na	na
S6h	0.0003814	0.18	na	na	na
S12h	na	lla	12.5	na	na
S24h	na	na	0.39	na	na
O1h	0.0003814	0.39	na	6.25	na
O2h	0.0003814	0.39	na	na	na
O3h	12.5	12.5	25	na	na
O6h	0.0003814	0.39	na	na	na
O12h	112	na	na	na	na
O24h	25	na	na	na	na

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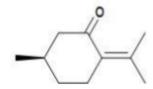
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### **Anti-biofilm Activity of EOs**

<u>Calamintha glandulosa</u>

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.



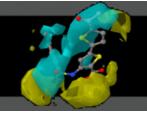
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Sample <sup>1</sup>	S. epidermidis 047	S. epidermidis RP02A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
J1h	0.0003814	0.0003814	na	na	na
J2h	0.0003814	0.0003814	na	na	na
J3h	12.5	12.5	na	na	na
J6h	12.5	25	1.55	na	na
J12h	25	25	na	na	na
J24h	25	25	3.125	na	na
A1h	0.0003814	3.125	na	na	25
A2h	0.0003814	0.18	na	na	na
A3h	0.0003814	0.18	na	na	25
A6h	0.0003814	0.39	na	na	25
A12h	25	na	25	na	na
A24	12.5	na	6.25	12.5	na
S1h	0.0003814	na	25	na	na
S2h	0.0003814	0.18	25	na	na
S3h	0.0003814	0.18	na	na	na
S6h	0.0003814	0.18	na	na	na
S12h	na	lla	12.5	na	na
S24h	na	na	0.39	na	na
O1h	0.0003814	0.39	na	6.25	na
O2h	0.0003814	0.39	na	na	na
O3h	12.5	12.5	25	na	na
O6h	0.0003814	0.39	na	na	na
O12h	112	na	na	na	na
O24h	25	na	na	na	na

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### **Anti-biofilm Activity of EOs**

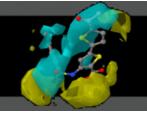
Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
1h	25	25	0.0003814	25	na
2h	25	25	0.0488	na	na
3h	na	25	3.125	12.5	na
6h	0.0003814	0.0003814	na	na	na
12h	25	25	12.5	na	na
24h	0.0003814	0.0003814	na	na	na

#### Ridolfia segetum

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.









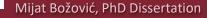
### **Anti-biofilm Activity of EOs**

Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
1h	25	25	0.0003814	25	na
2h	25	25	0.0488	na	na
3h	na	25	3.125	12.5	na
6h	0.0003814	0.0003814	na	na	na
12h	25	25	12.5	na	na
24h	0.0003814	0.0003814	na	na	na

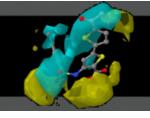
#### Ridolfia segetum

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.

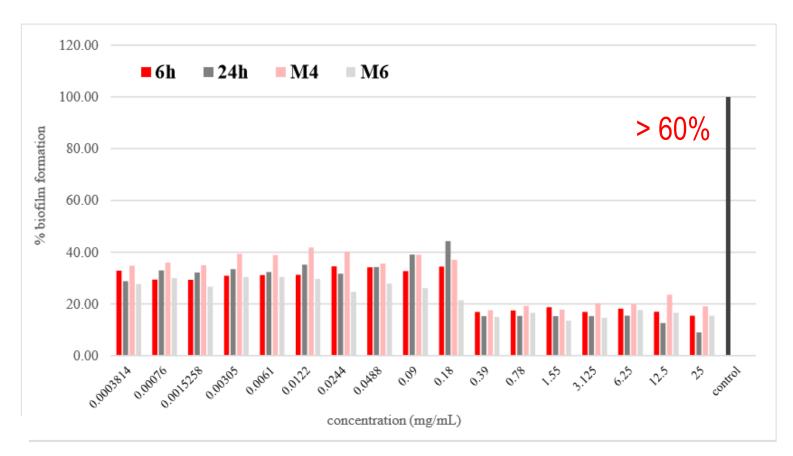




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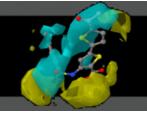
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Effects of selected Ridolfia segetum samples treatment on biofilm formation for S. epidermidis RP62A



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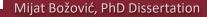
### **Anti-biofilm Activity of EOs**

Sample <sup>1</sup> S. epidermidi O47		S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
1h	25	25	0.0003814	25	na
2h	25	25	0.0488	na	na
3h	na	25	3.125	12.5	na
6h	0.0003814	0.0003814	na	na	na
12h	25	25	12.5	na	na
24h	0.0003814	0.0003814	na	na	na

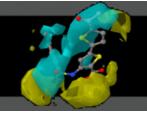
#### Ridolfia segetum

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.











### **Anti-biofilm Activity of EOs**

Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
1h	25	25	0.0003814	25	na
2h	25	25	0.0488	na	na
3h	na	25	3.125	12.5	na
6h	0.0003814	0.0003814	na	na	na
12h	25	25	12.5	na	na
24h	0.0003814	0.0003814	na	na	na
		Ridolfia so	aotum		0

#### <u>Ridolfia segetum</u>

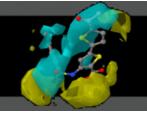
The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.





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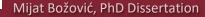
### **Anti-biofilm Activity of EOs**

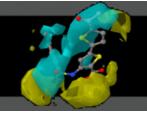
Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
1h	25	25	0.0003814	25	na
2h	25	25	0.0488	na	na
3h	na	25	3.125	12.5	na
6h	0.0003814	0.0003814	Па	na	na
12h	25	25	12.5	na	na
24h	0.0003814	0.0003814	na	na	na

#### Ridolfia segetum

The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.









### **Anti-biofilm Activity of EOs**

Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
1h	25	25	0.0003814	25	na
2h	25	25	0.0488	na	na
3h	na	25	3.125	12.5	na
6h	0.0003814	0.0003814	Па	na	na
12h	25	25	12.5	na	na
24h	0.0003814	0.0003814	na	na	na

#### **Ridolfia segetum**

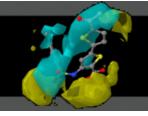
The lowest concentration (mg/mL) of samples causing at least 35% biofilm inhibition.





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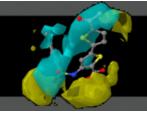




	Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
	A1h	na	3.125	0.0003814	25	na
	A2h	na	na	0.0003814	na	na
<u>Foeniculum</u>	A3h	25	25	0.0003814	na	na
vulgare	A6h	25	25	0.0003814	25	na
	A12h	25	12.5	0.0003814	25	na
The lowest	A24h	12.5	12.5	0.0003814	na	na
concentration	S1h	na	na	0.0003814	12.5	na
concentration	S2h	na	na	6.25	12.5	0.78
(mg/mL) of	S3h	25	25	na	12.5	na
samplas causing	S6h	25	25	12.5	12.5	25
samples causing	S12h	25	25	na	12.5	na
at least 35%	S24h	25	25	na	na	na
biofilm inhibition.	O1h	0.0003814	0.0003814	na	na	na
	O2h	na	na	na	na	na
	O3h	0.0003814	0.0003814	na	na	na
•	O6h	0.0003814	0.0003814	na	na	na
	O12h	na	na	25	na	na
	O24h	0.0003814	0.0003814	na	na	na





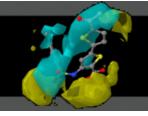




	Sample <sup>1</sup>	S. epidermidis	S. epidermidis	P. aeruginosa	S. aureus	S. aureus
	Sample -	Q47	RP62A	PaO1	6538P	25923
	A1h	na	3.125	0.0003814	25	na
	A2h	na	na	0.0003814	na	na
<u>Foeniculum</u>	A3h	25	25	0.0003814	na	na
vulgare	A6h	25	25	0.0003814	25	na
	A12h	25	12.5	0.0003814	25	na
The lowest	A24h	12.5	12.5	0.0003814	na	na
concentration	S1h	na	na	0.0003814	12.5	na
	S2h	na	na	6.25	12.5	0.78
(mg/mL) of	S3h	25	25	na	12.5	na
samples causing	S6h	25	25	12.5	12.5	25
i c	S12h	25	25	na	12.5	na
at least 35%	S24h	25	25	na	na	na
biofilm inhibition.	O1h	0.0003814	0.0003814	na	na	na
	O2h	na	na	na	na	na
	O3h	0.0003814	0.0003814	na	na	na
An	O6h	0.0003814	0.0003814	na	na	na
	O12h	na	na	25	na	na
	O24h	0.0003814	0.0003814	na	na	na





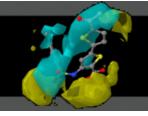




	Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
	A1h	na	3.125	0.0003814	25	na
	A2h	na	na	0.0003814	na	na
<u>Foeniculum</u>	A3h	25	25	0.0003814	na	na
vulgare	A6h	25	25	0.0003814	25	na
	A12h	25	12.5	0.0003814	25	na
The lowest	A24h	12.5	12.5	0.0003814	na	na
concentration	S1h	na	na	0.0003814	12.5	na
CONCENTIATION	S2h	na	na	6.25	12.5	0.78
(mg/mL) of	S3h	25	25	na	12.5	na
samples causing	S6h	25	25	12.5	12.5	25
, ,	S12h	25	25	na	12.5	na
at least 35%	S24h	25	25	na	na	na
biofilm inhibition.	O1h	0.0003814	0.0003814	na	na	na
	O2h	na	na	na	na	na
	O3h	0.0003814	0.0003814	na	na	na
An	O6h	0.0003814	0.0003814	na	na	na
	O12h	na	na	25	na	na
	O24h	0.0003814	0.0003814	na	na	na





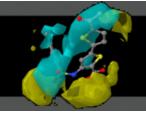




	Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923
	A1h	na	3.125	0.0003814	25	na
	A2h	na	na	0.0003814	na	na
<u>Foeniculum</u>	A3h	25	25	0.0003814	na	na
vulgare	A6h	25	25	0.0003814	25	na
	A12h	25	12.5	0.0003814	25	na
The lowest	A24h	12.5	12.5	0.0003814	na	na
concentration	S1h	na	na	0.0003814	12.5	na
CONCENTIATION	S2h	na	na	6.25	12.5	0.78
(mg/mL) of	S3h	25	25	na	12.5	na
samples causing	S6h	25	25	12.5	12.5	25
	S12h	25	25	na	12.5	na
at least 35%	S24h	25	25	na	na	na
biofilm inhibition.	O1h	0.0003814	0.0003814	na	na	na
	O2h	na	na	na	na	na
	O3h	0.0003814	0.0003814	na	na	na
An	O6h	0.0003814	0.0003814	na	na	na
	O12h	na	na	25	na	na
	O24h	0.0003814	0.0003814	na	na	na

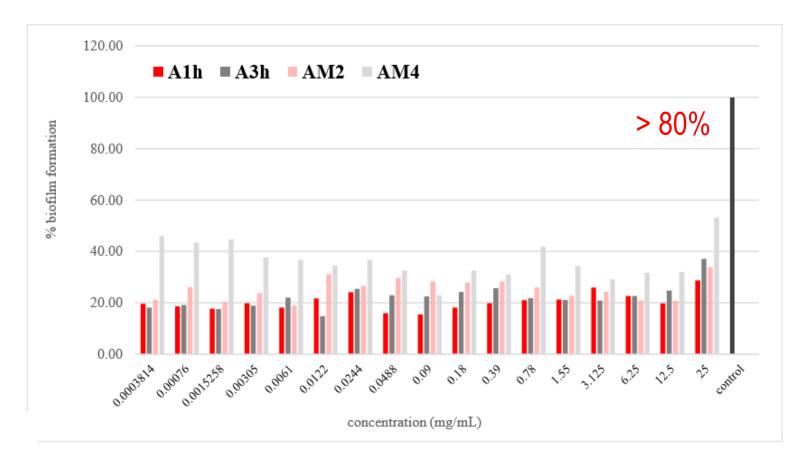








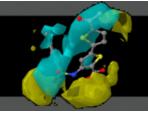
### **Anti-biofilm Activity of EOs**



Effects of selected Foeniculum vulgare samples treatment on biofilm formation for P. aeruginosa PaO1





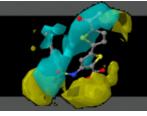




	Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923	
	A1h	na	3.125	0.0003814	25	na	
	A2h	na	na	0.0003814	na	na	
<u>Foeniculum</u>	A3h	25	25	0.0003814	na	na	
vulgare	A6h	25	25	0.0003814	25	na	
	A12h	25	12.5	0.0003814	25	na	
The lowest	A24h	12.5	12.5	0.0003814	na	na	
concentration	S1h	na	na	0.0003814	12.5	na	
CONCENTIATION	S2h	na	na	6.25	12.5	0.78 na	
(mg/mL) of	S3h	25	25	na	12.5		
samples causing	S6h	25	25	12.5	12.5	25	
, ,	S12h	25	25	na	12.5	na	
at least 35%	S24h	25	25	na	na	na	
biofilm inhibition.	O1h	0.0003814	0.0003814	na	na	na	
	O2h	na	na	na	na	na	
	O3h	0.0003814	0.0003814	na	na	na	
An	O6h	0.0003814	0.0003814	na	na	na	
	O12h	na	na	25	na	na	
	O24h	0.0003814	0.0003814	na	na	na	







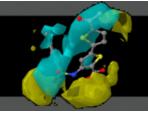


### **Anti-biofilm Activity of EOs**

	Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaO1	S. aureus 6538P	S. aureus 25923	
	A1h	na	3.125	0.0003814	25	na	
	A2h	na	na	0.0003814	na	na	
<u>Foeniculum</u>	A3h	25	25	0.0003814	na	na	
vulgare	A6h	25	25	0.0003814	25	na	
	A12h	25	12.5	0.0003814	25	na	
	A24h	12.5	12.5	0.0003814	na	na	
	S1h	na	na	0.0003814	12.5	na	
	S2h	na	na	6.25	12.5	0.78	
	S3h	25	25	na	12.5	na	
	S6h	25	25	12.5	12.5	25	
	S12h	25	25	na	12.5	na	
estragole	S24h	25	25	na	na	na	
8	O1h	0.0003814	0.0003814	na	na	na	
	O2h	na	na	na	na	na	
	O3h	0.0003814	0.0003814	na	na	na	
	O6h	0.0003814	0.0003814	na	na	na	
	O12h	na	na	25	na	na	
	O24h	0.0003814	0.0003814	na	na	na	
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	Sample <sup>1</sup>	S. epidermidis O47	S. epidermidis RP62A	P. aeruginosa PaOl	S. aureus 6538P	S. aureus 25923	
	A1h	na	3.125	0.0003814	25	na	
	A2h	na	na	0.0003814	na	na	
<u>Foeniculum</u>	A3h	25	25	0.0003814	na	na	
vulgare	A6h	25	25	0.0003814	25	na	
	A12h	25	12.5	0.0003814	25	na	
The lowest	A24h	12.5	12.5	0.0003814	na	na	
concentration	S1h	na	na	0.0003814	12.5	na	
CONCENTRATION	S2h	na	na	6.25	12.5	0.78	
(mg/mL) of	S3h	25	25	na	12.5	na	
samples causing	S6h	25	25	12.5	12.5	25	
•	S12h	25	25	na	12.5	na	
at least 35%	S24h	25	25	na	na	na	
biofilm inhibition.	O1h	0.0003814	0.0003814	na	na	na	
	O2h	na	na	na	na	na	
	O3h	0.0003814	0.0003814	na	na	na	
A.	O6h	0.0003814	0.0003814	na	na	na	
	O12h	na	na	25	na	na	
	O24h	0.0003814	0.0003814	na	na	na	









							0.08	-	July	AugustSep	otember						
			5.0	mple			0.07				1						
Name	Jlh	J2h	J3h	J6h	J12h	J24h	0.05 %				;		MIC	ng/mL		MIC r	ng/mL
3-octanol 4-terpineol	2.2 0.6	0.4 0.5	0.3 0.4	0.4	0.5 0.4	-	% 0.04 0.03	1	N.		i	Sample <sup>1</sup>	24h	48h	Sample <sup>1</sup>	24h	48h
caryophyllene cinerolone	0.3	-	1.3	2.9 2.9	2.3 5.8	-			N.Y.			A1h	na	na	S1h	na	na
crysanthenone $\delta$ -cadinene	4.4	10.5	20.3	22.7 0.6	33.9 0.8	27.3 2.4	0.01		Cir	'	_	A2h	12.48	na	S2h	6.24	12.48
d-limonene germacrene D	5.9	0.6	0.2 1.5	0.1	0.1	-	0					A3h	na	na	S3h	6.24	12.48
isocaryophyllene	-	-	-	-	-	3.8		1h	2h 3h	6h 12h	24h	A6h	na	na	S6h	6.24	na
isopiperitenone isopulegone	0.6	0.5	0.5	0.5	0.4	2.2			EXTRA	ACTION TIME		A12h	na	na	S12h	6.24	na
linalool menthone	0.3 3.1	0.8	0.2 0.6	0.4 0.6	0.5 0.5	-						A24h	na	na	S24h	12.48	12.48
methylisopulegone myrcene	0.4	-	-	-	-	12.6			diffe	erent		AM1	12.48	na	SM1	3.12	12.48
p-cymen-8-ol p-mentha-1,8-dien-3-one	-	- 0.6	0.7	0.7 1.2	1.5 2.0	2.2						AM2	12.48	12.48	SM2	3.12	12.48
p-menthene pulegone	76.8	77.7	64.3	0.2 53.2	41.1	37.7			l vie	eld		AM3	12.48	na	SM3	6.24	6.24
sabinene	0.6	- 0.5	- 0.7	- 0.8	- 1.2	-					)	AM4	12.48	na	SM4	6.24	12.48
terpineo1 trans-p-mentha-2,8-dieno1 Unidentified compounds		- 7.9	- 9.0	0.8 0.2 9.5	0.1 8.1	- 11.8						AM5	na	na	SM5	6.24	na
		fere con				al									ent re ical a		
							6 0	liffe	erent	EO fr	actions	5					

Mijat Božović, PhD Dissertation

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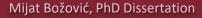


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- A selection of 6 Lamiaceae and Apiaceae plant species has been analyzed leading to a conclusion that no rule can be given about the appropriate duration of steam distillation process;
- Different plant species have different EO yields, and the dynamic of oil extraction from plant material could be considered a species specific;
- Harvesting period is also very important, directly affecting these parameters: it could be more related to the plant family than to the species;

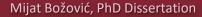






- The extraction method applied resulted in EO fractions that differ greatly in their chemical compositions;
- Although the main characterizing compounds are usually present in every fraction, variations in their amount are particularly evident between the first 3 fractions and the last ones;
- Furthermore, some compounds appear only with the development of the extraction process, and gradually increase in amount, being significantly present only in the last few fractions;
- Concerning the period of harvest, the chemical profile of an EO has been found to be heavily influenced by this factor.









 In order to monitor the biological variability, EOs of selected plant species were assayed by means of antimicrobial activity;

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- Having in mind the processes of synergism and antagonism between EO compounds, overall potential of the isolated oil fractions was evaluated;
- Analyses of antifungal activity have shown the significant efficacy of some samples, particularly in the case of *Mentha suaveolens*;
- Analyses of anti-biofilm activity against 5 bacterial pathogens showed S. aureus as the most resistant one, whereas 2 strains of S. epidermidis have demonstrated the highest susceptibility.

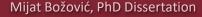






- The process duration is always dependent on what the study is conducted for;
- Prolonged and/or fractionated distillation may surely give more complete and chemically more diverse EO;
- As a delicate structure, its chemical profile can be easily altered or destroyed by adverse distillation conditions (e.g. thermal, hydrolytic), leading to the possible accumulation of artifactual formations;
- However, all of that may have a very curious effect on its biological activities: this concept has been clearly proved by our results.







## **RCMD** Lab

Rino Ragno Antonello Mai Manuela Sabatino Francesca Borzacchi Alberto Navarra

### External Collaborations Rome Stefania Garzoli (GC-MS) Federico Pepi (GC-MS) Laura Selan (Microbiology) Rosanna Papa (Microbiology) Ferrara Stefano Manfredini (Microbiology)

by www.

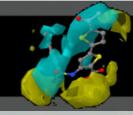
Herbal companies:

Minardi & Figli srl (Modena) Orto La Rocca (Sperlonga) Vivaio 98.3 (Tarquinia)





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Thank you! Grazie! Hvala!

> Natural habitat of the investigated species, Tarquinia countryside (Viterbo, Italy)



Mijat Božović, PhD Dissertation

30/01/2017

