

COMPARATIVE STUDY OF ESSENTIAL OILS OBTAINED FROM TWO BASIL TAXA

Cătălina STAN (TUDORA)^{1, 2}, Adriana MUSCALU², Valentin Nicolae VLĂDUȚ²,
Carmen POPESCU³, Floarea BURNICHI⁴, Florentina ISRAEL-ROMING¹

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

²National Institute of Research-Development for Machines and Installations Designed for
Agriculture and Food Industry - INMA, 6 Ion Ionescu de la Brad Blvd, District 1, Bucharest,
Romania

³SC Hofigal Export Import SA, 2 Intrarea Serelor, Bucharest, Romania

⁴Vegetable Research and Development Station Buzau, 23 Mesteacanului Street, Buzau, Romania

Corresponding author email: cmc_tudora@yahoo.com

Abstract

The objective of this study was to evaluate the essential oils (EOs) obtained from two medicinal plants belonging to the genus *Ocimum* sp. These were compared in terms extraction yield and analysis of EOs compounds using GC/MS (content, chemical composition and quantitative dosage of some compounds: linalool, estragole and eugenol). *Ocimum basilicum* L., fam. Lamiaceae with 2 cultivars: Yellow basil (*Ocimum basilicum* L., 'Aromat de Buzău' variety), Purple basil (*Ocimum basilicum* cv. *purpurascens*, 'Seraphim' variety) and Holy basil (Tulsi) was used in the experiments. Extraction yields obtained: 5 ml EO/1 kg plant material for Yellow basil, 1 ml EO/1 kg plant material for Purple basil and 0.9 ml EO/1 kg plant material for Tulsi. Twenty-six compounds of the Yellow basil, sixteen compounds of the Purple cultivar and twenty-eight compounds in Tulsi and were identified using GC/MS. Linalool (46.70%-48.52%), Estragole (31.50% for the 'Aromat de Buzău' variety) and Eugenol (13.18% for the 'Seraphim' variety and 35.85% for the Tulsi) were dosed quantitatively and identified as the main and common compounds, for the essential oils obtained from the two species of Basil.

Key words: *Ocimum basilicum*, *Ocimum sanctum*, yield, essential oils, GC/MS.

INTRODUCTION

The genus *Ocimum* belongs to the *Lamiaceae* family, commonly called Basil, is known and appreciated for its diversity throughout the world. It includes more than 30 species of plants and shrubs, originating in the tropical and subtropical regions of Asia, Africa, Central and South America (Simon et al., 1999). Basil is an important source of essential oils and aromatic compounds (Simon et al., 1990), it is an attractive culinary and ornamental plant with specific aroma and fragrance (Morales et al., 1996). For the future, a growth of the *Ocimum* essential oil market is expected to grow, from 86.5 million USD from 2019 to 2023, with an annual growth rate of 8%, and Europe will have the largest market share (Technavio, 2019). With such a high market potential, *Ocimum* essential oil, is of particular economic

importance to developing countries in terms of foreign exchange earnings (Gurav et al., 2021).

Basil has been classified according to different geographical origins into several chemotypes:

- European chemotype (found in Italy, France, Bulgaria, Egypt, South Africa), with the main compounds linalool and methyl chavicol (estragole);

- Tropical chemotype (found in India, Pakistan and Guatemala), rich in methyl cinnamate;

- Reunion chemotype (found in Thailand, Madagascar, Vietnam), characterized by high concentrations of methyl chavicol (estragole);

- Eugenol-rich chemotype (found in North Africa, Russia) (Simon et al., 1999; Telci et al., 2006).

Among the species of this genus, *Ocimum basilicum* L. - Basil, is cultivated in many countries and is the most important crop for obtaining essential oils worldwide. Basil is

used as a medicinal plant, and the essential oil is widely used in the food industry as a flavouring agent and in the perfumery industry. It is considered an important source of aromatic compounds that has a number of biological activities (repellent, insecticides, nematocides), antibacterial and antifungal activity, antioxidant activity (Lee et al., 2005; Telci et al., 2006).

Ocimum sanctum (The Queen of Herbs) has been known and cultivated in India for over 3,000 years. It is known as Tulsi and is considered a sacred plant, with special importance in Ayurvedic medicine (Khair-ul-Bariyah, 2013).

Basil essential oils are complex mixtures that can contain over a hundred chemical compounds. However, only a few of them are found in relatively high concentrations: citral, 1,8-cineole, linalool, methyl chavicol (estragole), eugenol, methyl eugenol and methyl cinnamate, while the mixture and the ratio of the compounds contained give the plant specific aroma (Simon et al., 1990; Korocha et al., 2017). Also, the concentration of the main and secondary compounds, the specific ratio between them is important and can form the basis for the commercial production of the specific essential oil (Zheljazkov et al., 2016).

Three major compounds are found in sweet basil grown in the United States: Linalool (7-59%), Estragole (5-29%) and Eugenol (2-12%). *Linalool* is a compound found in many essential oils and has the ability to alter the permeability and function of proteins in the cell membrane (Blejan et al., 2021). Has a wide range of biological activities, it has a sedative effect that helps relieve stress and has neurological effects.

Estragole has a sweet, herbaceous, anise-fennel like smell. It is used in the composition of perfumes offering an aromatic note of fruit and anise.

Eugenol is also used in perfumery, but as a dental medicine (local antiseptic) too.

The essential oil obtained from basil has a good antimicrobial and antioxidant activity, it is a good food preservative. With a strong attractiveness to many insects, it can be used as a potential green insecticide used to control harmful insects.

The aim of this study was to evaluate EOs obtained from two medicinal plants belonging

to the genus *Ocimum* (*Ocimum basilicum* and *Ocimum sanctum*). They were compared in terms of extraction yield and analysis of compounds using GC/MS (chemical composition, listing of compounds and quantitative dosing of specific compounds: linalool, estragole and eugenol).

MATERIALS AND METHODS

The biological material was represented by 2 Basil taxa:

- Common basil (*Ocimum basilicum* L.) with two Romanian cultivars: Yellow basil - "Aromat de Buzău" variety and Purple basil - "Seraphim" variety;

- Holy basil or Tulsi (*Ocimum sanctum* sin. *Ocimum tenuiflorum*), of Indian origin, the cultivar with purple leaves.

The study included varieties that have a relatively high yield of herba, supported by medium-tall plants with a high number of branches. All varieties were grown on experimental plots within INMA Bucharest (Băneasa area), on a reddish brown forest soil, in the climatic conditions of 2019, and the seedlings necessary for the establishment of crops were purchased from SCDL Buzău.

EOs were obtained by hydrodistillation method (distillation with water vapor under pressure) using equipment of French origin. The aerial part of the plants, which were in the flowering phase at the time of harvest, was processed. Extraction yield was calculated using the formula:

$$\text{Extraction yield (ml/kg)} = V/M$$

V = volume of essential oil obtained from the green plant sample (ml);

M = mass of medicinal plant sample (kg).

The chemical composition of EOs, the concentration of the compounds was determined by gas-chromatography coupled to mass spectrometry (GC-MS). The conditions for performing the gas-chromatography were the following: Agilent Technologies gas-chromatograph type 7890 A GC system, MS Agilent Technologies type 5975 C Mass Selective Detector; HP 5MS 30 m x 0.25 mm x 0.25 μm (5% Phetylmethylsiloxane) column; injector temperature 250°C, detector temperature 280°C; temperature regime 25°C

(10 degrees/min) up to 280°C (const. 5.5 min); mobile phase - helium 1 ml/min; injected volume - 0.1 µl essential oil; split ratio - 1:100.

RESULTS AND DISCUSSIONS

Extraction yield of EOs

For processing, the harvested plant material was left to wither for 5 hours, so as to reduce its moisture and volume. However, in order not to diminish the quality of the oil and the extraction yield, the material must be processed within 6-8 hours after harvesting, as an additional delay can cause significant losses. Hydrodistillation was used as method, using a plant of French origin, and the whole process took place at Horting Bucharest. Inflorescences, leafy branches and shoot tips, without foreign and organic bodies, were used for processing; with impurities max. 3% represented by browned or discoloured flowers, content in active substances min. 35%.

From the plant product was obtained using the distillation plant, the hydrolate (mixture of essential oil and flower water). The obtained EOs were decanted and filtered, then stored in dark bottles, in a cool, dry place.

Extraction yields obtained:

- 5 ml oil/1 kg plant material for “*Aromat de Buzău*” variety;
- 1 ml oil/1 kg plant material for “*Seraphim*” variety;
- 0.9 ml oil/1kg plant material for *Tulsi*.

It should be mentioned that both the quantity but especially the quality of the essential oil and the flower waters obtained differ, depending on the variety, the type of soil but also on the agroclimatic conditions in the year of production.

Data from the literature show that for basil varieties in Romania, the level of essential oil in the plant is between 0.2% and 0.6% (Benedec et al., 2009). The yield of essential oil obtained in Bosnia and Herzegovina was 0.4% (Stanojević et al., 2017)

EOs compositions

The biochemical characterization of the three essential oils of basil was performed based on the retention time (RT) and was compared with the main compounds using the Kovats retention rate (Skoog et al., 2004). The compounds of

interest (linalool, estragole and eugenol) have been confirmed (Adams, 2007) using standards Linalool (97% purity), Estragole (98% purity) and Eugenol (99% purity), purchased from Sigma-Aldrich and are presented as percentages in Table 1.

Table 1. Quantitative dosing of 3 compounds (Linalool, Estragole, Eugenol) from essential oils obtained from 2 Basil taxa

Compound name ^a	Yellow basil Variety “ <i>Aromat de Buzău</i> ” (g, %)	Purple basil Variety “ <i>Seraphim</i> ” (g, %)	Holy basil <i>Tulsi</i> (g, %)
Linalool	46.70	48.52	2.27
Estragole	31.50	1.35	2.92
Eugenol	-	13.18	35.85

^a - identification based on the co-injection of high purity compounds

The characterization of the 3 essential oils was done by GC-MS, allowing the identification and quantification of the compounds, and the results are presented as percentages in Table 2.

The chemical composition of the essential oil obtained from Yellow Basil cultivar, “*Aromat de Buzău*” variety is presented in table 2. By means of GC-MS, 26 compounds were identified, representing 99.74% of the total separated compounds. Linalool (27.59%) and Estragole (34.42%) were the major compounds. It should be noted the presence of other compounds as well, but in smaller quantities: Germacrene (4.62%), β-Caryophyllene (4.22%), Azulene (3.94%), Isoledene (3.06%). The main classes of compounds identified were: monoterpenes oxygenated (48.96%), monoterpenes hydrocarbons (25.51%), sesquiterpenes (25.07%), but also phenols (0.20%).

Subsequent dosing of the 3 compounds of interest (linalool, estragole and eugenol) using high purity standards showed that Linalool is the major compound with 46.70% and Estragole 31.50%. Eugenol could not be dosed because it was not identified in the chromatogram (Table 2).

The chemical composition of the essential oil obtained from Purple basil cultivar, the “*Seraphim*” variety is shown in Table 2. By means of GC-MS, 16 compounds were identified, representing 99.99% of the total separated compounds. Here, too, the compound Linalool was the majority with 32.56%, followed by β-caryophyllene with 24.83% and Eugenol (12.28%). It should also be noted the presence of other compounds, but in smaller

quantities: Eudesmadiene (7.91%), Guaiene (5.94%), Cineole (4.12%), etc. The main classes of compounds identified were: sesquiterpenes (53.76%), monoterpenes oxygenated (33.22%) but also phenols (13.01%).

The dosage of the 3 target compounds (linalool, estragole and eugenol), using high purity standards showed that Linalool is the major compound with 48.52% followed by Eugenol 13.18% and Estragole 1.35% (Table 1).

The chemical composition of the essential oil obtained from *Tulsi* is presented in Table 2. By means of GC-MS, 28 compounds were identified, representing 94.10% of the total separated compounds. Here, the majority compound was β -caryophyllene with 47.16%, followed by Eugenol (23.24%) and Guaiene with 10.24%. The presence of the compounds α -caryophyllene (2.45%), Germacrene-D (1.19%), Elemene (1.13%) should be noted, but in a smaller amount. The main classes of compounds identified in *Tulsi* oil were: sesquiterpenes (70.13%), phenols (20.80%) but also monoterpenes oxygenated (1.68%). The dosage of the 3 compounds followed (linalool, estragole and eugenol), using high purity standards showed that this time the compound Eugenol is the majority compound with 35.85%, and Estragole and Linalool quantitatively, were dosed 2.92% respectively 2.27% (Table 1).

The literature (Qing et al., 2016) mentions that in basil oil were identified over 200 chemicals such as monoterpenes, sesquiterpenes, triterpenes, flavones and aromatic compounds. The major compounds for basil oil include linalool, estragole (methyl chavicol), anethole, eugenol and methyl eugenol, which vary in quantity depending on the chemotype, which also confirms the results obtained in this study. It should be mentioned that for the 2 basil taxa analysed, all three varieties contain in the essential oil the following 6 compounds: cineole, linalool, estragole, β -caryophyllene, germacrene, elemene. But their concentration varies, both between the two basil taxa but also between the analysed varieties. Thus, the concentration for linalool varies from 2.27% in *Tulsi*, to 46.70% in "*Aromatul de Buzău*" and 48.52% in "*Seraphim*". On the other hand, regarding the Estragole concentration, there are significant differences both between the two

taxa (2.92% in *Tulsi*, but also between the two varieties of Basil (31.50% in the "*Aromat de Buzău*" variety and only 1.35 % in the purple variety "*Seraphim*"). Eugenol was dosed most quantitatively (35.85%) in *Tulsi*, and in the "*Seraphim*" variety the concentration was 13.18%. Another 7 compounds were identified as common (α -Pinene, Cineol, α -, β -Caryophyllene, Germacrene-D, Elemene, Eudesmadiene) in all three essential oils analysed, but in small quantities.

GC/MS analysis of essential oils obtained from the two taxa of basil (leaves and inflorescences), led to the identification of three main classes of compounds:

for the Yellow basil cultivar ("*Aromat de Buzău*" variety), out of a total of 99.74%, the main classes of compounds were oxygenated monoterpenes with 48.96%, monoterpene hydrocarbons with 25.51% followed by sesquiterpenes with 25.07%;

for the Purple basil cultivar ("*Seraphim*" variety) out of a total of 99.99%, the following classes of compounds were identified: sesquiterpenes 53.76%, monoterpenes oxygenated 33.22%, but also phenols 13.01%;

for *Tulsi* out of a total of 94.10% of the identified compounds, the main classes were 70.13% sesquiterpenes followed by 20.80% phenols.

Based on the study conducted by Zheljzkov in 2016, on 38 essential oils obtained from Basil, according to their composition, they were divided into 7 groups/chemotypes. According to this classification and based on the results obtained and presented in Tables 1 and 2, the basil varieties analysed in this study can be classified as follows:

The Yellow basil cultivar, "*Aromat de Buzău*" variety, can be included in:

Linalool chemotype (19-73%) - with dosed linalool 46.70%;

Chemotype Estragole (8-29%) - Linalool (8-53%) - dosed 31.5% and 46.70% respectively.

Purple basil cultivar, "*Seraphim*" variety:

Linalool chemotype (19-73%) - with 48.52% dosed linalool;

Linalool chemotype (28-66%) - *Eugenol* (5-29%), with dosed linalool 48.52% and 13.18% eugenol.

Tulsi with the *Eugenol chemotype* - dosed 35.85% in the essential oil.

Similar studies by Carović-Stanko et al., 2011; Koroch et al., 2017, on different varieties of *O. basilicum*, showed that they fall into two

main groups rich in methyl chavicol (estragole) and linalool, which confirms the results obtained in this study.

Table 2. Chemical composition of essential oils obtained from two Basil taxa (*Ocimum basilicum* vs. *Ocimum sanctum*)

No.	Compound name ^a	Yellow basil EO "Aromat de Buzău" variety		Purple basil EO "Seraphim" variety		Holy basil EO "Tulsi"	
		RT	Area %	RT	Area (%)	RT	Area (%)
1	<i>α</i> -Pinene	9.11	0.24	9.17	0.49	9.15	0.16
2	Myrcene	13.17	0.18	nd	Nd	nd	nd
3	Limonene	14.43	0.19	nd	Nd	nd	nd
4	Cineol	14.70	2.22	14.73	4.12	14.68	0.24
5	Carene	17.02	0.44	nd	Nd	nd	nd
6	Octanal	nd	nd	nd	Nd	18.37	0.24
7	Cubebene	24.33	0.19	nd	Nd	nd	nd
8	Camphor	24.97	1.17	24.95	1.01	nd	nd
9	Pinocamphene	nd	nd	nd	Nd	24.98	0.43
10	Pinanone	nd	nd	nd	Nd	25.74	1.14
11	Linalool	26.05	27.59	26.04	32.56	26.02	0.17
12	Alanine	nd	nd	nd	Nd	26.67	0.16
13	Bornyl acetate	26.73	0.74	nd	Nd	nd	nd
14	Farnesene	nd	nd	26.83	0.53	nd	nd
15	Azulene	26.95	3.94	nd	Nd	nd	nd
16	Guaiene	nd	nd	26.96	5.94	nd	nd
17	<i>β</i> -Caryophyllene	27.05	4.22	27.05	24.83	27.04	47.16
18	<i>α</i> -Caryophyllene	28.82	1.22	28.81	1.80	28.82	2.45
19	Estragole	28.98	34.42	28.99	0.64	28.97	0.26
20	J-Terpineol	nd	nd	29.59	0.60	nd	nd
21	<i>Germacrene-D</i>	29.77	4.62	29.75	2.94	29.74	1.19
22	Guaiodiene	29.91	2.65	29.90	2.11	nd	nd
23	Eudesma	nd	nd	nd	Nd	29.95	0.53
24	Selinene	nd	nd	nd	Nd	30.08	0.62
25	<i>Elemene</i>	30.33	1.34	30.32	1.52	30.30	1.13
26	Cariofilene	30.46	0.16	nd	Nd	nd	nd
27	Chamigrene	nd	nd	nd	Nd	30.71	0.75
28	Isodene	30.91	3.06	nd	Nd	nd	nd
29	Guaia	nd	nd	nd	Nd	30.95	10.24
30	<i>Eudesmadiene</i>	30.98	2.89	30.97	7.91	30.96	0.53
31	2-Hexanoilfuran	nd	nd	nd	Nd	31.86	0.30
32	Nerol	32.94	0.28	nd	Nd	nd	nd
33	Phenol	nd	nd	nd	Nd	34.72	0.15
34	Caryophyllene oxid	nd	nd	35.67	0.71	35.65	1.19
35	Cubenol	37.18	0.55	nd	Nd	37.62	0.72
36	Spathulenol	38.45	0.16	nd	Nd	38.43	0.17
37	J-Cadinol	39.33	5.67	nd	Nd	nd	nd
38	Eugenol	nd	nd	39.33	12.28	39.31	23.24
39	Guaiol	nd	nd	nd	Nd	40.23	0.18
40	Eudesmenol	nd	nd	nd	Nd	40.37	0.25
41	Mururol	40.41	0.24	nd	Nd	nd	nd
42	2-Allylphenol	42.42	0.20	nd	Nd	nd	nd
43	Ledene oxid	nd	nd	nd	Nd	42.96	0.15
44	Aromadendrene	nd	nd	nd	Nd	43.86	0.17
45	Ethylene oxid	nd	nd	nd	Nd	45.99	0.18
46	Phytol	46.95	1.16	nd	Nd	nd	nd
Total compounds		99.74%		99.99%		94.10%	
Monoterpene hydrocarbons		25.51		-		-	
Monoterpenes oxygenated		48.96		33.22		1.68	
Sesquiterpenes		25.07		53.76		70.13	
Phenols		0.20		13.01		20.80	
Others		-		-		1.50	

^a - Compounds identified based on retention index; nd - not detectable.

Chemotaxonomic studies of essential oils from European basil varieties (Gray et al., 1996; Ahlam et al., 2017) and two different varieties of basil from Iran (Pirmoradi et al., 2013) have shown that methyl chavicol (estragole) and linalool are the main compounds.

In other studies, the main compound found in essential oils from other varieties of basil growing in different geographical areas is methyl-eugenol (Parmeshwar et al., 2021), while the presence of methyl cinnamate among the main chemical constituents of basil oil "either as a chemotype or as a subtype" has been detected in basil varieties in Serbia (Beatovic et al., 2015).

Comparing the data obtained in this study with those in the literature (Ilic et al., 2019), the results showed the complex chemical composition of essential oils and highlighted the presence of biologically active compounds important for various branches of the pharmaceutical, chemical and food industries. Although there were differences between essential oils, the results showed that all are rich in biochemical compounds that are responsible for biological activities, but further studies are needed to determine their biological activities and their applicability as possible food additives.

The differences in the chemical composition of the analysed essential oils can be explained as a consequence of the morphological characteristics of the studied plants but also of the agroclimatic conditions of 2019.

CONCLUSIONS

In this study, the essential oil obtained from two basil taxa was researched: common basil with two cultivars (Yellow basil and Purple basil) and Holy basil or *Tulsi*.

The content of essential oil for the evaluated varieties varied from 5 ml of oil/1 kg of vegetable material for the "*Aromat de Buzău*" variety, to 1 ml for the "*Seraphim*" variety and 0.9 ml of oil for *Tulsi*.

GC/MS analysis showed differences in the chemical composition of each essential oil and in the concentration of each chemical compound in the chromatogram. As number, the compounds identified ranged from 26 in the "*Aromat de Buzău*", 16 in the purple variety

("Seraphim"), and 28 compounds in the *Tulsi* oil. This variation did not depend on the values of the quantitative characters, the colour of the leaves or flowers.

The concentration of the main compounds in the 3 essential oils varied as follows: in the "*Aromat de Buzău*" variety the main compounds are linalool (46.70%) and estragole (31.50%), in the "*Seraphim*" variety the linalool (48.52%) and eugenol (13.18%) compounds predominated and for *Tulsi* the main compound was eugenol, dosed 35.85%.

Ocimum sp. genotypes evaluated are divided into the following chemotypes:

- Yellow basil cultivar, "*Aromat de Buzău*" variety falls into the Linalool chemotype, but also into the Linalool-Estragole chemotype;
- Purple basil cultivar, "*Seraphim*" variety falls into the Linalool chemotype and the Linalool-Eugenol chemotype;
- *Tulsi* cultivar falls into the Eugenol chemotype.

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