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CHEMICAL COMPOSITIONS AND SEDATIVE ACTIVITIES OF THE *DRACOCEPHALUM MOLDAVICA* L. AND *OCIMUM AMERICANUM* L. ESSENTIAL OILS

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Abstract

Use of the natural sedatives is very promising for the elimination of anxiety and emotional stress. The aim of the study was to evaluate the chemical compositions and sedative effects of the *Dracocephalum moldavica* L. and *Ocimum americanum* L. (*Lamiaceae* Martinov) essential oils. The chromatographic analyses of essential oils revealed the high amounts of compounds with sedative actions in both of them. Thus. linalool (49.84%) dominated in *Ocimum americanum* and geranyl acetate (41.08%) prevailed in *Dracocephalum moldavica.* The *in vivo* investigations revealed the significant anxiolytic activities of the tested essential oils in a dose-dependent manner. They induced the sedative actions by decreasing animals' horizontal and orienteering research movies as well as emotional reactions. The inhalations of the essential oils of plants in the maximum concentrations (4.0%) did not differ significantly in many indicators from the action of reference drug Diazepam. Therefore, the results of this study found that investigated essential oils may represent a new natural source of sedative herbal medicinal products for aromatherapy.

Keywords: *Lamiaceae, essential oils, GC-MS analysis, sedative effect, in vivo*

Introduction

The increased anxiety, emotional stress, and depression are common causes of the deterioration of health and well-being in the 21st century [1, 2]. It is caused a growing need to expand the range of sedatives [3, 4]. Synthetic sedatives have a number of disadvantages, including the development of addiction, impaired attention and memory, interaction with other drugs, etc.

Use of the natural sedatives is very promising for studying possibility to eliminate the anxiety. Timely intake of sedative drugs could mitigate the impact of stressful situations on the human body, reduce the risk of psychosomatic diseases and improve the quality of life generally. Decreasing of anxiety could be a valuable instrument for the correction of mental state due to the high rhythm of modern life style in human society.

Aromatherapy uses essential oils for many medical purposes, e.g. relaxation [5–7]. Volatile terpenoids with anxiolytic properties are key compounds in the aromatherapy of anxiety and depression [8]. The *Lamiaceae* Martinov species are valuable sources of biologically active compounds including essential oils, triterpenoids and polyphenols with promising healing functions [9–15].

Lavender (*Lavandula angustifolia* Mill.) and lemon balm (*Melissa officinalis* L.) are the most popular sources of essential oils with a sedative effect among the officinal medicinal plants of the *Nepetoideae Burnett.* subfamily of the *Lamiaceae* family [16–20]. Their essential oils are used for inhalations or with massage. However, there is no scientific information about the influence of essential oils from the non-officinal species of this subfamily on the of animals' behavioral responses. Such species as *Dracocephalum moldavica* L. and *Ocimum americanum* L. are the annual essential oil-bearing plants which are applied in a folk medicine of many countries for the treatment of different health problems including anxiety and depression [11, 21, 22].

The aim of the study was to evaluate the chemical compositions and sedative effects of the *Dracocephalum moldavica* L. and *Ocimum americanum* L. (*Lamiaceae* Martinov) essential oils due to their application by inhalations in rats.

Methods

Plant material

The herbs of the *Dracocephalum moldavica* and *Ocimum americanum* were harvested from the experimental plots in Ternopil region (Ukraine) during their flowering period and shade dried.

Isolation and GC-MS analysis of the essential oils

Isolation of essential oils was performed using hydrodistillation. The components of essential oils were investigated by gas chromatography-mass spectrometry (GC-MS) method with the Agilent Technologies 6890 system. The chromatographic column was DB-5 capillary (length 30 m, inner diameter 0.25 mm). Helium (99.999% of purity) was used as carrier gas.

The chromatographic procedures were carried out in the programmed mode (temperature increased from 50 to 320 °C at a rate of 4 °C/min). The library of mass spectra of the reference compounds NIST (USA) was applied for the identification of components.

Sedative activity

The investigations of sedative effects of the inhaled essential oils were carried out by the "open field" method as it is described in [23, 24]. All the *in vivo* studies were conducted according to [25]. 48 non-linear white male rats weighing 220–240 g were used for the study. Each animal was placed in the center of "open field" and tested once for 3 min. The different discrete behavioral responses of rats were recorded, for instance: the horizontal motor activity (number of crossed squares), the orienteering research activity (vertical racks and inspected holes) and the anxiety indicators (emotional reactions, e.g. number of grooming acts, urinations and fecal bolus).

The "open field" test consisted of a light area of 1 m^2 surrounded on the perimeter by an opaque board and divided into 25 equal squares. There were holes with a diameter of 4 mm at the intersection of the squares to evaluate the sniffing reflex. The lighting was carried out using a lamp of 100 W installed at a height of 100 cm above the center of the field.

The experimental animals were divided into 8 groups: 1 – control (they received the purified water); 2–4 – experimental groups (they were subjected to the inhalations of *Dracocephalum*

moldavica essential oil): 1.0% (group 2); 2.0% (group 3); 4.0% (group 4); 5–7 – experimental groups (they were subjected to thr inhalations of *Ocimum americanum* essential oil): 1.0% (group 5); 2.0% (group 6); 4.0% (group 7). The animals of 8th group were injected subcutaneously with the reference drug "Diazepam" (manufacturer "Elegant India") in a dose 0.5 mg/kg.

There were used 4 plastic boxes with a volume of 27 L: 1) for the control group of the animals; 2) for the reference drug group; 3) for the groups treated with the *Dracocephalum moldavica* essential oil; 4) for the groups treated with the *Ocimum americanum* essential oil. The essential oils were emulsified in the 1% Tween-80 to obtain 1%, 2%, and 4% emulsions. 0.1 mL of each emulsion was applied to 4 disks of filter paper glued to the walls of the chamber to saturate it by the natural diffusion. After 30 min of the inhalation, the experimental animals were transferred to the "open field" for monitoring their behavioral responses.

The obtained data of experimental studies were processed using the standard package Statistica software, version 13.1.

Results and Discussion

The GC-MS analysis of the tested essential oils revealed that the amounts of several components were higher than 1% (Table 1). The typical GC-MS chromatogram of essential oil is presented on the example of *Ocimum americanum* (Figure 1).

It could be supposed, that the sedative effects of the studied essential oils could be due the high contents of components with anxiolytic properties. Thus, linalool as the molecule with proven anxiolytic action [24, 27] dominated in *Ocimum americanum* (49.84%) (Figure 2). This acyclic monoterpenoid was also one of the dominant components of the *Lavandula angustifolia* essential oil regarded as a famous sedative drug [20, 27]. Geranyl acetate (41.08%), neral (18.85%) and geranial (18.25%) were the predominant compounds of the tested *Dracocephalum moldavica* essential oil, as well as *Melissa officinalis* studied by [29, 30]. The abovementioned oxygenated monoterpenoids possess the very nice aromatic smells besides the significant sedative effects [18]. Thus, they are very promising for the application in aromatherapy. Generally, the tested *Dracocephalum moldavica* and *Ocimum americanum* essential oils could be regarded as an alternative to essential oils of such officinal medicinal plants as *Melissa officinalis* and *Lavandula angustifolia*, respectively.

All the used concentrations of the *Dracocephalum moldavica* and *Ocimum americanum* essential oils demonstrated the dose dependent sedative effects (Table 2). The anxiolytic actions of the studied essential oils on the behavioral reactions of experimental animals were determined by the decrease in their horizontal motor activity, orienteering research activity and emotional reactions. The essential oils of both plants significantly decreased the rats' spontaneous locomotor activity. The inhalation of maximum concentrations (4.0%) of their essential oils did not differ from the action of reference-drug Diazepam in the most indicators of sedative effect. The essential oil (4%) of *Dracocephalum moldavica* had the strongest action on the emotional reactions of rats.

Researchers proven the dose-dependent sedative effect of inhalations by the lavender essential oil which was similar to the reference drug "Chlordiazepoxide" [26]. Its predominant components such as linalool, citral, etc. have a central mechanism of action [27]. It is believed that there is a relationship between the perception of certain odors and the emotional behavioral response provided by a number of central nervous system structures and mediators [5]. The components of essential oils as compounds with low molecular weight have the direct effect on chemoreceptors [18, 26]. They are well adsorbed by the mucous membrane during inhalation which ensures their circulation in the blood for some time [18].

The synergistic effects of various components of the essential oils as the complex mixtures of volatile terpenoids were revealed by [18, 28]. Some of them exhibit the biological activity, whilst others facilitate the absorption of phytomedicines and their bioavailability.

Concerning the sedative effect of essential oils isolated from the raw materials of the *Ocimum* representatives, it should be noted that anxiolytic and antidepressant-like influences of the inhalation by the Cameroonian *Ocimum gratissimum* L. essential oil were found by Tankam et al. [32].

Anxiolytic effects of the *Ocimum basilicum* L. essential oil used by inhalation from the headspace air of living basil plants were revealed by Hirai et Ito [33]. Ayuob et al. [34] supposed that the essential oil of *Ocimum basilicum* relieved the chronic stress-induced changes in the olfactory system possibly through the up-regulation of gene expressions. The significant sedative effect was found for the tincture from the *Ocimum americanum* herb prepared using 70% ethanol and by adding 0.5% essential oil of this plant [35]. The revealed actions might be attributed to the linalool as the predominant bioactive compound of the essential oils of *Ocimum* species [35, 36].

Golparvar et al. [31] revealed that the major constituents of the *Dracocephalum moldavica* essential oil from Iran were neranyl acetate (36.62 %), geraniol 24.31%) and neral (16.25%). It was similar to the results of the conducted experiment (see Table 1). Many researchers [7, 30] described the valuable health-promoting properties of the abovementioned molecules including sedative effects. Besides of this, Martínez-Vázquez et al. [22] found that the aqueous extract of the *Dracocephalum moldavica* herb could decrease the spontaneous activity in mice.

Conclusions

This is the first research of sedative activities of the *Dracocephalum moldavica* and *Ocimum americanum* essential oils studied by inhalation in rats. The *in vivo* investigations revealed the significant sedative effects of the tested essential oils in a dose-dependent manner. They induced the sedative actions by the decrease of animals' emotional reactions, horizontal motor activity and orienteering research activity. The chromatographic analyses of the essential oils revealed the highest amount of linalool (49.84%) in *Ocimum americanum* and geranyl acetate (41.08%) in *Dracocephalum moldavica*. Therefore, the results of this study indicate that investigated essential oils may represent the new natural sources of herbal medicinal products with the significant sedative effects for aromatherapy.

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Table 1. The predominant compounds of the *Dracocephalum moldavica* and *Ocimum americanum* essential oils revealed by GC-MS

Compound	Retention time, min	Content in the essential oil, %	
		Dracocephalum moldavica	***Ocimum americanum***
1,8-cineole	9.12	5.86	6.75
Linalool	11.42	2.06	49.84
Camphor	12.97	-	3.09
α-Terpineol	14.6	-	2.40
Estragole	14.8	-	8.27
Nerol	15.96	0.54	-
Neral	16.46	18.85	-
Geraniol	16.83	6.97	-
Geranial	17.53	18.25	-
Bornyl acetate	17.64	-	2.39
β-Elemen	19.7	-	2.09
Neryl acetate	20.25	2.95	-
Eugenol	20.36	-	2.52
Geranyl acetate	21.3	41.08	0.80
Germacrene D	23.91	1.07	2.06
α-Bulnesene	24.6	-	1.86
α-Amorphene	24.81	-	1.49
epi-cubenol	27.33	-	1.09
epi-γ-cadinol	27.9	-	5.86

Note. "-" - compound not detected

Table 2. Sedative effects of the inhaled essential oils of *Dracocephalum moldavica* and *Ocimum americanum* in the "open field" test in rats

Group of the animals	Concentration of essential oil and reference drug	Horizontal motor activity (number of crossed squares)	Orienteering research activity		Anxiety indicators (emotional reactions)		
			vertical racks	inspected holes	bolus	urination	grooming
Control	-	35.40±0.82	10.27±0.28	4.50±0.12	1.17±0.06	0.67±0.03	2.17±0.06
Dracocephalum moldavica	1.0 %	29.67±0.51[1,2]	9.33±0.19[2]	4.33±0.15[2]	1.0±0.05[2]	0.50±0.04[1, 2]	2.0±0.05[1, 2]
	2.0 %	25.17±0.49[1,2]	7.67±0.26[1,2]	4.0 ±0.13[2]	0.67±0.03[1, 2]	0.33±0.02[1, 2]	1.67±0.08[1,2]
	4.0 %	22.83±0.39[1,2]	6.83±0.33[1]	3.83±0.18[1, 2]	0.33±0.02[1, 2]	0[1]	1.17±0.08[1]
Ocimum americanum	1.0 %	30.33±0.64[1,2]	9.50±0.25	4.33±0.14[2]	0.83±0.04[1, 2]	0.67±0.03 [2]	1.83±0.07[1, 2]
	2.0 %	26.17±0.47[1,2]	7.83±0.31[1,2]	4.17±0.18[2]	0.50±0.03[1]	0.17±0.01[1, 2]	1.50±0.05[1, 2]
	4.0 %	22.97±0.43[1,2]	6.33±0.16[1]	3.33±0.12[1]	0.17±0.01[1]	0[1]	1.0±0.03[2]
Diazepam	0.55 mg/kg	18.50±0.54[1]	6.50±0.18[1]	3.17±0.09[1]	0.50±0.02[1]	0[1]	1.17±0.04[1]

Note: [1] – significantly difference compared to control group ($p \leq 0.05$);
[2] – significantly difference compared to Diazepam group ($p \leq 0.05$)

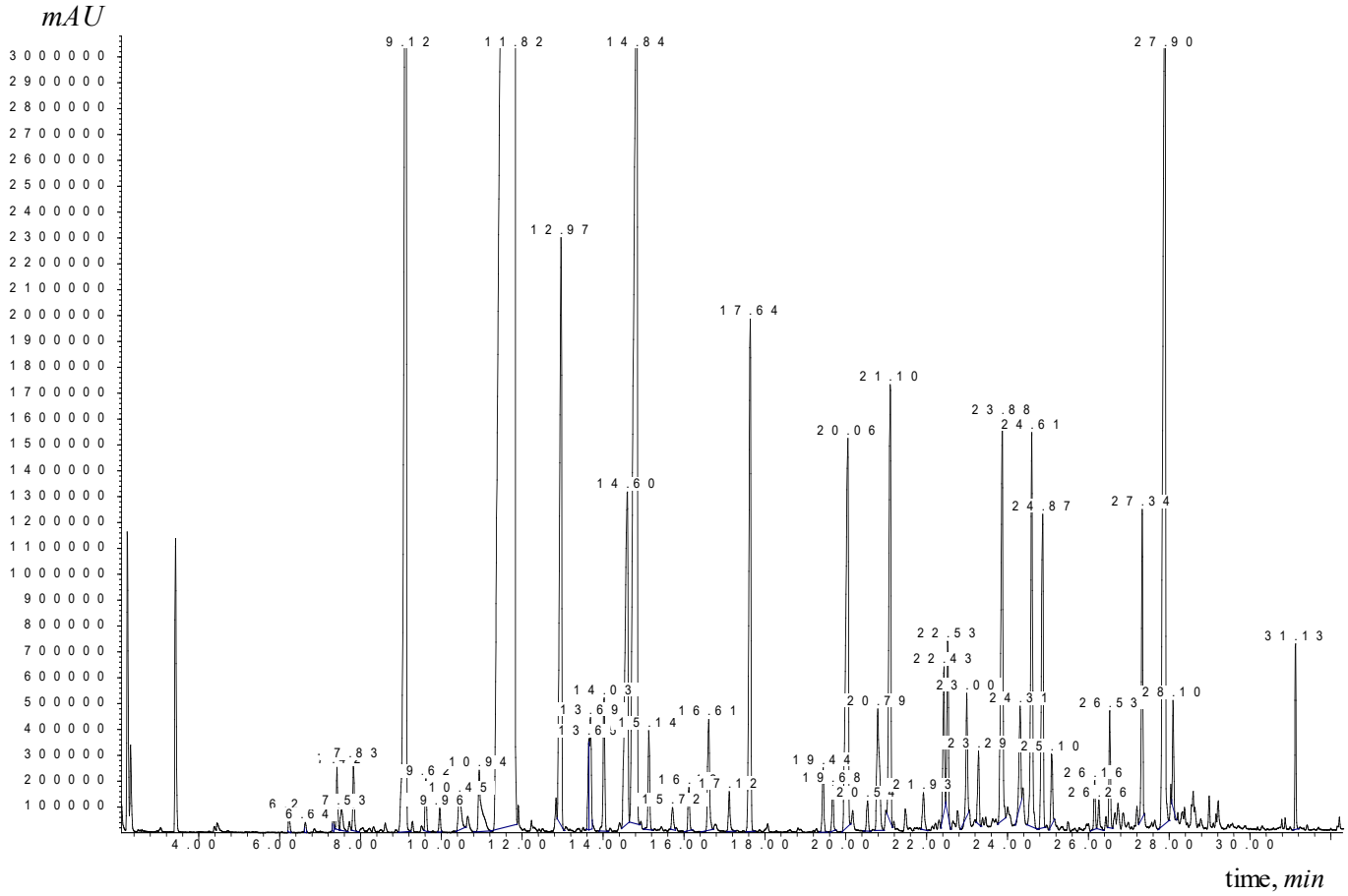
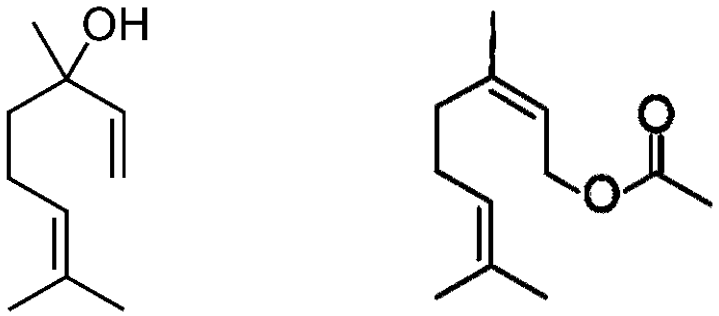


Figure 1. Typical GC-MS chromatogram of the *Ocimum americanum* essential oil



Linalool
A

Geranyl acetate
B

Figure 2. Structural formulas of the major compounds of the *Ocimum americanum* (A) and *Dracocephalum moldavica* (B) essential oils