Sedative Effect and Standardization Parameters of Herbal Medicinal Product Obtained from the *Ocimum americanum* L. Herb

Original Paper

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Abstract Sedative phytomedications continue to play an important role in the management of a considerable amount of anxiety symptoms because of the various side effects of synthetic sedatives and tranquilizers. However, developing new herbal drugs needs their appropriate quality control according to the relevant requirements. The aim of the study was to determine the sedative properties of the tinctures obtained from the American basil (*Ocimum americanum* L., *Lamiaceae* Martinov family) herb and to develop the standardization parameters for the promising herbal medicinal product. The open field test was used to evaluate the sedative effect of the prepared tinctures: (1) with the added of *O. americanum* essential oil (OATEs) and (2) without adding *O. americanum* essential oil (OAT). The standardization parameters for the OATEs were developed using validated High-Performance Thin Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) methods. The HPTLC analysis was used for the chromatographic fingerprints of polyphenols and for identifying linalool in the OATEs. The HPLC analysis found the significant content of rosmarinic acid (RA) (0.26%) in the OATEs. In conclusion, the developed OATEs can be considered as the new herbal medicinal product with significant sedative properties.

Keywords American basil – herb – tincture – sedative activity – polyphenols – linalool – chromatographic analysis

INTRODUCTION

Rational phytotherapy is a modern concept of herbal medicines that uses the standardized herbal medicinal products (Choudhary et al., 2011). A significant number of components in the tinctures and extracts from the plant raw materials are accompanied by the problem of their standardization. Quality control of herbal drugs should be done according to the requirements of relevant regulations (American Herbal Pharmacopoeia, 2011; European Pharmacopoeia, 2016).

Anxiety and depression are the important health problems in a modern society leading to low quality of life (Gutiérrez et al., 2015). Sedatives continue to play an important role in managing many anxiety symptoms in the context of medical illness. However, the side effects of synthetic anxiolytic drugs such as various syndromes of withdrawal, dependencies, and impairment of cognitive function and memory are of concern to

physicians and scientists. Searching of new sources of plant raw material with anxiolytic action among the *Lamiaceae* Martinov species is a promising direction of scientific investigations in the area of phytochemistry and ethnopharmacology (Rabbani et al., 2015; Caputo et al., 2018; Bittner Fialová et al., 2019). Pharmaceutical industries produce a lot of phytomedications of a sedative action using the aerial parts of the *Lamiaceae* representatives such as *Leonurus cardiaca*, *Melissa officinalis*, *Mentha piperita*, *Lavandula angustifolia*, and so on (Kowalczyk et al., 2012; Derzhavnyi rejestr, 2020).

Tincture, as a water-ethanol extract of a plant material being prepared at room temperature, preserves the complex as non-volatile so as volatile compounds (Doughari, 2012). The quality of tinctures or any herbal medicinal products primarily depends on the presence and content of the certain ingredients with proven biological activity (Hudcz et al., 2019).

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High-Performance Thin Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) methods have become increasingly practiced for routine analysis of herbal medicinal products obtained from the representatives of Mint Family (Staszek et al., 2013; Shafqatullah et al., 2014; Asha et al., 2015) as well as synthetic drugs (Logoyda et al., 2018).

American basil (*O. americanum* L.) belongs to the genus *Ocimum* L., subfamily *Nepetoideae* Burnett, family *Lamiaceae*. It is an annual essential oil-bearing plant native to Africa and Southeast Asia used locally as a spice or ornamental plant as well as a sedative, digestive, and anti-inflammatory remedy (The Genus *Ocimum*, 2006; Shanaida et al., 2017). Nowadays, this species is successfully naturalized on most continents. The aerial part of *O. americanum* accumulates several valuable groups of primary and secondary metabolites such as terpenoids, polyphenols, and amino acids, which are perspective for the sedative purpose (Koteswar and Babu, 2011; Shanayda, 2012; Shanaida et al., 2017; Shanaida and Golembiovskaya, 2018; Shanaida et al., 2018). Linalool (49.84%) that possesses significant sedative properties (Linck et al., 2009; Aprotosoia et al., 2014) was the major component of essential oil hydrodistilled from the *O. americanum* herb collected in Ukraine (Shanayda, 2012). Rosmarinic acid (19.59 mg/g) was revealed by HPTLC as a predominant polyphenolic compound in the methanol extract of this plant raw material (Shanaida et al., 2020). However, the raw material of this species is not included in any Pharmacopoeias (American Herbal Pharmacopoeia, 2011; European Pharmacopoeia, 2016). Thus, studying the sedative properties of tinctures obtained from the herb of *O. americanum* and developing their quality parameters can be considered as quite a perspective.

The aim of the study was to determine the sedative effects of the tinctures obtained from the *Ocimum americanum* herb and to develop the standardization parameters for the promising herbal medicinal product by the validated HPTLC and HPLC methods.

MATERIALS AND METHODS

Plant material and preparing of tinctures

The herb of *O. americanum* was collected from the experimental plots in Ternopil region (Ukraine). Plants were grown from the seeds obtained from the collection of M. Hryshko National Botanical Garden (Kyiv, Ukraine). A voucher specimen (OM: 10011) has been deposited in the Herbarium of **I. Horbachevsky Ternopil National Medical University** for further references. The herb was harvested during the flowering stage, then dried in the shadow at 25–35°C. Two tinctures were prepared from the *O. americanum* herb using 70% ethanol (V/V): (1) with added *O. americanum* essential oil (OATEs) and (2) without adding *O. americanum* essential oil (OAT).

The powdered herb of the plant (20.0 g) was macerated with 200 mL ethanol (70%, V/V) at room temperature for 24 hours using forced periodic stirring. The supernatant was separated and filtered with a Buchner funnel, then placed for 2 days in the refrigerator (at 8–10 °C) for the sedimentation of ballast substances. The total ratio of raw material and tincture obtained by the described method was 1:10 (if necessary, 70% ethanol was added to get 200 mL of OAT). The OATEs was obtained by the adding 0.5 mL of *O. americanum* essential oil hydrodistilled from its herb to 100 mL of OAT. The volume of *O. americanum* essential oil chosen for dissolving into basic ethanol extract was analogous to the dose of *Mentha × piperita* (*Lamiaceae*) essential oil, which is added to the '*Menthae piperitae* tincture' to enhance its pharmacological effect (Derzhavnyi reiestr, 2020). The essential oil of *O. americanum* was first dissolved in ethanol (96%, V/V) in the ratio 1:4, and then this solution was added to 100 mL of OAT to obtain a homogeneous solution.

Drugs and chemicals

Ethanol, ethyl acetate, and formic acid were purchased from Merck (Kennborough, NJ, USA). Aluminium chloride, toluene, Tween-80, anisaldehyde were purchased from Sigma-Aldrich (Germany). HPLC-grade acetonitrile, methanol, and trifluoroacetic acid were purchased from Sigma-Aldrich (USA). Double-distilled water prepared using a Milli-Q water purification system (Millipore system). Silica gel plates for HPTLC and reference standards such as linalool, rutin, caffeic acid, and rosmarinic acid (RA) were purchased from Merck (Germany). Diazepam (5 mg/mL) was obtained from the Elegant (India). All the chemicals used in this study were of analytical grade.

Open field test

The spontaneous locomotor activities of experimental animals were assessed according to Linck et al. (2009) and Sarker Apu et al. (2013). Open field device consisted of a wooden field of one square meter with a series of squares and holes at their intersection; the floor was divided into 25 marked small squares of equal dimensions (20 × 20 cm). This device was placed in a dimly lit room.

Animals: male albino rats (220–240 g) were obtained from the Vivarium of I. Horbachevsky Ternopil National Medical University (Ukraine). Animals were kept in the standard laboratory conditions: relative humidity 55±5%; room temperature 22±2°C under a 12 h light/dark cycle. The animals were provided with standard diet and clean water *ad libitum* during acclimatization period. They were fasted overnight, with free access to water before the experiments. All the experimental animals were conformed to internationally accepted standards, which were approved by the Bioethical Committee of I. Horbachevsky Ternopil National Medical University, in accordance with 'Ethical Principles and Guidelines for Experiments on Animals' (2005).

The tested animals were randomly divided into four groups ($n = 6$). The rats were fasted for 12 h before the experiment. The tinctures were dissolved in the vehicle (1% solution of Tween-80 in distilled water) to obtain the dose 100 mg/kg body weight, calculated in terms of dry residue of tincture. Each group of animals received a particular treatment. Group I: negative control (vehicle). Group II: treatment with OAT. Group III: treatment with OATs. Group IV: positive control (reference drug Diazepam at a dose of 0.55 mg/kg body weight). After one hour of the treatment, each animal was placed individually at the centre of the device and observed for 3 min to count the number of actions. The open field arena was thoroughly cleaned between each test to avoid the influence of the urine odours and faeces of the previous animal.

HPTLC analysis of OATs

The HPTLC method was performed as described by Shanaida et al. (2020) with some modifications. 5 μ L of OATs and 10 μ L of standard solutions (0.25 mg/mL of phenolic compounds and 5 μ L/mL of linalool) were applied to HPTLC plates using an automatic HPTLC application device (Linomat 5, CAMAG, Muttenz, Switzerland). The chromatographic separation was performed on 20 $\times$ 10 cm HPTLC plates. Two mobile phases were used for eluting process: the ethyl acetate – toluene (5:95, V/V) was used for method A, and the ethyl acetate – formic acid – water (15:1:1, V/V) was applied for method B. The chromatographic plates were evaluated after derivatization with anisaldehyde reagent (method A) or with 1% aluminium chloride solution in methanol (method B). The resulting bands were observed under the daylight (method A) and with UV at 254 and 366 nm. The obtained chromatograms were analysed using HPTLC software (vision CATS, CAMAG). The compounds were identified using their R_f values and colours of the fluorescence comparatively to those of the reference standards. The applied HPTLC method was validated using specificity and robustness parameters.

HPLC analysis of OATs

The quantitative analysis of RA in the OATs was performed with Shimadzu HPLC-DAD system (Shimadzu, Japan). Good separation was achieved by a Phenomenex Luna C18 column (Phenomenex Inc., 250 $\times$ 4.6 mm i.d., 5 μ m particle size) at 35°C. The UV absorption spectra of the reference standards and test samples were recorded in the range from 190 to 400 nm. The mobile phase consisted of two solutions: A (0.1% trifluoroacetic acid in water) and B (0.1% trifluoroacetic acid in acetonitrile). The flow rate was 1.0 mL/min and injection volume was 5 μ L. The developed HPLC method for analysis of RA content in the OATs was validated according to the linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and robustness parameters.

Statistical analysis

The variables for parametric data were analysed by one-way analysis of variance (ANOVA). Statistical analyses were performed using Statistica 12 software (StatSoft Inc., Tulsa, OK, USA). The data were expressed as mean $\pm$ standard error of the mean (SEM). The statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Sedative activity of OAT and OATs

The obtained OATs demonstrated the noticeable sedative effect when compared to OAT for all the studied parameters in the open field test (Table 1). Anxiety level of animals after the single administration of OATs decreased significantly. Sedative effect for OAT compared to Diazepam was observed only for the influence on defecation. Thus, adding essential oils of *O. americanum* to OAT led to increasing sedative activity. It was established that linalool as a predominant compound of *O. americanum* essential oil (Shanayda, 2012) added to tincture to obtain OATs, possess significant sedative properties (Linck et al., 2009). As it can be seen from the Table 1, the number of squares crossed by the rats was the smallest after the treatment with OATs. The frequency of defecation as an important indicator of anxiety after the OATs influence was at the same level as a reference drug. The levels of tentative research activities such as vertical racks and examined holes were also the least numerous in the OATs group compared to OAT and control groups.

Chromatographic analyses and standardization procedures for OATs

HPTLC analysis

The HPTLC method for the qualitative determination of phenolic compounds and linalool in the OATs was developed for the first time. A number of solvent systems were tried for OATs chromatographic analysis. The satisfactory resolutions were reached using such solvent systems as ethyl acetate – toluene (5:95, V/V) for linalool (method A) and ethyl acetate – formic acid – water (15:1:1, V/V) for phenolic compounds (method B).

The validation of HPTLC procedure for the identification of linalool in the OATs (method A) included the specificity and robustness studies. During the specificity study, it was revealed that chromatograms of the reference solution had the violet zone of linalool at $R_f = 0.29$ at daylight (Fig. 1, Table 2). The chromatogram of the test solution showed the weak violet zone at the level of linalool spot. The additional violet bands of unknown compounds possessing the specific for terpenoids violet shades were also available at R_f values 0.03, 0.11, 0.19, and 0.85 in the chromatogram of test solutions. The maximum difference in the values of the R_f zones of linalool

Table 1. The influence of tinctures prepared from *Ocimum americanum* herb on the spontaneous behavior of rats in the open field test ($n = 6$).

Group	Number of squares crossed	Tentative research activity		Indicators of anxiety (emotional reactions)		
		Vertical racks	Examined holes	Defecations	Urinations	Grooming
Control	32.61 ± 2.29	9.52 ± 0.89	5.21 ± 0.47	1.03 ± 0.1	0.51 ± 0.05	2.32 ± 0.21
OAT	29.17 ± 1.09	8.83 ± 0.28	4.83 ± 0.35	0.67 ± 0.09 ¹	0.5 ± 0.05	2.33 ± 0.24
OATEs	26.0 ± 1.02 ¹	8.0 ± 0.42 ¹	4.5 ± 0.21	0.5 ± 0.05 ¹	0.33 ± 0.02 ^{1,2}	2.17 ± 0.19
Diazepam	18.5 ± 0.54 ¹	6.5 ± 0.18 ¹	3.17 ± 0.09 ¹	0.5 ± 0.02 ¹	0 ¹	1.17 ± 0.04 ¹

Notation. ¹ – the differences are statistically significant for the values of the control group ($p \leq 0.05$); ² – the differences are statistically significant for the values of the Diazepam group ($p \leq 0.05$)

Table 2. The results of HPTLC analyses of biologically active compounds in OATEs.

Compound	R _f value	Colour	Presence in OATEs	Presence in essential oil of <i>O. americanum</i>
Method A				
Unknown compound	0.03	Violet	+	-
Unknown compound	0.11	Violet	+	-
Unknown compound	0.19	Violet	+	-
Linalool	0.29	Violet	+	+
Unknown compound	0.85	Violet	+	+
Method B				
Rutin	0.09	Yellow	+	*
Unknown compound	0.14	Blue	+	*
Unknown compound	0.27	Yellow	+	*
Unknown compound	0.29	Blue	+	*
Unknown compound	0.43	Dark blue	+	*
Unknown compound	0.64	Blue	+	*
Rosmarinic acid	0.75	Blue	+	*
Caffeic acid	0.79	Blue	+	*

Notation. '+' compound is presented; * – parameter was not studied

Table 3. The robustness testing for the identification of linalool in OATEs by HPTLC.

Indicator	Level of changes	Rf variability (RSD, %)
Mobile phase composition (ethyl acetate – toluene, V/V)	5:95	0.6
	5.2:95	1.3
	4.8:95	0.8
Development distance, cm	8	1.3
	18	1.8
Chamber	3 different containers	1.3
Analysts	2 different analysts	0.8

corresponding the test and reference solutions within one plate was not exceed 0.01 units of R_f. The robustness studies for method A are presented in Table 3.

The specificity study of the polyphenols identified in OATEs (method B) showed that the chromatograms of reference and test solutions had the prominent blue fluorescent zone of RA (at $\lambda = 366$ nm) in the upper part at R_f = 0.75 (Fig. 2, Table 2). The less intense blue zone of caffeic acid was found at R_f = 0.79 and the yellow band of rutin was revealed at R_f = 0.09. The unknown compounds of blue shades were identified at 0.14, 0.29, 0.43, and 0.64 of R_f values, and yellow one corresponding to flavonoids was found at 0.27 in the chromatogram of test solutions. The differences in the values of the R_f zones of RA as a predominant component corresponding the test and

Figure 1. HPTLC chromatograms of the OATes (1), *Ocimum americanum* essential oil (2), and linalool standard (A) after the derivatization with anisaldehyde solution at day light. Mobile phase: ethyl acetate – toluene (5:95).

Figure 2. HPTLC chromatograms of the OATes (A) and polyphenols standards (1 – rutin; 2 – caffeic acid; 3 – RA) after the derivatization with 1% methanolic AlCl_3 at $\lambda=366$ nm. Mobile phase: ethyl acetate – formic acid – water (15:1:1).

reference solutions within one plate did not exceed 0.01 units of R_f . The robustness studies of the RA identification in the OATes are presented in Table 4.

Summarizing the abovementioned results, it was considered that the validated techniques for the identification of linalool and polyphenols in OATes by the HPTLC method is a suitable procedure to control its quality in the 'Identification' test.

Table 4. The robustness testing of RA identification in OATes by HPTLC.

Indicator	Level of changes	Rf variability (RSD, %)
Mobile phase composition (ethyl acetate – formic acid – water, V/V)	15:1:1	0.8
	15:1.05:1	1.3
	15:0.95:1	1.7
	15:1:1.05	1.3
	15:1:0.95	1.3
Development distance, cm	8	0.8
	18	1.3
Chamber	3 different containers	1.7
Analysts	2 different analysts	0.6

HPLC analysis

The content of RA as a predominant biologically active ingredient in the OATes was assayed and validated by the HPLC for the first time. The mobile phase for HPLC consisted of two solutions: A (trifluoroacetic acid 0.1% in water), and B (trifluoroacetic acid 0.1% in acetonitrile) at 1.0 mL/min flow rate, volume of injection: 5 μL . All the solvents should be filtered through a 0.45 μm Millipore filter before use and degassed in an ultrasonic bath. Detection was made with spectrophotometer at 330 nm. HPLC was performed at 35°C column temperature. RA was determined using external standard method. The gradient elution was provided by mixing mobile phases A and B.

The validation of the HPLC test procedure for the analysis of RA content in the OATes included specificity, linearity, accuracy, precision, and robustness studies. The specificity of the test was confirmed by the coincidence of retention time of the RA peak on the chromatogram of OATes test solution with the retention of this peak on the chromatogram of RA standard solution (Fig. 3, 4). The chosen chromatographic conditions allowed to separate the peak of the RA from the peaks of other phenolic components. The values for the system suitability were an average of 6 replicates (Table 5). The linearity, recovery, accuracy, and range of HPLC method were defined on model mixtures with known content of active ingredients in the range of 80% to 120% of the RA nominal value (Table 6). The regression equation was calculated from the calibration curve for nine concentrations of the RA reference standard. The LOD and LOQ calculated for signal to noise ratio of 3 and 10, respectively. The aliquots of OATes test solution was split and stored at 4°C. The aliquots were compared against the fresh-prepared reference standards at various intervals to determine the standard stability. It was recommended to perform all the experiments during a maximum of 3 days and to store stock and standard solutions in a refrigerator.

Figure 3. UV absorption spectrum of RA in the HPLC method.

Figure 4. HPLC chromatogram of the RA standard solution (A) and OATEs (B) ($\lambda=330$ nm).

Table 5. The system suitability parameters for the determination of RA by HPLC.

Standard	Retention time (Rt)	Tailing factor (T)	Theoretical plates (N)
Rosmarinic acid	38.0	1.2	2399586

Table 6. The linearity, regression equation, limit of detection, limit of quantitation, precision parameters of RA standard in the HPLC analysis.

Standard	Linearity range [$\mu\text{g/ml}$]	Regression equation	R^2	LOD [$\mu\text{g/ml}$]	LOQ [$\mu\text{g/ml}$]	RSD [%]	
						Inter-day	Intra-day
Rosmarinic acid	5–50	$Y = 0.235x + 0.164$	0.9998	0.005	0.015	0.16	0.04

According to the results of the robustness study, the column temperature and the mobile phase flow within $\pm 5\%$ did not affect the test results significantly.

Standardization procedure

The standardization of the OATEs as a perspective herbal medicinal product has been carried out in accordance with the requirements of European Pharmacopoeia (2016) using such a scheme: definition (source of a plant material); production (method of extraction and solvent); characters

(appearance, taste, odour), identification (by HPTLC); assay (content of the main biologically active compound by HPLC). **Definition.** Tincture is produced from the *O. americanum* herb and its essential oil (OATEs).

Production. Tincture is produced from one part of dried herb using ethanol (70%, V/V) by a maceration method to obtain 10 parts of the finished product. Essential oil was dissolved first in ethanol (96%, V/V) in ratio 1:4, and then added to the tincture until 0.5% solution was obtained.

Characters. Greenish-brown transparent liquid with a specific aromatic small and bitter-burning taste.

Identification. Method A

Test solution. The tincture (OATs) to be examined (10 µL).

Reference solution. Dissolve 25 µL of linalool in 5 mL of toluene.

Plate: TLC silica gel F₂₅₄ plate (10 × 20 cm).

Mobile phase: ethyl acetate – toluene (5:95, V/V); **application:** 5 µL, as bands; **development:** 8 cm from the line of start; **drying:** in air.

Detection: treat with anisaldehyde solution, heat at 100–105°C for 5 min and examine in daylight immediately.

Results of TLC analysis. The chromatogram of the test solution in the lower third should be characterized by the weak violet zone of linalool ($R_f = 0.29$). Furthermore, other faint violet zones may be present in the chromatogram obtained with the test solution. Sequence of the zones present in the chromatograms obtained with the reference and test solutions (comparatively with the essential oil of *O. americanum*) are shown in Fig. 1.

Identification. Method B

Test solution. The tincture (OATs) to be examined (10 µL).

Reference solutions. Dissolve (separately) 1 mg of rutin, 1 mg of caffeic acid, and 1 mg of RA in 4 mL of methanol.

Plate: TLC silica gel F₂₅₄ plate (10 × 20 cm).

Mobile phase: ethyl acetate – formic acid – water (15:1:1, V/V); **application:** 5 µL, as bands; **development:** 8 cm from the line of start; **drying:** in air.

Detection: Visualization should be performed under UV light (254 nm, 366 nm) after spraying with 1% aluminium chloride solution and heating at 100–105°C for 5 min.

Results of TLC analysis. The chromatogram of the test solution should be characterized by intense blue zone of RA ($R_f = 0.75$) and less intense blue zone of caffeic acid ($R_f = 0.79$) just above it. The yellow zone of rutin should be evaluated at the bottom ($R_f = 0.09$). Furthermore, other faint blue and yellow zones may be present in the chromatogram obtained with the test solution. Red zones of chlorophyll may be present at the top. Sequence of the zones present in the chromatograms of the reference and test solutions are shown in Fig. 2.

HPLC analysis of RA content

Test solution. Introduce 1 mL of OATs into a volumetric flask (100 mL) and dilute it by adding 70% ethanol to the mark. Filter the obtained solution through 0.45 µm Millipore filter.

Reference solution. Dissolve 15 mg of RA standard in 70% ethanol and dilute to 100.0 mL with the same solvent.

Column: Phenomenex Luna C18 column (250 × 4.6 mm i.d., 5 µm particle size) at 35°C as appropriate.

Mobile phase: Trifluoroacetic acid 0.1% in water (solution A), and trifluoroacetic acid 0.1% in acetonitrile (solution B) at 1.0 mL/min flow rate in a gradient elution mode (Table 7).

Detection: DAD (330 nm).

Table 7. The gradient mode in the HPLC analysis.

Time (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0–5	95	5
5–35	95 → 75	5 → 25
35–40	75	25
40–60	75 → 50	25 → 50
60–65	50 → 20	50 → 80
65–70	20	80
70–85	95	5

Results of HPLC analysis: $0.26 \pm 0.01\%$ of RA. Identification of RA achieved by comparison of the retention time on the chromatogram and the absorption spectra obtained for its peak in tincture with those obtained for the standard. The quantification of RA made by measuring its peak area at $\lambda = 330$ nm. The content of RA in tincture should be at minimum 0.2%.

As it is known, the healing properties of the herbal medicinal products can be viewed as a result of the synergistic effect of a lot of biologically active compounds (Yang et al., 2014). As the complexes of active compounds in the obtained tinctures can interact synergistically, the dominating of both RA and linalool in the OATs could be the reason for the manifestation of its noticeable sedative action. A number of different pharmacologically active compounds with the possible sedative action was established in the *Ocimum americanum* water-ethanol extracts and essential oil (Shanayda, 2012; Shanaida and Golembiovska, 2018). The noticeable reduction in locomotor activity of the OATs could either be due to the effect of its biologically active compounds on the central nervous system or a direct influence on the periphery as it was established for the related species *Ocimum sanctum* (Richard et al., 2016). Researchers observed a marked anxiolytic dose-dependent effect, similar to Diazepam, after using the water-ethanol extracts and essential oil of *Ocimum basilicum* herb (Rabbani et al., 2015). The sedative influence of the essential oil released by *Ocimum basilicum* plants was found in mice using open field test (Hirai and Ito, 2019). The sedative, anxiolytic, and antidepressant-like effects were established after the inhalation of *Ocimum gratissimum* essential oil in mice (Tankam et al., 2014).

The obtained data about the prevalence of RA among phenolic compounds in the OATs revealed by the chromatographic methods correlates with the results of phytochemical analyses of the *O. americanum* herb (Shanaida et al., 2018; Shanaida et al., 2020). RA was also the 'marker component' of the ethanol extracts obtained from a lot of plants belonging to the *Lamiaceae* family such as *Origanum vulgare*, *Melissa officinalis*, *Rosmarinus officinalis*, *Ocimum basilicum*, *Salvia officinalis*, and *Hyssopus officinalis* (Staszek et al., 2013;

Benedec et al., 2015). RA can potentiate the pentobarbital-induced sleeping through the activation of GABA_A-ergic systems (Yeong et al., 2017). It can also demonstrate the antioxidant effect and other valuable biological activities similar to the other polyphenols containing more than 3 OH-groups in their molecules (Gontova et al., 2016; Rytsyk et al., 2020). Polyphenols possess free radicals scavenging activity, which can prevent neurodegenerative diseases (Cory et al., 2018). The methanol extract of *Stachytarpheta cayennensis* (order *Lamiales*) and its butanol, aqueous, and ethylacetate fractions demonstrated the sedative effect in a dose-dependent manner (Olayiwola et al., 2013).

Linalool as a key volatile molecule of many essential oils of the *Ocimum* species possess the sedative activity (Aprotosoie et al., 2014). Linalool and other terpenoids can modify the function of GABA_A receptors (Kessler et al., 2014). It was found that linalool and linalool-containing essential oil obtained from *Lavandula angustifolia* (*Lamiaceae*) aerial part were useful alternative tools to the available traditional treatments for social stress-induced mental illnesses such as anxiety and depression (Caputo et al., 2018; Agatonovic-Kustrin et al., 2020). The relationships between the contents of certain bioactive compounds of terpenoid and polyphenol nature in the *Leonurus cardiaca* (*Lamiaceae*) tinctures and their sedative effect were revealed by Romanenko et al. (2018).

The sedative properties of the obtained tinctures can be enhanced by some amino acids, which are extracted from the plant raw material with water and water-ethanol solutions (Shafaei et al., 2017; Shanaida et al., 2017). Besides γ -aminobutyric acid and glycine as the famous inhibitory neurotransmitter in the central nervous system, it was also established that the sedative effect of a lysine, arginine, alanine, serine, and cysteine acting via γ -aminobutyric acid

receptors (Yamane et al., 2009). All these amino acids were found in the *Ocimum americanum* herb (Shanaida et al., 2017). The triterpenic ursolic acid possessing sedative action (Colla et al., 2015) was revealed in the 70% ethanol extract of *Ocimum americanum* (0.12%) (Shanaida and Golembiovska, 2018). Thus, the revealed sedative properties of the OATs can be due to synergistic effects of RA and linalool as well as some amino acids and triterpenoids extracted from the *Ocimum americanum* raw material with water-ethanol solutions.

CONCLUSIONS

This study demonstrates the significant sedative effect of the OATs developed from *O. americanum* herb using 70% ethanol and essential oil isolated from this plant. Such sedative property could be due to the common influence of RA and linalool as the predominant biologically active compounds in OATs as well as some amino acids and triterpenoids. More tests and studies are needed to clarify the exact components with sedative activity in the developed herbal medicinal product. The necessary standardization parameters for the chromatographic analysis of the OATs were established. The validated HPTLC method was successfully applied for identifying linalool and RA as the predominant compounds of the OATs. The HPLC method was developed and validated to quantify the RA content in the OATs. The current study provides a basis for the assessment of developed OATs as the new perspective herbal medicinal product with sedative action.

CONFLICT OF INTEREST

There are no conflicts of interest declared by the authors.

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