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Original research

Comparative analysis of essential oils from three species of the genus *Artemisia* cultivated in Ukraine

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Abstract

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This study aimed to determine the composition of essential oils in the aerial part of three species of the genus *Artemisia* L.: *Artemisia argyi* H. Lév. & Vaniot*, Artemisia austriaca* Jacq. and *Artemisia ludoviciana* Nutt*.* These species of different origins were introduced at the M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). The conducted gas chromatography-mass spectrometry analysis revealed that the main components of *Artemisia argyi* essential oil were *alpha*-terpinolene (45.984%), pinocarvone (25.651%), *alpha*-phellandrene (11.446%) and 1,8-cineole (5.895%), whereas 1,8-cineole (19.921%), sobrerol (15.162 %), pinocarvone (14.535%) and 1-nitrophenyl propene (7.738%) dominated in the essential oil of *Artemisia austriaca*. Such volatile compounds as *beta*-ocimene (40.307 %), hexyl butyrate (18.291%), mirtenol (10.785%), mirtanal (10.174%), *cis*-sabinol (6.025%) and verbenene (5.779%) prevailed in the *Artemisia ludoviciana* essential oil. The domination of 1,8-cineole and pinocarvone revealed similarities between the essential oils of *Artemisia argyi* and *Artemisia austriaca*. Considering that many of the predominant compounds have proven therapeutic properties, it could be supposed that essential oils and the raw materials of the three studied species are potentially suitable for future use in the pharmaceutical industry and aromatherapy.

Keywords: *Artemisia argyi, Artemisia austriaca, Artemisia ludoviciana,* gas chromatography-mass spectrometry, herb, volatile compounds.

INTRODUCTION

The genus *Artemisia* L. comprises about 500 species of aromatic plants spread worldwide (Trifan et al., 2022). The species of the genus *Artemisia* grow abundantly in the Northern Hemisphere, and a small number of species are distributed in the Southern Hemisphere (Sharifi-Rad et al., 2022). Many *Artemisia* species are recognised medicinal plants, especially *Artemisia absinthium* L., *Artemisia annua* L. and *Artemisia vulgaris* L. (Trifan et al., 2022; Sharifi-Rad et al., 2022; Ekiert et al., 2022). These three representatives are the most scientifically studied regarding their chemical composition, biological activity and history of use in healing (Ekiert et al., 2022).

Many species of the genus *Artemisia* originating from different continents are used in traditional medicine. The species of *Artemisia* are widespread throughout the world. Still, they are popular in traditional Chinese medicine, frequently used for treating malaria, inflammation, hepatitis, cancer, and microbial infections (Abad et al., 2012). Essential oils from different *Artemisia* species exhibited strong antimicrobial effects against plant pathogens as well as insecticidal and antioxidant activities (Pandey & Singh, 2017; Hrytsyk et al., 2021). Generally, essential oils from *Artemisia* plants have a broad spectrum of pharmacological effects owing to several main bioactive ingredients demonstrating various modes of action (Abad et al., 2012; Sharifi-Rad et al., 2017).

Some representatives of *Artemisia* have successfully been introduced in the botanical gardens of Ukraine, and several of them are of great scientific interest because their phytochemical properties are poorly studied. In particular, this applies to species such as *Artemisia austriaca* Jacq., *Artemisia argyi* H. Lév. & Vaniot and *Artemisia ludoviciana* Nutt.

The aim of the study was the chromatographic analysis of essential oils obtained from three species of the genus *Artemisia* (*Artemisia argyi*, *Artemisia austriaca* and *Artemisia ludoviciana*) under their cultivation in Ukraine.

MATERIALS AND METHODS

Characteristics of the studied species

Artemisia austriaca is native to central Europe and some Asian regions and grows primarily in the temperate biome. It is a semi-shrub up to 60 cm tall (Adekenov, 2021; Kovaleva et al., 2010). *Artemisia ludoviciana*, is a North American species (Anaya-Eugenio et al., 2016; Shultz, 2006). It is a rhizomatous perennial plant growing up to 100 cm tall. *Artemisia argyi* is native to Asian regions. It is a herbaceous perennial plant with a creeping rhizome and upright, greyish branches up to 110 cm tall (Huang et al., 2012; Korablova et al., 2021). All the species, as mentioned above, possess a specific bitter smell, perceptible when the aerial parts of the plants are rubbed.

Plant raw materials

The herbs of three studied species were harvested in August 2022 at the start of the flowering period from the experimental plots at M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). It is located in the Forest-Steppe zone (50°24′45″ N and 30°33′44″ E).

Conditions for obtaining essential oil

The above-ground part of the plants was cut off, chopped into 1.5–2 cm pieces and left for 24 hours at room conditions. After this, the raw material was dried on an *Eridri ULTRA FD1000* dryer at 35 °C. Essential oils were hydrodistilled from the dried plants using a Clevenger apparatus. The weight of each sample of raw material was 35 g.

The exposure time during hydrodistillation was 2 hours (from when the water boils). The contents of essential oils in the raw plant material of the studied species were determined in terms of absolute dry weight. All experiments were performed in triplicate.

Chromatographic conditions

The gas chromatography-mass spectrometry (GC/MS) was applied to analyse essential oils. The *Agilent Technologies 7890* gas chromatograph with (5%-phenyl)-methylpolysiloxane) 25 m long capillary column (internal diameter of 0.25 mm and the stationary phase thickness of 0.33 µm) was used (Kovtun-Vodyanytska et al., 2023). The following chromatographic conditions were applied: carrier gas (helium) velocity was 1.0 mL/min; flow split ratio was 1 : 20; evaporator temperature was 250 °C; the column was gradually heated from 60 °C to 185 °C; detector temperature was 280 °C. Sample injection (1 µL) was without flow split. A mass spectrometric detector *Hewlett Packard 6890* with a mass spectrometric detector 5973 was used. The National Institute of Standards and Technology (NIST, 1994) mass spectrum library was combined with programmes to identify essential oil components.

Each chromatographic analysis was repeated three times. The mean value of the relative composition of the percentage of essential oils was calculated based on values obtained from the evaluation of peaks.

RESULTS

During the GC/MS analysis, a total of 48 compounds were identified in the essential oils of the studied species (Table 1). The examples of the GC/MS chromatograms are provided in Figs 1–3. The essential oil yields in *Artemisia argyi, Artemisia austriaca* and *Artemisia ludoviciana* were 0.73 ± 0.03%, 0.75 ± 0.03 and 1.08 ± 0.06%, respectively. The essential oil of *Artemisia argyi* and *Artemisia austriaca* was colourless, while *Artemisia ludoviciana* had a blue colour.

It was found that the predominant components of *Artemisia argyi* essential oil were monoterpenoids: *alpha*-terpinolene (45.984%), pinocarvone (25.651%), *alpha*-phellandrene (11.446%) and 1,8-cineole (5.895%). Dominant compounds 1,8-cineole (19.921%), sobrerol (15.162%) and pinocarvone (14.535%) belonging to monoterpenoids were in the essential oil of *Artemisia austriaca*. Such volatile compounds as *beta*-ocimene (40.307%), hexyl butyrate (18.291%), mirtenol (10.785%), mirtanal (10.174%), *cis*-sabinol (6.025%) and verbenene (5.779%) prevailed in the *Artemisia ludoviciana* essential oil. It should be noted that hexyl butyrate (butyric acid hexyl ester) is an aliphatic hydrocarbon, while all other components belong to compounds of the monoterpene group.

It could be mentioned that the domination of 1,8-cineole and pinocarvone points to some similarities between the essential oils of *Artemisia argyi* and *Artemisia austriaca*. Such compounds as *alpha*-terpinene*, p*-cymene*, gamma*-terpinene, thujone, hexyl butyrate, germacrene D, D-verbenone and methyl jasmonate were detected in small amounts in the essential oils of all three studied species.

DISCUSSION

Essential oils contain a variety of volatile molecules such as terpenoids, phenol-derived aromatic compounds, and aliphatic components (Abad et al., 2012). Most detected predominant components from the investigated essential oils of the *Artemisia* species belong to the monoterpenoids, except hexyl butyrate, a fatty acid ester. At the same time, the essential oils of *Artemisia absinthium*, *Artemisia annua,* and *Artemisia vulgaris* as the best-known species of this genus contain essential oil where monoterpenoids commonly with sesquiterpenoids (*αlpha*- and *beta*-thujone, 1,8-cineole, sabinene, guaiazulene, artemisinin, etc.) dominate (Trifan et al., 2022; Sharifi-Rad et al., 2022).

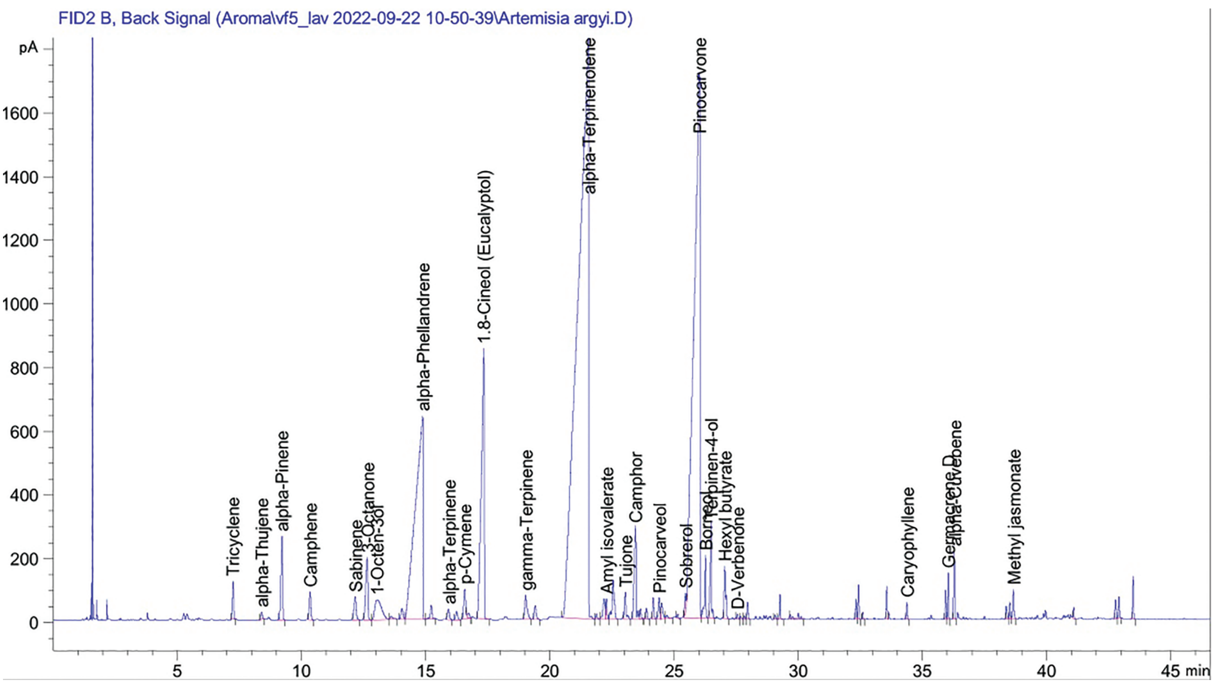

Fig. 1. The characteristic gas chromatography-mass spectrometry chromatogram of the *Artemisia argyi* essential oil

Table 1. Chemical compositions of essential oils isolated from three *Artemisia* species

Compound	Retention time, min	Relative quantity (%)		
		Artemisia argyi	*Artemisia austriaca*	*Artemisia ludovociana*
Tricyclene	7.239	0.349	–	–
alpha-Thujene	8.391	0.081	–	0.316
alpha-Pinene	9.208	1.018	–	0.606
Camphene	10.357	0.362	–	–
Verbenene	10.742	–	–	5.779
Sabinene	12.241	–	–	0.332
3-octanone	12.661	1.029	–	0.709
1-octen-3ol	13.057	1.008	0.202	–
beta-Myrcene	13.702	–	0.219	–
alpha-Phellandrene	14.895	11.446	0.218	–
alpha-Terpinene	15.925	0.142	0.393	0.088
p-Cymene	16.585	0.446	1.161	0.025
1,8-Cineole (eucalyptol)	17.352	5.895	19.921	–
Trans-*beta*-ocimene	18.243	–	0.193	40.307
gamma-Terpinene	19.042	0.411	0.651	1.668
Sabinene hydrate	20.001	–	0.417	0.077
Citral	20.986	–	0.527	0.111
2-carene	21.260	–	0.155	–
alpha-Terpinolene	21.562	45.984	–	–
trans-*p*-Mentha-2,8-diene-1-ol	22.080	–	0.483	0.301
Amyl isovalerate	22.286	0.213	0.228	–
Thujone	23.076	0.335	0.703	0.042
Camphor	23.678	1.313	–	0.334
Pinocarveol	24.666	0.225	8.501	–
cis-Sabinol	24.882	–	8.801	6.025
Sobrerol	25.452	0.166	15.162	–
Pinocarvone	26.037	25.651	14.535	–
Borneol	26.276	0.609	3.295	–
Terpinen-4-ol	26.505	1.006	1.642	–
Mirtanal	26.646	–	0.295	10.174
Hexyl butyrate	27.058	0.668	0.856	18.291
Mirtenol	27.208	–	0.523	10.785
D-verbenone	27.571	0.046	0.559	1.859
Cuminaldehyde	28.491	–	0.316	0.283
Thujol	28.854	–	0.264	–
Linalyl acetate	29.430	–	–	0.138
1-nitrophenyl propene	30.511	–	7.738	0.800
Phenylproppene	30.702	–	0.729	0.086
Eugenol	31.780	–	0.234	0.033
Copaene	33.089	–	0.236	0.045
Caryophyllene	34.389	0.131	–	0.075
alpha-Bergamotene	35.140	–	0.135	–
Germacrene D	36.089	0.318	2.370	0.287
alpha-Cuvebene	36.301	0.500	–	0.066
Germacrene B	36.740	–	0.505	0.040
Caryophyllene oxide	36.963	–	0.070	0.071
Methyl jasmonate	38.698	0.261	0.520	0.215
Chamazulene	42.124	–	0.563	0.033

Ochkur (2014) conducted the GC/MS analysis of the essential oils of 14 *Artemisia* species growing in Ukraine, including *Artemisia ludoviciana*, and determined that such components as 1,8-cineole, copaine, thujone, caryophyllene oxide, etc. are quite characteristic for almost all the studied species. This is consistent with the data obtained by us regarding three studied species. As for chromatographic studies of essential oils of such species as *Artemisia argyi* and *Artemisia austriaca*, we did

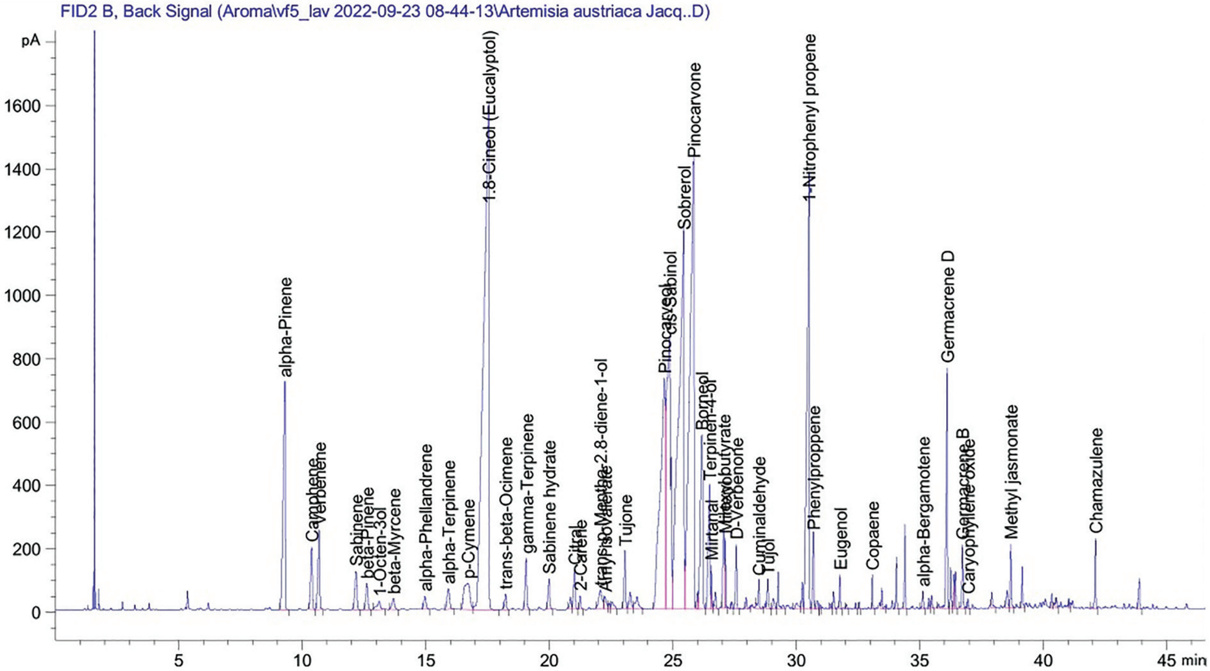

Fig. 2. The characteristic gas chromatography-mass spectrometry chromatogram of the *Artemisia austriaca* essential oil

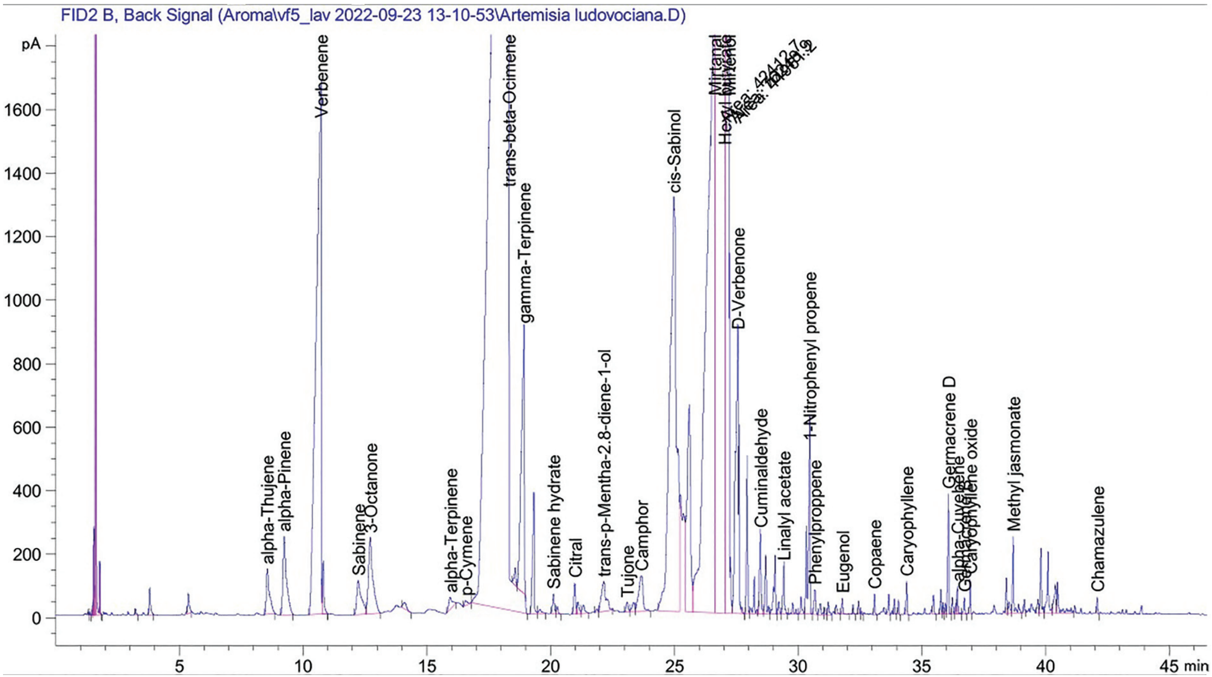

Fig. 3. The characteristic gas chromatography-mass spectrometry chromatogram of the *Artemisia ludoviciana* essential oil

not find such information in the scientific sources available.

Many of the revealed predominant compounds from the three studied species have proven therapeutic properties. For instance, 1,8-cineole (eucalyptol) has antiseptic and anti-inflammatory effects (Arooj et al., 2023). According to Radice et al. (2022), *alpha*-phellandrene has promising biological properties, including antibacterial, insecticidal, antitumoral, antinociceptive, etc. Recent studies (Ciprandi & Varricchio, 2023) have demonstrated that monoterpenoid sobrerol, which belongs to the significant components of the *Artemisia austriaca* essential oil, has antioxidant and mucolytic properties which are necessary for managing children with frequent respiratory infections. Antibacterial and antileishmanial activity of *beta*-ocimene has been revealed (Sousa et al., 2023). It should be noted that the content of such a potentially toxic compound as thujone was less than 1.0% in the essential oil of each investigated species.

As it has recently been revealed, oxygenated monoterpenes (47.38 %), oxygenated sesquiterpenes (22.72%) and sesquiterpene hydrocarbons (12.70%) dominate in the essential oil from the *Artemisia argyi* leaves of Chinese origin, which has been obtained by hydrodistillation (Guan et al., 2019). Among monoterpenoids, *alpha*-thujone (7.99%) and borneol (6.48%) dominate, while neointermedeol (9.65%), caryophyllene oxide (8.71%) and *beta*-caryophyllene (7.49%) prevail among sesquiterpenoids (Guan et al., 2019). Monoterpenoids borneol (30.1%) and bornyl acetate (29.8%) dominate in the essential oils from the *Artemisia argyi* flowers collected in China (Li et al., 2008). Terpineol (10.1%), spathulenol (10.0%), caryophyllene oxide (6.5 %), juniper camphor (8.7%), 1,8-cineole (4.4 %) and borneol (3.5%) being considered as the significant component of *Artemisia argyi* flower-heads collected in another region of China (Wenqiang et al., 2006). Eucalyptol (23.66 %) and caryophyllene (10.19%) were the predominant components of the *Artemisia argyi* essential oil harvested in Taiwan (Huang et al., 2012). Jiang et al. (2016) have revealed that the predominant components of the *Artemisia argyi* essential oils are 1,8-cineole (16.2%), *beta*-pinene (14.3%), camphor (14.0%) and artemisia ketone (13.9%). Recently, it has been revealed that the main volatile components of essential oils from different *Artemisia argyi* germplasm resources are cineole, thujarone, artemisia ketone, and *beta*-caryophyllene (Guo et al., 2023). Zhan et al. (2014) have found that monoterpenoids eucalyptol (22.03%), *beta*-pinene (14.53%) and (-)-camphor (5.45%) as well as sesquiterpenoid *beta*-caryophyllene (9.24 %) are the major components of essential oil isolated from Chinese *Artemisia argyi*. It has also been revealed that this essential oil and the four isolated predominant compounds exhibit some toxic effects against *Lasioderma serricorne* in adults (Zhan et al., 2014). The best LD_{50} value has been found for whole essential oil (6.42 µg/adult) followed by camphor (11.30 µg/adult) and eucalyptol (15.58 µg/adult). Thus, it can be concluded that different researchers are dealing with varieties of chemotypes of *Artemisia argyi*.

The anticancer, antiviral, antioxidant, anti-inflammatory and neuroprotective effects of the *Artemisia argyi* essential oil have recently been discovered (Wang et al., 2023a). Zhao et al. (2021) have reported that *Artemisia argyi* can be used for contagion prevention of coronavirus infection in hospital corridors by aromatherapy. Wang et al. (2023b) have described the antioxidant effects of the *Artemisia argyi* extracts. Zhang et al. (2023) have found the antihepatitis B activity of *Artemisia argyi* essential oil nanoparticles. The hepatoprotective effect of *Artemisia argyi* essential oil on bisphenol A-induced hepatotoxicity has recently been revealed (Cui et al., 2023).

According to Kikhanova et al. (2013), the essential oil of *Artemisia austriaca* is mainly comprised of camphor, 1,8-cineole and camphene. Thus, these scientists have dealt with another chemotype of *Artemisia austriaca* compared to that studied by us (Table 1). Three sesquiterpene lactones (arborescin, austricin and artausin) of the guaian type have been identified in the acetone extract of *Artemisia austriaca* by Adekenov (2021). The *Artemisia austriaca* herb is traditionally used for its wound healing, anthelmintic, anticonvulsant, choleretic, and anti-inflammatory activities (Adekenov, 2021).

Regarding *Artemisia ludoviciana,* Mexican scientists Anaya-Eugenio et al. (Anaya-Eugenio et al., 2016) have revealed that the main volatile compounds of its essential oil are (±)-camphor, *gamma*-terpineol and 1,8-cineole (unlike the data that we received regarding this species). High contents of 1,8-cineole and camphor have been found in *Artemisia ludoviciana* essential oil obtained in Western Canada (Lopes-Lutz et al., 2008). The essential oil of *Artemisia ludoviciana* has also been characterised by a high content of oxygenated sesquiterpenes, with davanone (11.5%) as the predominant component (Lopes-Lutz et al., 2008). Anaya-Eugenio et al. (2016) revealed the antinociceptive activity of essential oil from *Artemisia ludoviciana*. We hypothesise that the blue colour of the studied *Artemisia ludoviciana* essential oil may be due to the sesquiterpenoid chamazulene (0.563%).

It could be supposed that the differences in the chemical compositions of essential oils of the studied species comparatively to those found in other scientific articles could be explained depending on their genetic chemotype as well as due to the different geographic location of plants, their stage of development, growth conditions, method of extraction, etc. (Hudz et al., 2020; Koshovyi et al., 2020; Kovaleva et al., 2010; Shanaida et al., 2021; Sharifi-Rad et al., 2017).

CONCLUSIONS

This study identified and quantified the major and minor components of essential oils from three *Artemisia* representatives under their cultivation in Ukraine. The obtained results regarding GC/MS chromatographic profiles and specific bioactive compounds of the essential oils could be taken into account for further studying their chemotaxonomical peculiarities and biological activities. We suppose that essential oils and the raw materials of *Artemisia argyi*, *Artemisia austriaca* and *Artemisia ludoviciana* are potentially suitable for future use in the pharmaceutical industry and aromatherapy.

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