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Chromatographic Profiles of Polyphenols in the Herbs of *Artemisia campestris* L. and *Artemisia ludoviciana Nutt*.

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This study aimed to analyze the compositions of flavonoids and phenolic acids in the aerial parts of two Artemisia L. species (Artemisia ludoviciana Nutt. and Artemisia campestris L.) when grown in Ukraine. High-performance liquid chromatography analysis (HPLC) detected the presence of 11 flavonoids and 10 phenolic acids in the plant materials under study. Among the phenolic acids, chlorogenic acid was the most abundant in the raw material of both species (14.503 mg/g in Artemisia ludoviciana and 4.504 mg/g in Artemisia campestris). The main flavonoids in the Artemisia ludoviciana herb decreased in the following order: flavanone-7-O-glycoside (naringin) (21.924 mg/g) > fisetin (13.068 mg/g) > kaempferol-3-b-glucoside (5.119 mg/g) > rutin (1.295 mg/g). In comparison, in Artemisia campestris raw material the order was: flavanone-7-O-glycoside (7.525 mg/g) > fisetin (2.933 mg/g) > rutin (1.355 mg/g). Since the predominant polyphenols have demonstrated valuable therapeutic potential, the data obtained could be considered for further exploration of their biological activities.

Keywords: Aerial part; Artemisia species; Flavonoids; High-performance Liquid Chromatography; Phenolic acids.

Medicinal plants are rich sources of valuable natural compounds with promising therapeutic potential, which play a crucial role in traditional and evidence-based medicine[1]. The gradual development of more advanced instrumental extraction techniques and analytical methods has allowed for the comprehensive identification of numerous bioactive plant metabolites[2]. There is no doubt that polyphenols, synthesized in plants, are a diverse and essential group of bioactive components[3-5].

The genus Wormwood (*Artemisia* L., family *Asteraceae* Dumort.) includes many species that have long been of interest to phytotherapists and researchers as valuable sources of healing substances. They are considered to be among the most well-known medicinal plants in the world[6]. These plants have significant potential in the treatment of numerous diseases, and more recently, in the treatment of malaria[7]. It's important to note that *Artemisia absinthium* L. was previously included in the European Pharmacopoeia (6.4)[8]. However, it was later removed from pharmacopoeial monographs due to the neurotoxic effects of thujone, one of the main compounds in its essential oil.

The aerial parts of several *Artemisia* plants have been used in traditional medicine in many countries due to their antimicrobial, antiparasitic, nematocidal, insecticidal, hypoglycemic, secretolytic, etc. effects. These effects are attributed to the diverse chemical compounds found in the essential oils and polyphenols of *Artemisia* species[9]. Researchers in the pharmaceutical industry have shown significant interest in the varied chemical compositions of *Artemisia* representatives, leading to numerous studies on their potential medical uses[6,7,9,10].

It is evident from the analysis of available scientific sources that there has been relatively limited study of the phytochemical profiles and pharmacological properties of certain *Artemisia* species. For instance, as of July 01, 2024, a search on the PubMed international database of scientific publications revealed only 38 articles for "*Artemisia ludoviciana*", 69 articles for "*Artemisia campestris*", and 6159 articles for "*Artemisia vulgaris*". This indicates the significant differences in the levels of scientific interest in studying the bioactive components of various *Artemisia* representatives. Therefore, there is a considerable key point in further exploring the phytochemicals of *Artemisia ludoviciana* Nutt. and *Artemisia campestris* L.

Recent studies have revealed compelling data regarding the chemical composition and medicinal properties of *Artemisia ludoviciana* and *Artemisia campestris,* plants with a history of traditional use in North American and Eurasian indigenous medicine, respectively[10-12]. Despite this, there has been limited scientific research on the biomedical potential of these plants. Their essential oils were much better studied than polyphenols or other bioactive compounds [6,10,12-15]. Besides it, *Artemisia campestris* is a polymorphic species consisting of many subspecies, varieties, and chemotypes that differ in the chemical composition of biocompounds[11].

It should be noted that *Artemisia ludoviciana* and *Artemisia campestris* have been successfully introduced to botanical gardens and research institutions, including in Ukraine. Considering this, we advocate for comprehensive phytochemical research attention for these two species.

MATERIALS AND METHODS

Plant raw material

The herbs of *Artemisia campestris* and *Artemisia ludoviciana* were harvested in 2022 during the flowering period from the plots in M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) which is located in the Forest-Steppe zone (30°332 443 east longitude and 50°242 453 north latitudes). The above-ground parts of plants were dried at 30-35°C.

The phytochemical research of flavonoids and phenolic acids in the studied raw materials was carried out by the method of high-performance liquid chromatography (HPLC). Before the HPLC analysis, the grinded raw material was extracted with 80% methanol using an ultrasonic bath (in sealed glass vials with Teflon caps for 2 hours at 70¨Ñ). The obtained extracts were centrifuged and then filtered through membrane filters (pores 0.22 ìm).

Chromatographic analysis

The HPLC analis was performed on an Agilent Technologies 1200 liquid chromatograph with a Zorbax SB-C18 column (3.5 ìm, 150 x 4.6 mm).

During the study of flavonoids, acetonitrile (A) and a 0.1% solution of formic acid in water (B) were used as the mobile phase. The elution was performed in the gradient mode: 0 min – A (5%) : B (95%); 20 min – A (30%) : B (70%); 30 min – A (60%) : B (40%); 50 min – A (100%) : B (0%); 60 min – A (100 %) : B (0 %). The flow rate was 0.25 mL/min. The injection volume was 4 ìL. The detection was carried out using a diode-matrix detector with signal registration at a wavelength of 280 and 365 nm. The research was carried out using standard solutions of flavonoids (rutin, quercetin, kaempferol, naringenin, naringin, neohesperidin, quercetin-3-*b*-glycoside, kaempferol-3-*b*-glucoside, apigenin, luteolin, baicalein, rhamnetin, fisetin and silibenin).

To analyse the phenolic acids, methanol (A) and a 0.1% solution of formic acid in water (B) were chosen as the mobile phase. The elution was carried out in the gradient mode: 0 min – A (10%): B (90%); 40 min – A (75%) : B (25%); 45 min – A (100%) : B (0%); 55 min – A (100 %) : B (0 %). The flow rate through the column was 0.6 mL/min. The injection volume was 3 ìL. The detection was carried out using a diode-matrix detector with signal registration at 275 and 330 nm. The identification and quantification were performed using standard solutions of phenolic acids (gallic, hydroxyphenylacetic, benzoic, quinic, syringic, sinapic, rosmarinic, caffeic, chlorogenic, *p*-coumaric, *trans*-cinnamic, and *trans*-ferulic).

The content of identified phenolic compounds (X) (mg/g) was calculated according to the formula:

$$X = C \times V/m,$$

Where: C – is the concentration of the compound determined chromatographically, mg/mL;
V – the volume of the extract, mL;
m – the weight of the extracted raw material, g.

RESULTS AND DISCUSSION

The conducted HPLC study revealed the composition and contents of phenolic acids and flavonoids in the raw materials of *Artemisia campestris* and *Artemisia ludoviciana*. In the analysis, 10 phenolic acids were identified (Table 1, Fig. 1). Hydroxycinnamic chlorogenic acid was the most abundant in the herbs of both species, with its content being 3.2 times higher in *Artemisia ludoviciana* compared to *Artemisia campestris.* Rosmarinic acid was the second most prevalent phenolic acid in the aerial parts of the studied plants. Other phenolic acids were found in much smaller quantities. It is important to note that caffeic and quinic acids were identified only in the *Artemisia campestris* herb while *trans*-ferulic acid was found only in the *Artemisia ludoviciana.*

Chlorogenic acid (Fig. 2) as the major phenolic acid of both studied herbs has various pharmacological activities such as antioxidant, anticancer, hepatoprotective, immunomodulating, antimicrobial, antidiabetic, etc.[16]. It finds applications in multiple industries, including healthcare, food, and chemicals[17]. As it was discovered recently, chlorogenic acid was responsible for the observed antioxidant activity of the water-alcohol extract of *Artemisia campestris*[18].

Using the HPLC method, Ochkur[19] identified chlorogenic acid in ethanolic extracts of 5 species of wormwood harvested in Ukraine: *Artemisia vulgaris* L., *Artemisia*

Table 1. The amounts of phenolic acids in the studied *Artemisia* herbs evaluated by the HPLC method

Phenolic acid	Retention time,min	Content, mg/g	
		Artemisia campestris	*Artemisia ludoviciana*
Gallic acid	5.9	0.081	0.117
Hydroxyphenylacetic acid	9.4	0.109	0.136
Chlorogenic acid	11.3	4.504	14.503
Caffeic acid	12.2	0.216	Not detected
Syringic acid	14.5	0.089	0.223
Benzoic acid	15.8	0.044	0.079
trans-Ferulic acid	17.6	Not detected	0.324
Sinapic acid	19.3	0.051	0.041
Rosmarinic acid	20.4	1.774	3.326
Quinic acid	23.1	0.117	Not detected

abrotanum L., *Artemisia austriaca* Jacq., *Artemisia dracunculus* L. and *Artemisia absinthium*. The liquid chromatography-mass spectrometry assessment of methanol extracts of five *Artemisia* representatives collected from Romanian flora (*Artemisia vulgaris*, *Artemisia absinthium, Artemisia austriaca, Artemisia pontica* L. and *Artemisia annua* L.) led to the identification

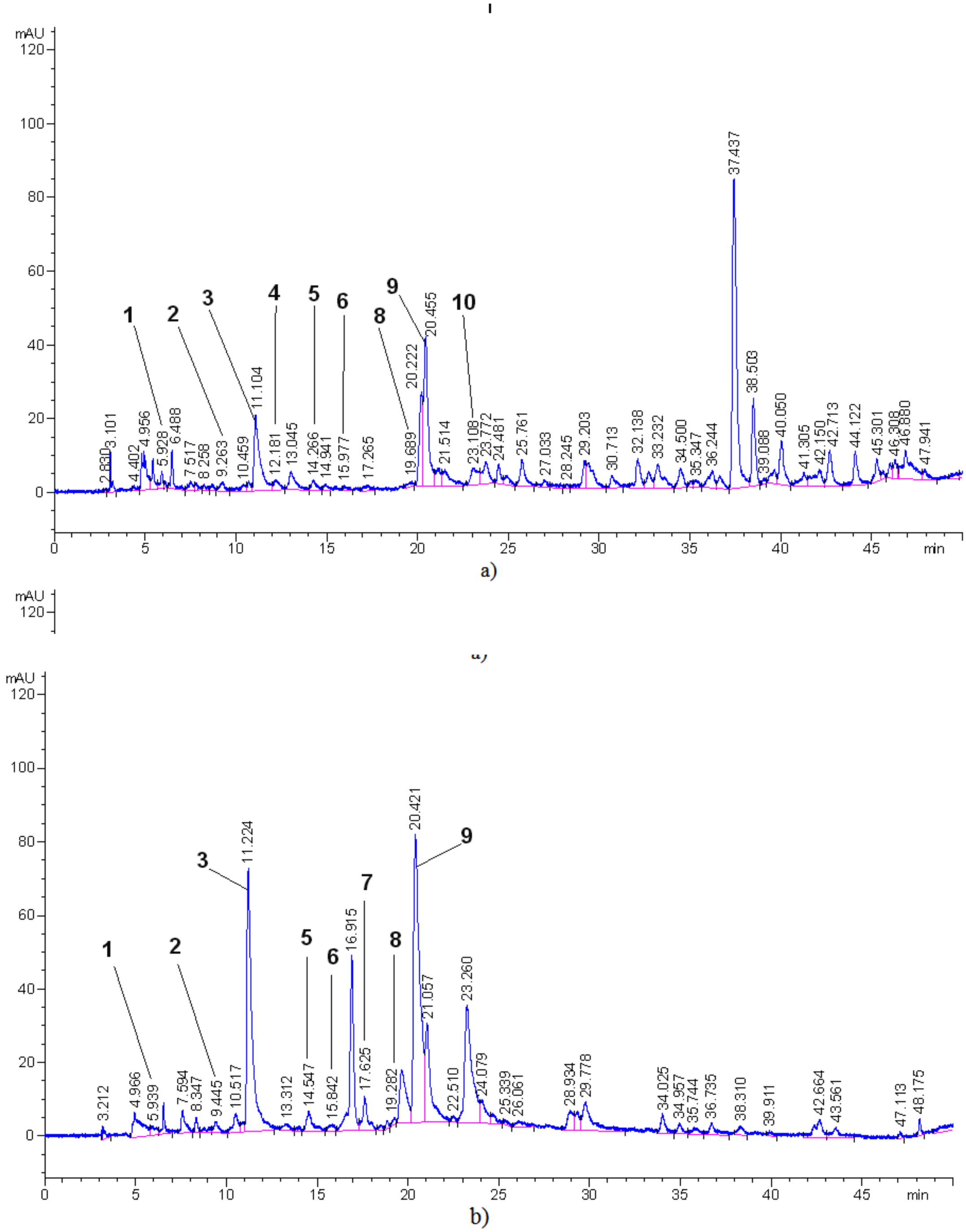


Fig. 1. HPLC chromatograms of phenolic acids detected in the *Artemisia campestris* (a) and *Artemisia ludoviciana* (b) herbs: 1 – gallic acid; 2 – hydroxyphenylacetic acid; 3 – chlorogenic acid; 4 – caffeic acid; 5 – syringic acid; 6 – benzoic acid; 7 – *trans*-ferulic acid; 8 – sinapic acid; 9 – rosmarinic acid; 10 – quinic acid

of 26 flavonoids (mainly flavone derivatives) and 15 phenolic acids[20]. It should be noted that chlorogenic acid was the common predominant compound of all studied *Artemisia* speciesfrom Romania[20]. Chlorogenic acid was also regarded as a predominant component with antioxidant activity among several caffeoylquinic acids detected in the *Artemisia absinthium* and *Artemisia ludoviciana* grown in Lithuania[9]. In confirmation of the above data, Ickovski and co-authors[21] found that chlorogenic acid was the most abundant phenolic acid in the methanolic extracts of *Artemisia vulgaris* and *Artemisia absinthium* aerial parts collected in Serbia.

The main biological activities of rosmarinic acid include anti-inflammatory, antioxidant, antidiabetic, antiviral, antitumor, neuroprotective, and hepatoprotective effects[22]. The accumulation of rosmarinic acid is very specific for some taxons of the *Lamiaceae* as well as *Asteraceae* families[23-24]. Interesting scientific data has been revealed regarding the rosmarinic acid, identified in *Artemisia annua* extracts. It showed a synergistic interaction with artemisinin when acting on a malarial plasmodium strain[25].

Among 11 identified flavonoids detected in the herbs of *Artemisia* species by us, it was determined the prevailing flavanone-7-*O*-glycoside (naringin) (Table 2, Fig. 3). Its content was 2.9 times higher in *Artemisia ludoviciana* compared to *Artemisia campestris*. Regarding flavonoid fisetin, its amount was 4.6 times higher in the *Artemisia ludoviciana* herb.

As can be seen from Table 2, the *Artemisia ludoviciana* herb accumulates apigenin and naringenin, while *Artemisia campestris* does not contain these flavonoids at all. Regarding quercetin and baicalein on the contrary, in

Table 2. The amounts of flavonoids in the studied *Artemisia* herbs evaluated by the HPLC method

Flavonoid	Retention time, min	Content, mg/g	
		Artemisia campestris	*Artemisia ludoviciana*
Rutin	22.6	1.355	1.295
Quercetin-3-*b*-glycoside	23.6	0.109	0.080
Kaempferol-3-*b*-glucoside	25.2	0.181	5.119
Flavanone-7-*O*-glycoside	26.1	7.525	21.924
Fisetin	27.7	2.933	13.068
Quercetin	33.3	0.296	Not detected
Luteolin	36.9	0.404	0.101
Naringenin	38.1	Not detected	0.304
Apigenin	38.7	Not detected	0.739
Baicalein	39.4	0.266	Not detected
Kaempferol	47.3	0.718	0.148

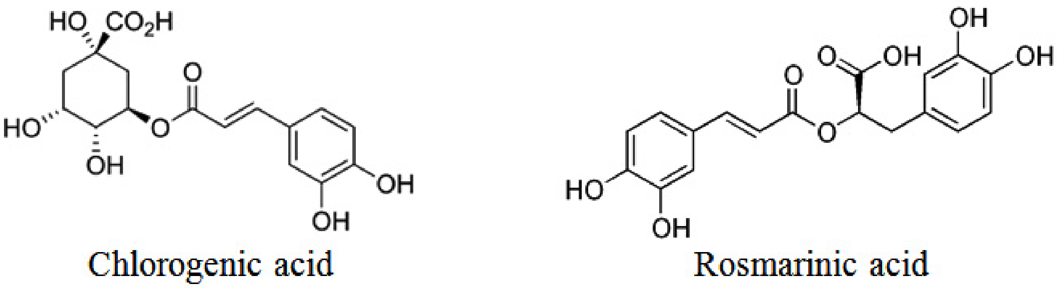


Fig. 2. Structural formulas of the predominant phenolic acids in the studied *Artemisia* raw materials

Artemisia campestris herb, some amounts of these flavonoids. The studied species differ quite significantly in terms of kaempferol-3-*b*-glucoside content, since in *Artemisia ludoviciana* herb it was detected 28.3 times more than in *Artemisia campestris* raw material. As for the content of rutin, it was found at the same level in the herbs of both species.

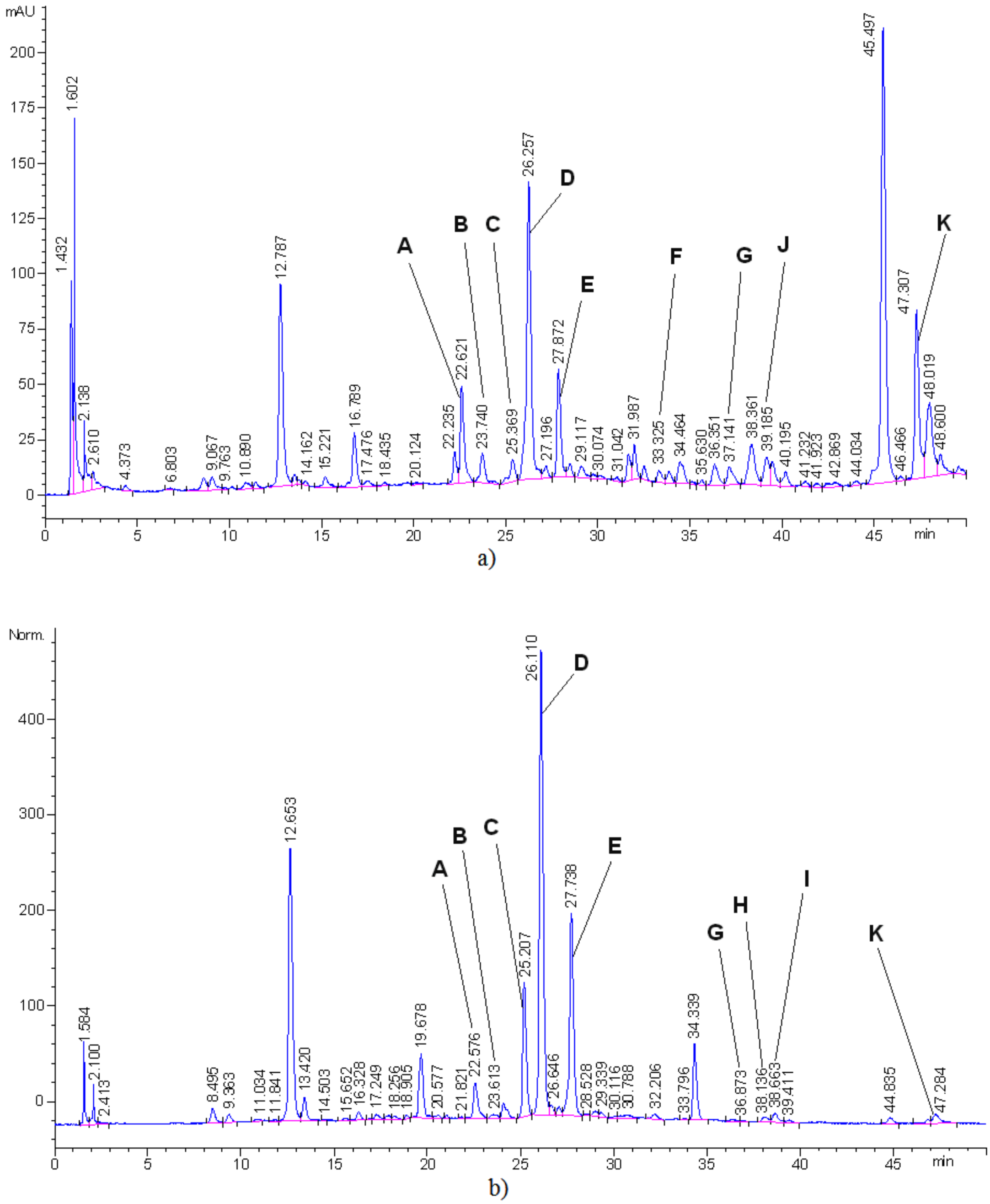
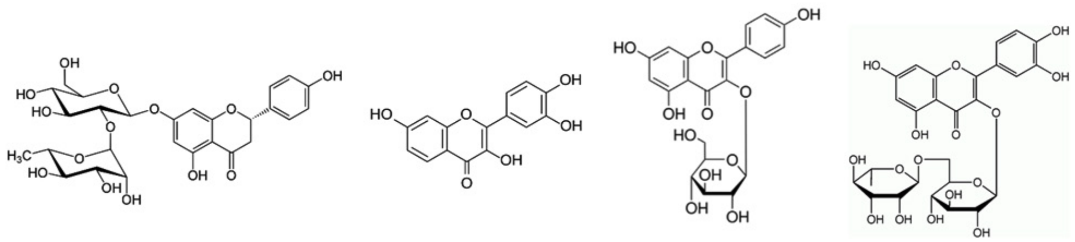


Fig. 3. HPLC chromatograms of flavonoids detected in the *Artemisia campestris* (a) and *Artemisia ludoviciana* (b) herbs: A – rutin; B – quercetin-3-*b*-glycoside; C – kaempferol-3-*b*-glucoside; D – flavanone-7-*O*-glycoside; E – fisetin; F – quercetin; G – luteolin; H – naringenin; I – apigenin; J – baicalein; K – kaempferol



Naringin Fisetin Kaempferol-3-*b*-glucoside Rutin

Fig. 4. Structural formulas of the predominant flavonoids in the studied *Artemisia* raw materials

Naringin (Fig. 4), the common predominant flavonoid compound in the herbs of both studied species, is one of the main polyphenols in grapefruits, giving them a bitter taste. Naringin is known for its anti-inflammatory, antioxidant, and antitumor properties[26]. Fisetin possesses an antioxidant effect and positively influences the brain cortex cells, improving cognitive functions[27]. Rutin possesses antioxidant, anti-inflammatory and capillary-strengthening properties[28].

Recently, it was revealed that the *Artemisia campestris* extract with high total phenolic content, demonstrated antioxidant activity as well as an anti-hyperglycemic effect via the inhibiting of *á*-amylase and *á*-glucosidase[29].

As it is known, significant differences in the chemical composition of raw material collected from the same species can exist due to geographical distribution, varying climatic conditions during the year of harvest, or the presence of genetic variations. Thus, Anaya-Eugenio and co-authors[30] identified an O-methylated flavone e*upatilin in the herb Artemisia ludoviciana* collected in Mexico. Eupatilin and sesquiterpene lactone estafiatin were isolated from the *Artemisia ludoviciana* subsp. *mexicana* aerial part demonstrated the anti-*Helicobacter pylori* activity[31]. Bioactive flavons eupatilin and dimethoxycentaureidin were revealed in the *Artemisia campestris* leaves collected in Tunis[14]. Eupatilin and 3-O-methylquercetin prevailed in the 80% aqueous methanol extracts of *Artemisia campestris* and *Artemisia herba-alba* collected in Algeria[32].

There is no doubt that the choice of solvent and extraction method substantially impacts the extraction level of polyphenols from plant raw materials. Thus, it was found[33] that methanolic extract from the leaves of Tunisian *Artemisia campestris* subjected to maceration under shaking conditions exhibited a higher content of polyphenols and antioxidant effects compared to the hexane extract. The methanolic extracts of aerial part of some endemic *Artemisia* representatives from Central Asia were the most promising source of polyphenolic compounds[34]. Similarly to our findings, 70% aqueous methanol extract from *Artemisia argyi* stems prepared using ultrasonic waves was considered optimal for obtaining high content of flavonoids[35].

CONCLUSIONS

The HPLC method was utilized to analyze the chromatographic profiles and quantitative content of flavonoids and phenolic acids in the *Artemisia ludoviciana* and *Artemisia campestris* herbs cultivated in Ukraine. The study revealed the presence of 10 phenolic acids, with the dominant compounds being hydroxycinnamic chlorogenic acid (14.503 mg/g in *Artemisia ludoviciana* and 4.504 mg/g in *Artemisia campestris*). Additionally, 11 flavonoid compounds were discovered, with the highest content observed for flavanone-7-*O*-glycoside (naringin) in both species. The predominant flavonoids in the herb of *Artemisia ludoviciana* decreased in the order of flavanone-7-*O*-glycoside (21.924 mg/g) > fisetin (13.068 mg/g) > kaempferol-3-*b*-glucoside (5.119 mg/g) > rutin (1.295 mg/g), while in the raw material of *Artemisia campestris* the following decline was observed: flavanone-7-*O*-glycoside (7.525 mg/g) > fisetin (2.933 mg/g) > rutin (1.355 mg/g). The major polyphenolic compounds revealed possess significant therapeutic potential, and the data obtained could be considered for further research into their biological activities.

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Conflict of interest

The authors declare no conflict of interest.

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