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ORIGINAL RESEARCH PAPER

STUDY OF CHROMATOGRAPHIC FINGERPRINT OF FLAVONOID COMPOUNDS OF THE BUR-MARIGOLD HERB

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Abstract: The main aim of this work was to determine the possibility of reliable identification of phenolic compounds, in particular flavonoids, specific for very popular type of medicinal plant material *Bidens* (*B. tripartita, B. frondosa, B. cernua*), their qualitative and quantitative determination by various methods: TLC, HPLC and absorption spectrophotometry (UV method). TLC method was used to isolate a specific compound from *B. cernua* sample, and by using HPLC method this compound was identified as quercitrin in comparison with a reference sample. A study of 8 series of the bur-marigold herb has shown that the presence of significant amounts of quercitrin in the sample indicates an unacceptable impurity of *B. cernua* in the pharmacopeial samples of *B. tripartita*. The main components of *B. tripartita* were identified as: luteolin-7-glucoside, unidentified luteolin glycoside, luteolin, derivatives of caffeic acid, in particular chlorogenic acid, polyacetylenes, chalcones. The correlation between the results of quantitative determination of the sum of flavonoids by the UV method and the results of HPLC determination of main components was shown.

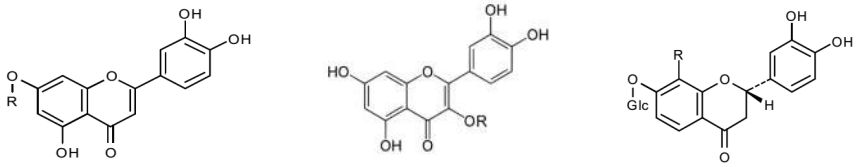
Keywords: *B. tripartita, B. cernua, B. frondosa, HPLC, luteolin-7-glucoside, quercitrin, TLC, UV method*

INTRODUCTION

The genus *Bidens* L. combines more than 200 species. In the flora of the Ukraine it is represented by 5 species: *B. tripartita* L., *B. cernua* L., *B. orientalis* Velen, *B. radiata* Thuill. and *B. frondosa* L. The first four species are native species of Ukrainian flora. *B. frondosa* is an adventitious species introduced from North America, which has been actively migrating to Ukraine over the last decades and has displaced native species, in particular *B. tripartita*. All of these species, except *B. orientalis*, which grows in the Crimea, are widespread within all botanical and geographical regions of the Ukraine. They mostly grow along the shores of various reservoirs. *B. tripartita* and *B. frondosa* also frequently occur in brownfields. The overlap of habitats and the identity of the places of growth often determine the presence of impurities of other species in the composition of the pharmacopoeial species (*B. tripartita*), which should not be harvested [1 – 4].

B. tripartita herb (bur-marigold) is very popular in folk medicine due to its diaphoretic, bactericidal, anti-allergic, anti-inflammatory effects. In national traditional medicine, an infusion of the aerial part of *B. tripartita* L. is widely used in the treatment of catarrhal rhinitis, angina, acute respiratory infection [5 – 7]. This plant is also used as an antiseptic and as a bath for children to treat diathesis (antiallergic action) [8]. Due to composition of the biologically active substances it belongs to adaptogens [9], has antioxidant properties [10, 11].

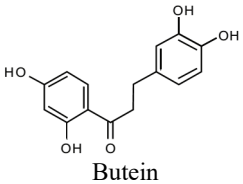
The chemical composition of the *B. tripartita* herb is represented by the following classes of biologically active substances: flavonoid compounds - flavonoids (luteolin, cynaroside/luteolin-7-glucoside), flavanones (isocoreopsin, flavanomarein), associated chalcones (including butein), as well as aurones (sulfuretin, sulfurein, etc.; more than ten substances); polysaccharides carbohydrates and related compounds (4.5-4.7 %), in particular in the hydrolyzate - arabinose, galactose, glucose, rhamnose and others. Also, the green parts of *B. tripartita* contain polyacetylenic compounds, essential oil (0.5-1.34 %), small amounts of vitamin C, also rich in coumarins, carotenoids, tannins [12 – 18]. Structural formulas of the main biologically active substances of bur-marigold are given in Figure 1.



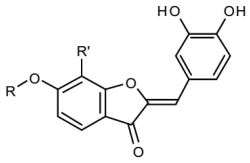
Luteolin R = H
Cynaroside R = Glc

Quercitrin R = Rha

Isocoreopsin R = H
Flavanomarein R = OH



Butein



Maritimetin R = H R' = OH; Maritimein R = H R' = H
Sulfuretin R = Glc R' = OH; Sulfurein R = Glc R' = H

Figure 1. *The main biologically active substances of bur-marigold herb*

The literature data on the presence of major flavonoids substances in three species of the *Bidens* are presented in the Table 1 [12 – 15].

Table 1. *The chemical composition of 3 species of the genus Bidens*

***Bidens* L.** / **Major flavonoid compounds**	***Bidens tripartita***	***Bidens frondosa***	***Bidens cernua***
Flavonoids	• luteolin (0.025 %) • luteolin-7-glucoside (cynaroside) (0.48 %) • isocoreopsin (0.025 %) • flavanomarein (0.015 %)	• luteolin, • luteolin-7-glucoside • coreopsin • marein	• luteolin • luteolin-7-glucoside • quercetin-3-rhamnoside (quercitrin) (0.6 %) • flavanomarein (0.09 %) • isocoreopsin (0.14 %) • coreopsin (0.025 %)
Chalcones	• butein (0.05 %) • butein-7-O-*β*-D-gluco-pyranoside	• butein • coreopsin • okanin	• butein (0.006 %) • butein-7-*O*-*β*-D-gluco-pyranoside
Aurones	• maritimetin-7-glucoside (sulfuretin) (0.4 %) • maritimein-7-glucoside (sulfurein) (0.2 %) • maritimetin (0.35 %) • maritimein (0.005 %)	• maritimetin • sulfuretin • sulfurein	• sulfuretin • maritimetin (0.45 %) • maritimein (0.01 %)

As seen from the data in Table 1, the chemical composition of different species of the bur-marigold is quite close. The only compound that distinguishes the *B. cernua* from the other two species is quercitrin, the amount of which according to Isakova (1980) [15] is quite significant.

The aim of this work was to study the profile of flavonoid compounds of bur-marigold samples by chromatographic methods, to identify specific compounds for each species and to clarify the possibility of standardizing of a pharmacopeia species by qualitative and quantitative content of specific flavonoid compounds.

MATERIALS AND METHODS

Plant material

For this experiment, we used 6 batches of the *B. tripartita* herb that was collected in 2017-2019 and registered in the State Enterprise "Pharmacopoeia Center": No. 1: collected near Dergachi, Kharkiv region (RS 707); No. 2: collected in the Zhytomyr region (RS 708); No. 3: collected near Merefa, Kharkiv region (RS 1053); No. 4: collected in the Zhytomyr region (RS 1081); No. 5: collected near city Kharkiv (RS 1090); and No. 6: collected in the Zhytomyr region (RS 1093) and also a sample of whole material of *B. frondosa*, collected near Ahtyrka, Sumy region (RS 700) and a

sample of whole material of *B. cernua* (RS 717) provided by the Kiev Botanical Garden.
The samples were dried at 40 °C in an air-forced dryer (Binder drying cabinet ED 53T, Germany), ground to a powder in an excelsior mill (electric coffee grinder, art. 346AU, China) and stored in closed containers. The identification and authentication of the plant material was carried out by Associate Prof., doct. A.G. Vovk, Department of Experimental Support the Elaboration of monographs on Herbal Drugs of the SE "Pharmacopoeia Center" (Kharkov, Ukraine).

Total flavonoid contents

The quantitative determination of the flavonoid content was carried out by absorption spectrometry using HP-8453 UV-VIS Spectrophotometer (Hewlett Packard, USA) according to The State Pharmacopoeia of Ukraine [19]. The total flavonoid content was estimated as luteolin-7-glucoside equivalents in g/100 g dry weight. About 0.500 g of the plant material was extracted first time with 40 mL of ethanol (60 %, v/v) and second - with 40 mL of ethanol (60 %, v/v), every time by heating for 10 min in a Nanbei water bath (Model HWS-12, Zhengzhou Nanbei Instrument Equipment Co., Ltd., China) at 60 °C. After cooling, the extract was filtered into 100 mL volumetric flask and diluted to 100 mL with the same solvent. 5 mL of the solution was transferred into a round-bottomed flask and was evaporated to dryness under reduced pressure. The residue was taken up with 8 mL of a mixture of 10 volumes of methanol and 100 volumes of anhydrous acetic acid and transferred to a 25 mL volumetric flask, added 10.0 mL of a solution containing 25 $g \cdot L^{-1}$ of boric acid and 20 $g \cdot L^{-1}$ of oxalic acid in anhydrous formic acid and was diluted to 25.0 mL with anhydrous acetic acid. Blanks were prepared as described above except solution of boric and oxalic acid was replaced by anhydrous formic acid. The absorption at 410 nm was measured after 30 min and compared to a luteolin-7-glucoside calibration curve.

Chromatographic fingerprint analyses by TLC

Thin-layer chromatographic (TLC) analysis of phenolic compounds was performed on precoated silica gel 60 F_{254} TLC plate (Merck, Germany) of glass and metal support. Aliquots (10 µL) of 10 % plant ethanolic-water extracts and 0.03 % methanolic solution of reference substances were manually applied on the plates which were then developed in vertical glass chamber previously saturated with the mobile phase anhydrous formic acid – water – ethyl acetate (10:10:80, v/v). After development, the plate was dried at a temperature of 100-105 °C, it was sprayed with a 1 % solution of diphenylboronic acid aminoethyl ether in methanol, then with a 5 % solution of macrogol 400 in methanol; after 30 minutes was viewed in UV light (CAMAG® TLC Visualizer, Switzerland) at a wavelength of 365 nm (visualization A) and in daylight (visualization B).

Chromatographic fingerprint analyses by HPLC

The liquid chromatographic apparatus Waters Alliance with separation module Waters 2690 and with detector 996 PDA (Waters, USA) was used. Separation was achieved on a column Kromasil 100-5-C18 4.6 × 250 mm (Nouryon Bohus, Sweden) with a

pre-column (2 mm) containing the same adsorbent. The temperature of the column was kept constant at 30 °C and the mobile phase was delivered at a flow rate of 1.0 mL/min and the detection wavelength set at 360 nm. UV spectra were carried out between the wavelengths of 200 and 800 nm. Sample volume was 10 mL and each sample was analyzed in triplicate. The components were identified by comparison of their retention times and UV spectra to those of authentic standards under identical analysis conditions and the published data UV spectra [20].

Preparation of sample solutions

5 g of the powdered herbal drug was placed in a 200 mL round-bottom flask, 100 mL of ethanol (70 %, v/v) was added, and heated in a water bath under reflux for 60 minutes, cooled and filtered into a round-bottom flask. 80 mL of ethanol (70 %, v/v) was added to the residue, heated in a Nanbei water bath (Model HWS-12, China) for 30 minutes, cooled and filtered into the same round bottom flask. The resulting filtrate was evaporated to a volume of 50 mL and settled for a day in the refrigerator. It was filtered into a separatory funnel and extracted first with 50 mL of butanol, then with 40 mL of butanol. The butanol extract was evaporated to dry on the Nanbei water bath. The residue, using portions of methanol, was quantitatively transferred to a 25 mL volumetric flask and adjusted to the mark with the same solvent. 5.0 mL of the resulting solution was transferred to a 20 mL volumetric flask and adjusted to the mark with methanol.

Preparation of reference solutions

The solutions of the pure compounds were prepared by dissolving 1 mg chlorogenic acid + 1 mg ferulic acid in 10 mL methanol; 1 mg luteolin + 1 mg luteolin-7-glucoside in 10 mL methanol; 1 mg hyperoside + 1 mg quercetin in 10 mL methanol, 1 mg quercitrin in 10 mL methanol and 1 mg rutin + 1 mg apigenin-7-glucoside in 10 mL methanol.

Mobile phase

The binary solvent system of the mobile phase consisted of solvent A (0.1 % trifluoroacetic acid in water) and solvent B (acetonitrile). A linear gradient program was applied as follows: 0–8 min, 5–15 % B; 8–30 min, 15–20 % B; 30–48 min, 20–40 % B; 48–58 min, 40–50 % B; 58–65 min, 50 %; 65–66 min, 50–95 % B. A 5-min equilibrium time was allowed between injections.

Reference standards and chemicals

The used reference compounds - chlorogenic acid (purity 100 %), ferulic acid (purity 98 %), luteolin-7-glucoside (purity 99 %), luteolin (purity 98 %), quercetin (purity 100 %), caffeic acid (purity 100 %), hyperoside (purity 100 %), quercitrin (purity 95 %), rutin (purity 100 %) were pharmacopoeial reference standards of the State Pharmacopoeia of Ukraine and apigenin-7-glucoside (purity 98 %) from Sigma–Aldrich (Germany).

The solvents (ethanol, methanol, butanol, acetonitrile, water, ethyl acetate) and chemicals (anhydrous acetic acid, trifluoroacetic acid, oxalic acid, anhydrous formic acid, boric acid, diphenylboronic acid aminoethyl ether, macrogol 400) used in the

experiments were of analytical grade and were purchased from Sigma-Aldrich (Scnelldorf, Germany) and from Merck (Darmstadt, Germany).

RESULTS

As we mentioned earlier, three species of the bur-marigold (*B. tripartita, B. frondosa* and *B. cernua*) differ in the chromatographic profile when studying its flavonoid compounds by TLC method [21]. The main diagnostic difference between *B. cernua* and 2 other species is the presence on the chromatogram of a distinguishing intense zone in the upper part of the chromatogram, while on the chromatograms of *B. tripartita* and *B. frondosa* solutions the described zone is absent (Figure 2), so this zone for the *B. cernua* is diagnostic.

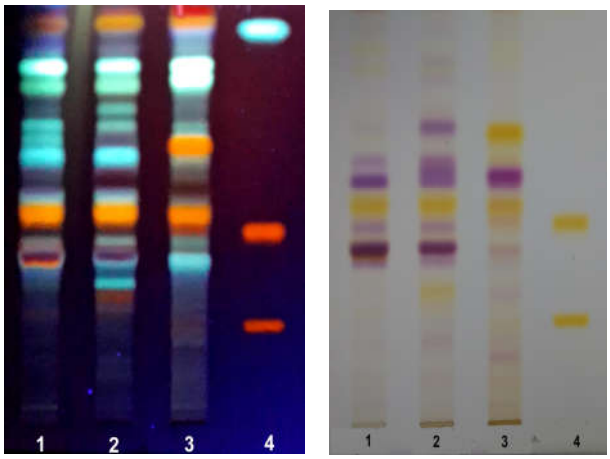


Figure 2*. TLC chromatographic profile obtained for: 1 – Bidens tripartita, 2 – Bidens frondosa, 3 – Bidens cernua, 4 – rutin+hyperoside+caffeic acid (in order of increasing R_f); left - in UV 365 nm, right - in daylight*

To clarify the nature of the substance, TLC chromatography was performed with a solution of *B. cernua* under the conditions described in the section "Chromatographic fingerprint analyses by TLC"; the area of silica gel corresponding to the level elution of the indicated zone was removed from the chromatographic plate, the silica gel was exhaustively extracted with methanol and the residue obtained after evaporation of the solvent was redissolved into methanol to a concentration of 1 mg/10 mL and analyzed by HPLC under the conditions of the technique described in the section "Materials and methods". In parallel, a solution of reference quercitrin (1 mg/10 mL) was analyzed.
To identify the substance, both the retention time of the peaks and their UV spectra were used (Table 2 and Figure 3).

Table 2. *Retention time and maximum UV absorption for the peak of a quercitrin and peak of a zone selected from TLC plate*

Quercitrin		**Zone, selected from TLC plate**	
Retention time [min]	**UV max**	**Retention time [min]**	**UV max**
21.0	256 nm, 349 nm	21.0	256 nm, 347 nm

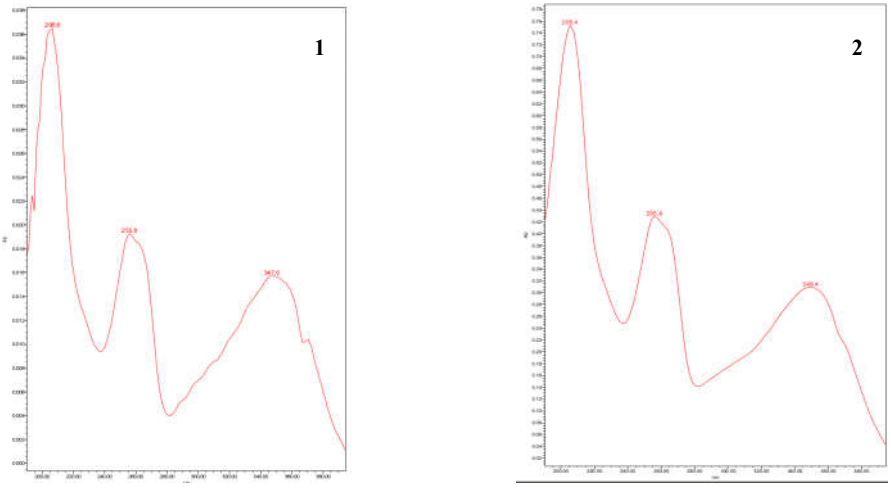


Figure 3. *UV spectra of 1 - the quercitrin peak; 2 - the peak of the zone which was isolated from the TLC plate*

Based on the obtained results, namely, from the overlap of both the retention times and the maxima of the absorption spectra, it is highly likely that the diagnostic zone in the chromatogram of the *B. cernua* belongs to quercitrin.

For a detailed study of the component composition of flavonoids and hydroxycinnamic acids, a HPLC study of 8 samples of the bur-marigold was carried out, 5 of which were pharmacopeial materials (samples of the *B. tripartita* RS 707, 1053, 1081, 1090, 1093), 1 sample was a sample of the *B. cernua* (RS 717), one was a sample of the *B. frondosa* (RS 700) and one - a mixture of the sample of the *B. tripartita* and *B. cernua* (RS 708).

Figure 4 shows the combined chromatogram of 9 reference samples and their retention times and Figure 5 shows the UV spectra of reference substances which peaks were found in the chromatograms of the studied samples.

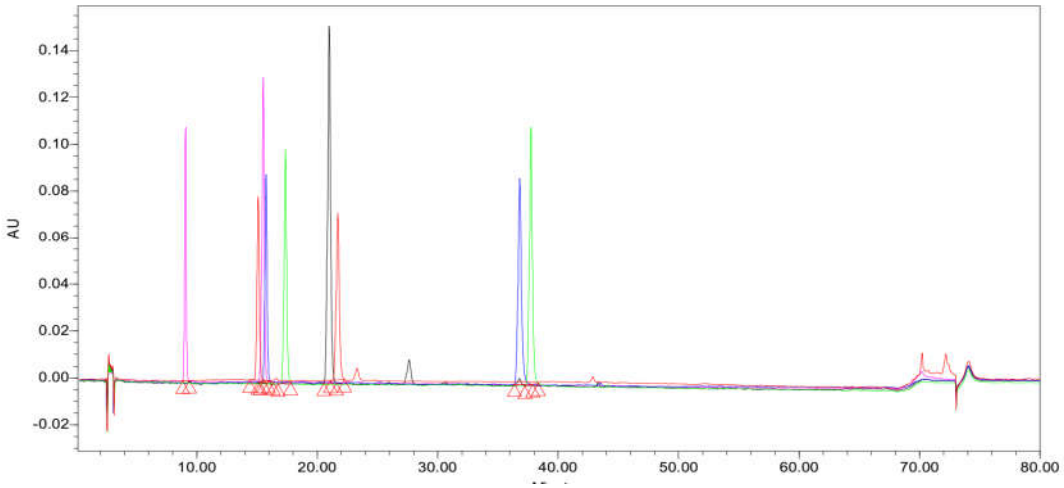


Figure 4. *The combined chromatogram of the reference substances and their retention times: chlorogenic acid (9.1 min) + ferulic acid (15.5 min) – pink color; rutin (15.1 min) + apigenin-7-glucoside (21.7 min) – red color; luteolin-7-glucoside (17.4 min) + luteolin (37.5 min) – green color; hyperoside (15.7 min) + quercetin (36.7 min) – blue color; quercitrin (21.0 min) – black color*

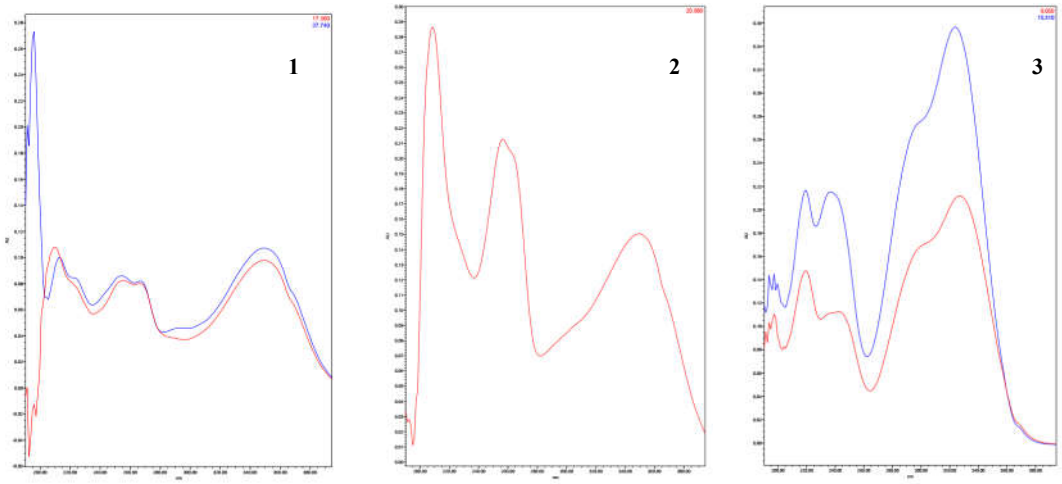


Figure 5. *UV spectra of 1 – luteolin-7-glucoside (red) + luteolin (blue); 2 – quercitrin; 3 – chlorogenic acid (red)+ ferulic acid (blue)*

Figure 6 shows the chromatographic profile for the samples of 3 species: RS 1081 – sample of *Bidens tripartita*, RS 700 - sample of *Bidens frondosa*, RS 717 - sample of *Bidens cernua* obtained under the conditions of the technique "Chromatographic fingerprint analyses by HPLC".

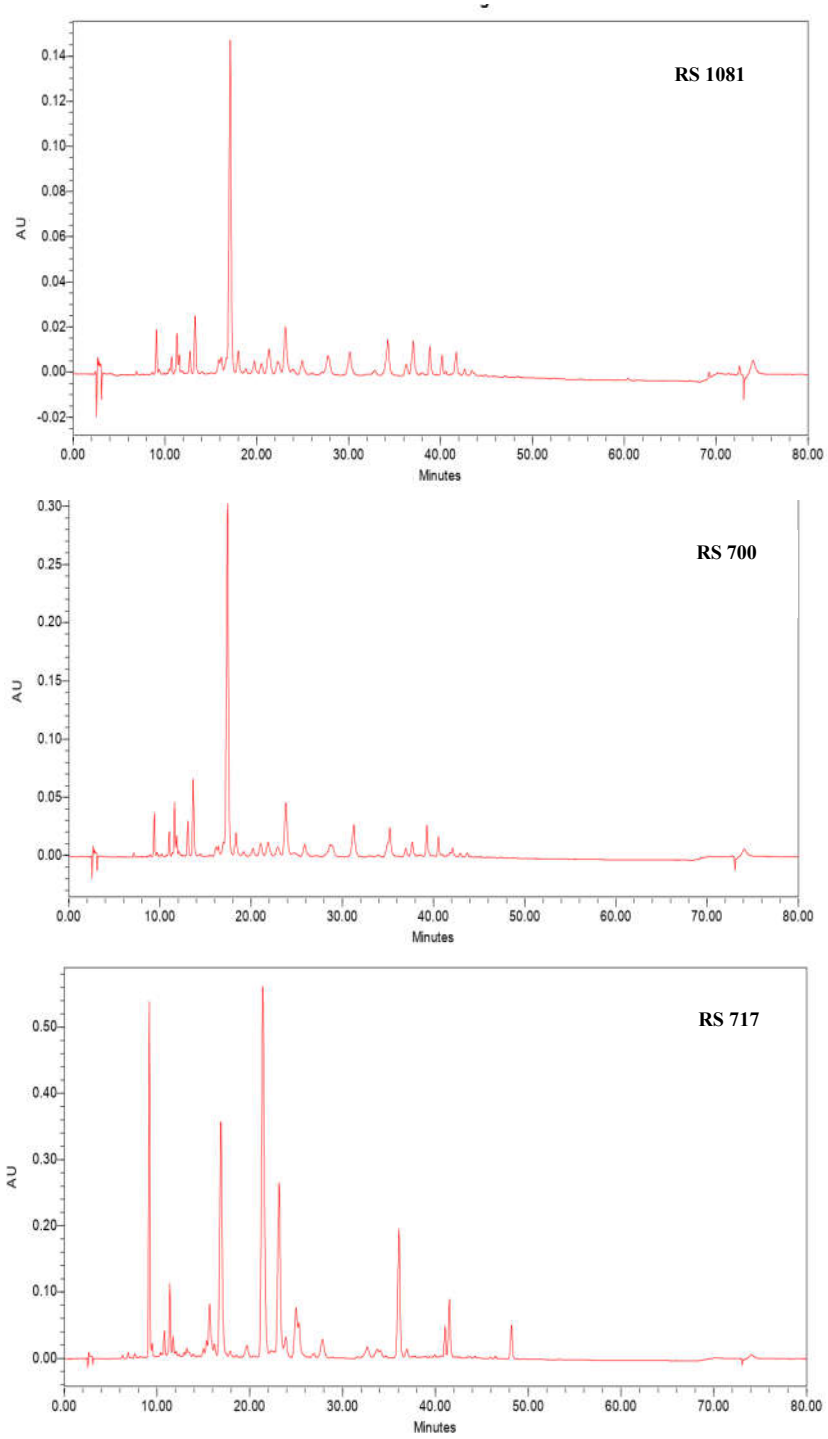


Figure 6. *The chromatographic profile for the samples of 3 species: RS 1081 – sample of Bidens tripartita, RS 700 – sample of Bidens frondosa, RS 717 – sample of Bidens cernua*

Figure 7 shows the UV spectra of the most specific peaks in the chromatograms of the bur-marigold, and Table 3 - the HPLC results obtained for all the samples studied.

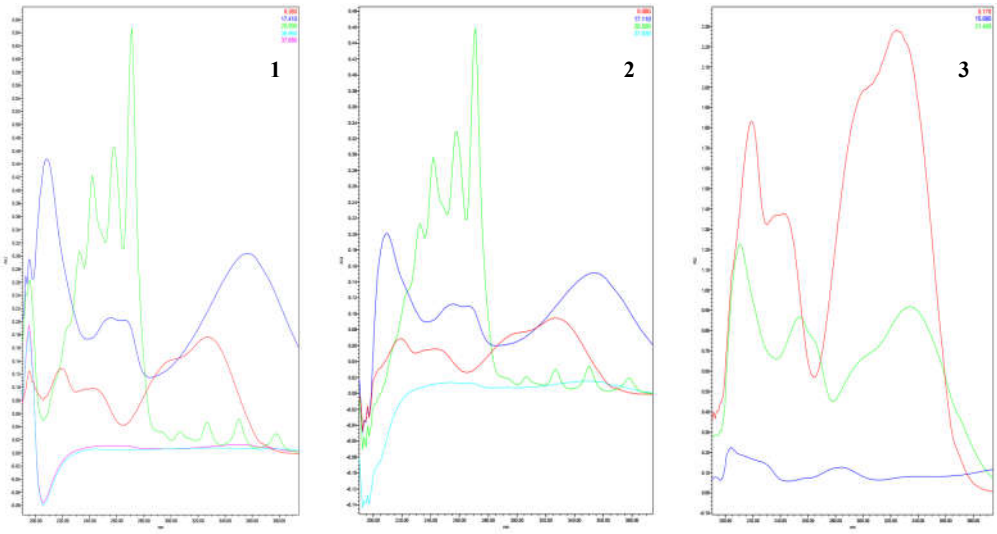


Figure 7. *UV spectra of the most specific peaks in the chromatograms of the bur-marigold: 1 (RS 700), 2 (RS 1081) - peak with the retention time of chlorogenic acid (red), peak with the retention time of luteolin-7-glucoside (blue), peak with the retention time of 20.5-20.9 min with a specific spectrum form for polyacetylenes (green), 3 (RS 717) - peak with retention time of quercitrin (green), peak with retention time of chlorogenic acid - red.*

To study the correlation between chromatographic profiles obtained by HPLC and TLC, some samples were additionally analyzed under the conditions of the procedure "Chromatographic fingerprint analyses by TLC" (Figure 8).

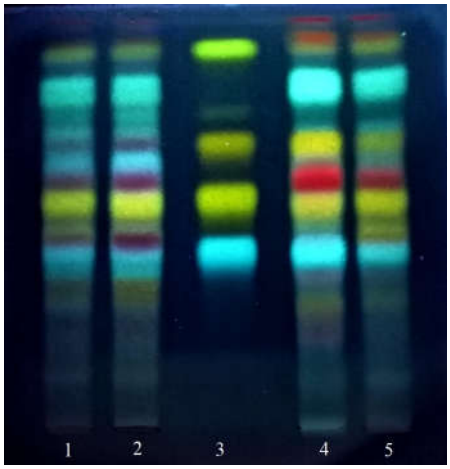


Figure 8. *TLC chromatographic profile obtained for: 1 – B. tripartita (RS 1081), 2 – B. frondosa (RS 700), 3 – chlorogenic acid + luteolin-7-glucoside + quercitrin + luteolin (in order of increasing R_f), 4 – B. cernua (RS 717), 5 – B. tripartita with impurity B. cernua (RS 708) (in UV 365 nm)*

Table 3. *HPLC results for 8 samples of the bur-marigold herb*

Reference	**Retention time, min/max***	***RS 700***	***RS 707***	***RS 708***	***RS 717***	***RS 1053***	***RS 1081***	***RS 1090***	***RS 1093***
		%**							
Chlorogenic acid	9.1-9.4/220 nm, 245 nm, 327 nm	3.4	4.1	5.9	11.4	2.2	3.2	6.7	2.6
Unknown acid	10.7-11.0/217 nm, 242 nm, 325 nm	1.9	1.2	0.7	0.8	1.9	0.9	2.0	1.5
Caffeic acid derivative	11.1-11.6/217 nm, 242 nm, 326 nm	3.4	2.0	1.0	2.1	2.6	2.3	3.9	6.0
Unknown	12.9-13.5/215 nm, 280 nm, 343 nm	7.5	3.5	-	-	5.9	5.3	-	-
Rutin	15.1	-	-	-	-	-	-	-	-
Hyperoside	15.5-15.8/ 254 nm, 353 nm	-	3.3	3.1	2.2	2.9		3.5	-
Luteolin-7-glucoside	17.0-17.3/254 nm, 267 nm, 350 nm	47.9	31.6	29.5	16.4	31.7	44.5	43.3	63
Luteolin-glycoside	17.4-17.8/253 nm, 267 nm, 348 nm	-	2.5	12.7	-	2.2	2.6	13.0	11.6
Unknown acid	19.7/242 nm, 295 nm, 325 nm	3.1	2.0	1.7	-	-	-	-	-
Polyacetylens	20.5-20.9/230 nm, 257 nm, 270 nm	1.5	1.1	0.9	-	3.7	1.0	1.1	-
Quercitrin	20.7-21.0/ 256 nm, 347 nm	-	-	15.7	29.2	3.4	-	-	2.8
Unknown acid	21.3-21.4/220 nm, 245 nm, 327 nm	-	4.5	-	-	1.9	4.6	6.9	-
Apygenin-7-glucoside	21.7-22.1	-	-	-	-	2.6	-	-	-
Unknown chalcone	23.0-23.5/ 263 nm, 380 nm	10.1	9.8	8.7	11.8	1.4	7.6	9.6	8.3
Unknown acid	25.0-26.7/ 220 nm, 327 nm	2.0	3.1	3.5	5.7	3.3	2.8	2.4	-
Unknown	27.7-28.7	2.9	3.3	2.2	1.8	4.3	4.0	-	-
Unknown	30.3-31.2	6.9	3.8	-	-	-	4.7	-	-
Unknown acid	34.5-35.0/242 nm, 325 nm	6.4	12.4	2.6	-	-	5.3	-	-
Quercetin	36.3-36.8/ 254 nm, 368 nm	0.6	1.8	3.4	8.2	-	-	-	-
Luteolin	37.0-37.5/252 nm, 267 nm, 347 nm	1.1	2.2	2.8	-	12.4	5.3	1.5	3.2
Unknown	38.0-38.7/310 nm, 382 nm	-	2.1	-	-	4.1	-	2.6	-
Unknown	40.5-41.7	1.9	1.6	2.2	3.0	2.9	3.0	1.1	1.0

*Note 1: max – UV absorption maximum
**Note 2: the content (%) was calculated as the ratio of the areas of individual peaks in the chromatogram to the total area of all chromatographic peaks

DISCCUSSION

As seen from the Figure 6 and data in the Table 3, a similarity of the chromatographic profile for the *B. tripartita* and *B. frondosa* was found. For the *B. cernua* solution (RS 717), in the first place, in contrast to the other samples, the presence of an intense peak, which retention time (about 21 min) is close to the retention time of quercitrin and which UV absorption maxima corresponds to quercitrin (Figure 7.3/green line and Figure 5.2) was found. On TLC chromatograms (Figure 8), the main difference between 3 species was the presence of an intense yellow fluorescent zone at the level quercitrin zone in the chromatogram of the *B. cernua*, which, as shown above, belongs to quercitrin.

In addition to sample RS 717, quercitrin peak was found in the chromatogram of sample RS 708, for which, during macroscopic analysis was found that it was a mixture of *B. tripartita* and *B. cernua*. The content of this substance in the indicated samples, found by internal normalization, was 29 % for the RS 717 and 16 % for the RS 708, that also was correlated with the chromatographic profile of these samples obtained by TLC analysis (Figure 8, presence intensive yellow fluorescent zone in the upper third of the chromatogram for sample RS 717 and less intense for sample RS 708).

In the chromatograms of all samples, a peak which retention time is close to the retention time of chlorogenic acid and which UV spectrum coincides the spectrum of the chlorogenic acid was identified (Figure 5.3/red line and Figure 7.1, 7.2/red line). The maximum content was detected for the sample of the *B. cernua*. The obtained data was correlated with the results of TLC analysis of the indicated samples. Intense blue fluorescent zones were found at the level of the chlorogenic acid zone and intensity of these zones in the chromatogram of the *B. cernu*a were higher than ones in chromatograms of other samples of the bur-marigold (Figure 8).

In addition, peaks with a retention time of about 11 min were found in the chromatograms of all samples, which, based on absorption maxima, also belong to caffeic acid derivatives. The content was in the range from 1 to 6 %.

The main component for all samples of the bur-marigold, except for sample RS 717, was a substance which retention time is close to the retention time of luteolin-7-glucoside and which UV absorption maxima coincides with luteolin-7-glucoside absorption maxima (Figure 5.1/red line and Figure 7.1, 7.2/blue line). Data obtained also was agreed with the TLC analysis of the indicated samples (Figure 8) - an intense orange/yellow zone at the level of the luteolin-7-glucoside zone was appeared in all chromatograms. Its content varied within 30 to 63 % for the sample RS 1093. For 3 samples (RS 708, RS 1090 and RS 1093), a peak was also found that eluted after the peak of the luteolin-7-glucoside which UV spectrum was similar to UV spectrum of the luteolin-7-glucoside, therefore, it can be assumed that this substance also belongs to luteolin glycosides.

In addition, in all samples, except for sample RS 717, a peak was revealed which retention time is close to the retention time of luteolin and which UV absorption maxima coincides with UV maxima of luteolin; the content of this substance ranged from 1 to 12 %.

In four samples of the bur-marigold, the flavonoid content was additionally determined by the spectrophotometric method (Total flavonoid contents described in the section “Materials and methods”). The Table 4 shows the results of the UV determination of the

total flavonoid content expressed as luteolin-7-glucoside and HPLC results of luteolin-7-glucoside and luteolin sum expressed as luteolin-7-glucoside for 4 samples of the bur-marigold. As seen from the comparison of results, there was also a correlation between results obtained by UV determination and results obtained by HPLC determination.
The maximum content of the luteolin-7-glucoside and aglycone (they are determined together by the UV method) was found for sample RS 1093 (2.3 %), which also was correlated with the results obtained by HPLC analysis - the maximum content of luteolin and its glucoside (1.3 %) was found for this sample.

Table 4. *The results of the total flavonoid content UV determination and HPLC determination of sum luteolin-7-glucoside and luteolin, expressed as luteolin-7-glucoside**

Assay	RS 1053	RS 1081	RS 1090	RS 1093
The total flavonoid content expressed as luteolin-7-glucoside, obtained by UV method	1.8 ± 0.06	1.0 ± 0.04	1.4 ± 0.05	2.3 ± 0.06
The content of the sum of luteolin-7-glucoside and luteolin expressed as luteolin-7-glucoside, obtained by HPLC	0.9 ± 0.04	0.4 ± 0.03	0.7 ± 0.04	1.3 ± 0.05

*The data (%) are presented as mean ± standard error of mean (n = 3)

Analyzing the data of HPLC results, it was found that in the chromatograms of all samples was absent a peak with the retention time of the rutin that was also corresponded with the results of TLC analysis above (Figure 2, the absence of a zone at the level of the rutin zone). It should be noted that our results do not coincide with the published data [22], where for samples of a *B. tripartita* growing in the Lithuania, rutin in samples was identified by HPLC.
Another characteristic peak in the chromatograms of all samples was a peak with a retention time of 20.5–20.9 min and with a specific spectral shape (Figure 7.1 and 7.2/green line), which, according to published data, is characteristic of polyacetylenes. The peak with a retention time of about 23 min, which was also detected in the chromatograms of all samples, had a specific long-wave maximum at 380 nm. According to published data, it may belong to chalcones having hydroxyl radicals at positions 3' and 4' (for example butein). These compounds are characterized by the presence of an absorption maximum at the wavelength range of 340-390 nm and a slightly pronounced maximum - at 220-270 nm [20, 23]. The content of this substance was varied in different samples from 8 to 11 % (except for sample RS 1053).

CONCLUSIONS

The component composition of flavonoid compounds for various species of the bur-marigold herb was studied by HPLC and TLC methods. The similarity of the component composition for *B. tripartita* and *B. frondosa*, and the difference for *B. cernua* was established. TLC-HPLC methods were used to isolate and identify quercitrin, a substance that is diagnostic for a *B. cernua*, impurity of which can be controlled by the described methods in the pharmacopeia species.

It was shown that the main component in the 6 studied samples of the *B. tripartita* was luteolin-7-glucoside with content from 30 to 63 %. In addition, among other components, chlorogenic acid (the maximum content was found in the sample of a *B. cernua*), derivatives of caffeic acid (from 2 to 6 %), luteolin glycoside (up to 13 %), luteolin (from 1 to 12 %), polyacetylenes, chalcones (from 8 to 11 %) were identified.
The correlation between the results of the sum flavonoids quantitative determination by the UV method and the HPLC results of the determination of main components - luteolin and luteolin-7-glucoside in samples of a *B. tripartita* was shown, as well as the correlation between the intensity of the zones in the TLC chromatograms for various samples and the content of the corresponding substances (chlorogenic acid, luteolin-7-glucoside, quercitrin) obtained by HPLC method.

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