UDK: 547.918:547.926

INULA RHIZOCEPHALA FLAVONOIDS OF THE AERIAL PARTS AND BIOLOGICAL ACTIVITIES

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Annotation. *Inula rhizocephala* is a plant species belonging to the *Asteraseae* family. The chemical components of flavonoids in *I. rhizocephala* were studied by column chromatography methods and six known compounds were isolated. **Keywords:** *I. rhizocephala*, chrysin (5,7-dihydroxyflavone), oroxylin A (5,7-dihydroxy-6-methoxy flavone), apigenin (5,7,4'-trihydroxyflavone), kaempferol (5,7,3',4'-tetrahydroxyflavone), tamiflazid, astragalin (kaempferol *3-O-β-D*-glucopyranoside), chrysoeriol, luteolin. HPLC, NMR-spectrum.

INULA RHIZOCEPHALA YER UATKI QISMINING FLAVONOIDLARI

VA BIOLOGIK AKTIVLIGI

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Annotatsiya. *Inula rhizocephala - Asteraseae* oilasiga mansub o'simlik turi. *I. rhizocephala* o‘simligidagi flavonoidlarining kimyoviy tarkibiy qismlari ustunli xromatografiya usullari bilan o‘rganildi va oltita ma’lum birikma ajratib olindi.

Kalit so'zlar: *Inula rhizocephala*, xrisin (5,7-digidroksiflavon), oroksilin A (5,7-digidroksi-6-metoksi flavon), apigenin (5,7,4'-trigidroksiflavon), kaempferol (5,7,3',4'-tetrahidroksiflavon), (kaempferol 3-O-b-D-glyukopiranozid), xrizoeriol, luteolin. HPLC, NMR spektri.

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ннотация. *Inula rhizocephala* — вид растений, принадлежащий к семейству *Asteraseae.* Химические компоненты флавоноидов из *I. rhizocephala* были изучены методами колоночной хроматографии и выделены шесть известных соединений.

Ключевые слова: *Inula rhizocephala*, хризин (5,7-дигидроксифлавон), ороксилин А (5,7-дигидрокси-6-метоксифлавон), апигенин (5,7,4'-тригидроксифлавон), кемпферол (5,7,3',4'-тетрагидроксифлавон), тамифлазид, астрагалин (кемпферола 3-O-*β*-D-глюкопиранозид), хризоэриол, лютеолин. ВЭЖХ, ЯМР-спектр.

INTRODUCTION

The genus *Inula* (family *Asteraceae*) includes about 78 species of plants growing in Europe, Asia and Africa [1,2]. *Inula* species are used to treat dysfunctions of the cardiovascular, respiratory, urinary, digestive and central nervous systems, as well as to treat asthma, diabetes, cancer, skin diseases, liver diseases, fungal and bacterial infections. In India, the dried roots of this plant *I. rhizocephala* are used to treat colds, coughs and chest diseases [3,4].

MATERIALS AND METHODS

UV spectra were measured on a Lambda-16 spectrophotometer (Perkin-Elmer). PMR and ^{13}C NMR spectra were recorded on a Unity-600 plus spectrometer (Varian) in $CDCl_3$, Py and DMSO-d_6 at a working frequency of 600 MHz using HMDS as an internal standard for the PMR spectra and the DMSO-d_6 resonance (relative to 39.5

ppm). TMS) for the ^{13}C NMR spectra. TLC was performed using Silufol UV 254 plates, illuminated with iodine vapor, ammonia vapor, 254 and 365 nm ultraviolet radiation and a vanillin solution (1%) in a concentration of H_2SO_4. The aerial part of *I. rhizocephala* was taken as the object. HPLC method for the determination of flavonoids. Extraction was carried out twice with 70% ethanol at 70-75 ^0C for 3 hours and the solvent ratio was 90:20. The solutions were filtered and a 1 ml aliquot was diluted with 9 ml of the solvent system (acetonitrile: buffer (acetate) 70:30). Centrifuged and filtered through a membrane filter. The analysis was carried out by HPLC using isocratic elution mode and diode array detector (DAD). Acetonitrile and buffer solution were used as mobile phases. Spectral data were studied in the spectral range from 200 to 400 nm.

RESULTS AND DISCUSSION

The chemical structure of the isolated compounds was determined by studying their ^1H and ^{13}C NMR, as well as by direct comparison with real samples of flavonoids and by comparison with literature data on these compounds. NMR analysis of individual substances isolated from the *Inula rhizocephala* plant revealed the presence of flavonoids and their glycosides (shown in Fig.1).

Chrysin (5,7-dihydroxyflavone) (1). $C_{15}H_{14}O_4$, mp 289–292 °C. NMR spectrum ^1H (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.45 (1H, d, J=2.0, H-6), 6.86 (1H, d, J=2.0, H-8), 6.98 (1H, s, H-3), 7.57 (2H, m, H-3 ',5'), 8.20 (2H, dd, J = 1.2, 7.9, H-2',6'), 12.77 (1H, s, 5-OH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 163.47 (2), 105.41 (3), 182.27 (4), 161.11 (5), 98.98 (6), 163.76 (7), 95.17 (8), 157.29 (9), 104.89 (10), 130.59(1'), 126.52 (2'), 129.21(3'), 132.27 (4'), 129.32 (5'), 126.57 (6') [5,6].

Oroxylin A (5,7-dihydroxy-6-methoxyflavone) (2). $C_{16}H_{12}O_5$ ([M]+ 284), mp. 215-217 °C. IR spectrum (v, cm–1): 3480–3290 (OH), 1685 (C=O), 1605, 1510 (C=C). ^1H NMR spectrum (400 MHz, $CDCl_3$, , ppm, J/Hz, 3.97 (3H, s, OCH3), 6.34(1H, s, H-8), 6.72 (1H, s , H-3), 7.51 (3H, m, H-3′,4′,5′), 7.83 (2H, d, J = 8.2, H-2′,6′), 12.47 (1H, s, 5-OH) [7].

Apigenin (5,7,4'-trihydroxyflavone) (3), $C_{15}H_{10}O_5$, mp 345–350°C. NMR ^1H (400 MHz, DMSO-d_6 + CCl_4, δ, ppm, J/Hz): 6.07 (1H, d, J=2.0, H-6), 6.33 (1H, d, J=2.0,

H-8), 6.53 (1H, s, 5-OH). ^{13}C NMR (100 MHz, DMSO-d_6 + CCl_4, δ, ppm): 164.01 (C-2), 103.04 (C-3), 182.04 (C-4), 162.21 (C-5), 99.71 (C-6), 164.51 (C-7), 94.25 (C -8), 157.69 (C-9), 104.54 (C-10), 121.46 (C-1'), 128.48 (C-2'), 116.12 (C-3'), 161.59 (C-4'), 116.12 (C-5'), 129.38 (C-6') [6]

Kaempferol (5,7,3',4'-tetrahydroxyflavone) (4), $C_{15}H_{10}O_6$, mp 277–279°C, was identified by comparison of ^{1}H and ^{13}C NMR spectral data with literary data [4,5] and also by direct comparison with authentic samples.

Tamiflazid (5), $C_{28}H_{32}O_{14}$ pale yellow powder, mp 264–268 °C (MeOH). IR spectrum (KBr, νmax, cm–1): 3466 (OH), 1659 (C=O), 1353, 906 (OCH_3). ^{1}H NMR spectra (600 MHz, DMSO-d_6 + CCl_4, δ, ppm, J/Hz). 12.77 (1H, s, 5'-OH), 7.94 (2H, d, J=9.0, H-2', 6'), 7.06 (2H, d, J=9.0, H-3', 5'), 6.74 (1H, s, H-3), 6.67 (1H, d, J=2.0, H-8), 6.38 (1H, d, J=2.0, H-6), 3.85 (OCH_3), 4.90 (1H, d, J=7.2, H-1''), 3.91, 3.46 (2H, m, m, H-6''), 3.52 (1H, m, H-5''), 3.45 (1H, m, H-2''), 3.44 (1H, m, H-4''), 3.26 (1H, m, H-3''), 4.56 (1H, d, J=1.7, H-1'''), 3.78 (1H, m, H-2'''), 3.42 (1H, m, H-5'''), 3.13 (1H, m, H-3'''), 3.11 (1H, m, H-4'''), 1.11 (1H, d, J=6.2, H-6'''). ^{13}C NMR spectrum (150 MHz, DMSO-d_6 + CCl_4, δ, ppm, J/Hz). 162.82 (2), 100.91 (C-3), 182.33 (C-4), 161.93 (C-5), 100.35 (C-6), 163.63 (C-7), 95.14 (C-8), 157.57 (C-9), 101.01 (C-10), 123.38 (C-1'), 128.48 (C-2',6'), 115.01(C-3',5'), 162.82 (C-4'), 55.80 (OCH_3), 100.91 (C-1''), 76.44 (C-2''), 73.47 (C-3''), 70.09 (C-4''), 76.97 (C-5''), 66.69 (C-6''), 101.01 (C-1'''), 71.51 (C-2'''), 70.84 (C-3'''), 72.77 (C-4'''), 68.69 (C-5'''), 18.26 (C-6''') [5,6].

Astragalin (Kaempferol 3-O-*β*-D-glucopyranoside). $C_{21}H_{20}0_{11}$, mp: 175-176.°C, NMR spectra ^{1}H (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.96 (1H, s, OH-5), 10.52 (1H, s, OH-7), 9.40 (1H, s, OH-4′), 7.91 (1H, d, J= 9.0, H-5′), 7.52 (1H, d, J= 9.0, H-3′), 7.42 (1H, dd, J = 9.0, 2.2, H-6′), 6.83 (1H, d, J = 2.0, H-2′) 6.31 (1H, d, J = 2.1, H-8), 6.11 (1H, d, J = 2.0, H-6), 5.42 (1H, d, J = 6.0, H-1''), 3.55 (1H, m, H -6''b), 3.46 (1H, dd, J = 11.5, 1.7 H -6''a), 3.25 (1H. m, H -5''), 3.23 (1H. m, H -2''), 3.17 (1H. m, H -4''), 3.09 (1H. m, H -3''). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 177.99 (C-4), 164.76 (C-7), 162.0 (C-5), 156.97 (C-2), 156.88 (C-9), 150.98 (C-4'), 147.35 (C-3'), 133.81 (C-3), 122.35 (C-6'), 121.59 (C-1'), 115.53 (C-5'),

114.06 (C-2'), 104.65 (C-10), 102.09 (C-l''), 99.22 (C-6), 94.00 (C-8), 77.50 (C-3''), 75.95 (C-5''), 74.77 (C-2''), 70.12 (C-4''), 61.12 (C6'') [7,8].

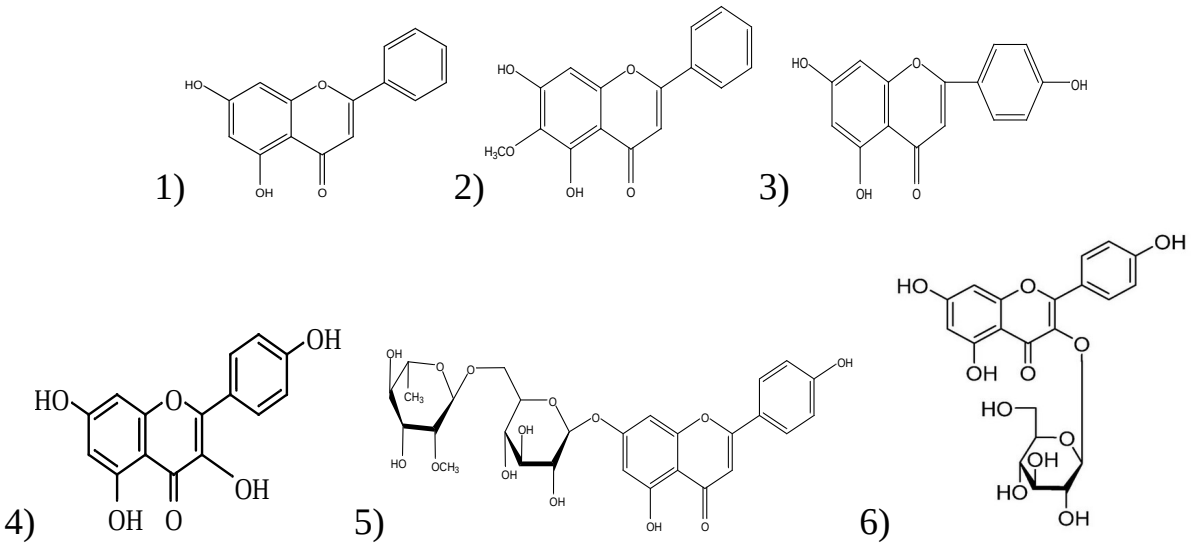


Fig. 1 NMR analysis of individual isolates from *Inula rhizocephala*, flavonoids and their glycosides.

1-6 flavonoids were individually isolated for the first time from the plant *Inula rhizocephalan*

High-performance liquid chromatography (HPLC) analysis

Extractions and fractions of *I. rhizocephala* plants growing in Uzbekistan were subjected to HPLC analysis and standard substances oroxylin, chrysoeriol, luteolin were comparatively analyzed. According to the results of HPLC analysis, it was determined that the extractions and fractions of *I. Rhizocephala* plants are rich in chemical composition. As a result of analysis by high-performance liquid chromatography, it was determined that they contain flavonoids (shown in Figures 2-9).

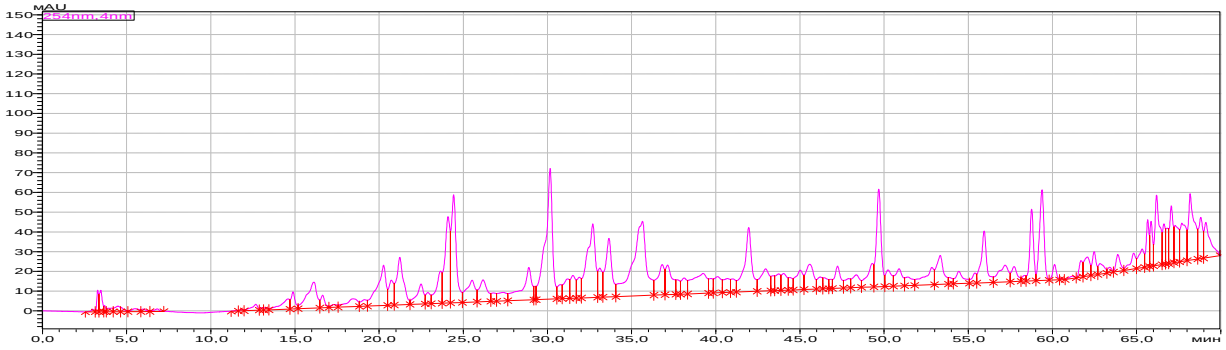


Fig. 2. HPLC chromatogram of the *I. rhizocephala* 70% ethanolic extract

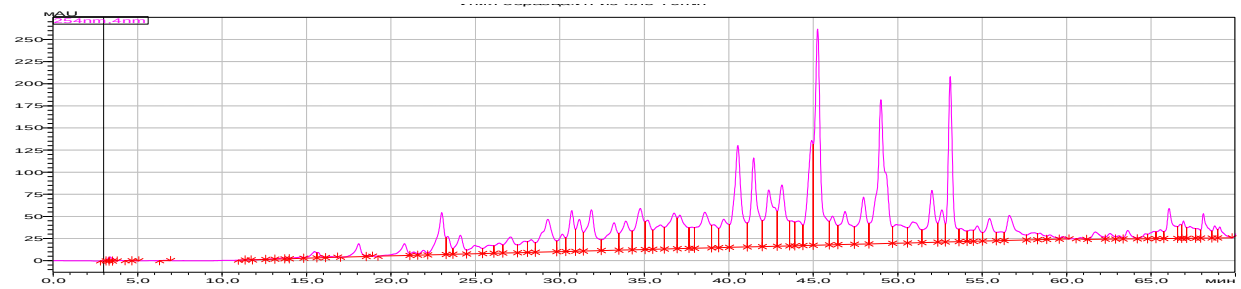


Fig. 3. HPLC chromatogram of the chloroform fraction of *Inula rhizocephala*..

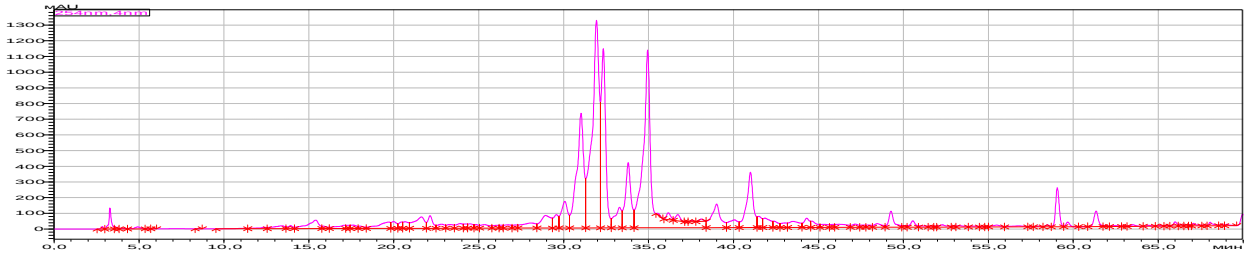


Fig. 4. HPLC chromatogram of the ethyl acetate fraction of *Inula rhizocep*

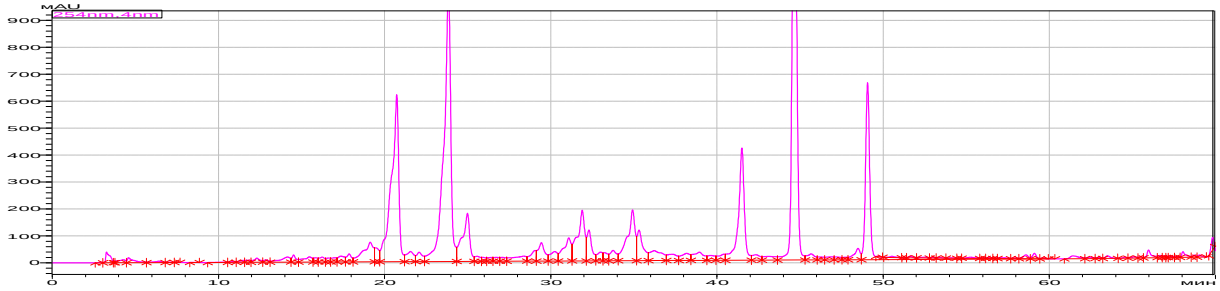


Fig. 5. HPLC chromatogram of the n-butanol fraction of *Inula rhizocephala*.

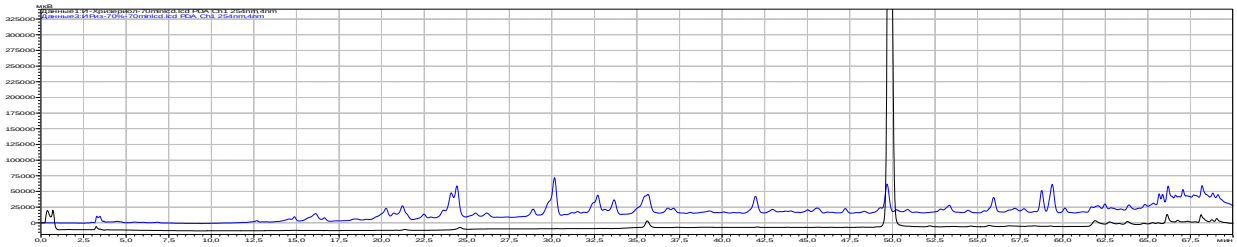


Fig. 6. HPLC chromatogram of 70% ethanol extract of *Inula rhizocephala*, chrysoeriol standard.

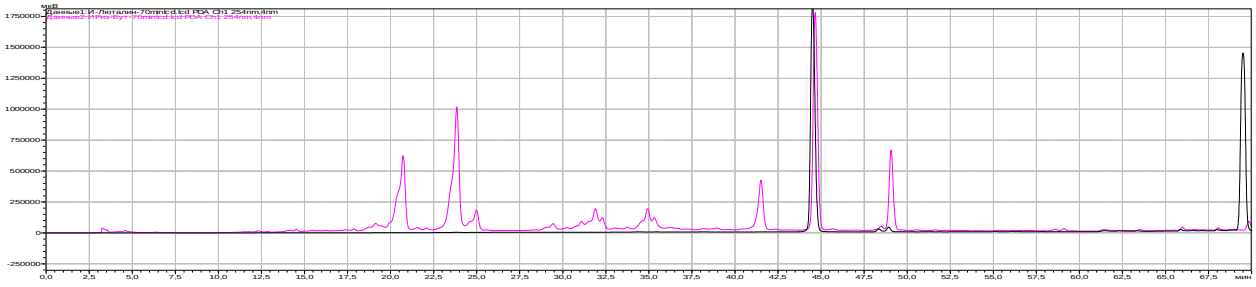


Fig.7. HPLC chromatogram of n-butanol fraction of *Inula rhizocephala*, luteolin standard

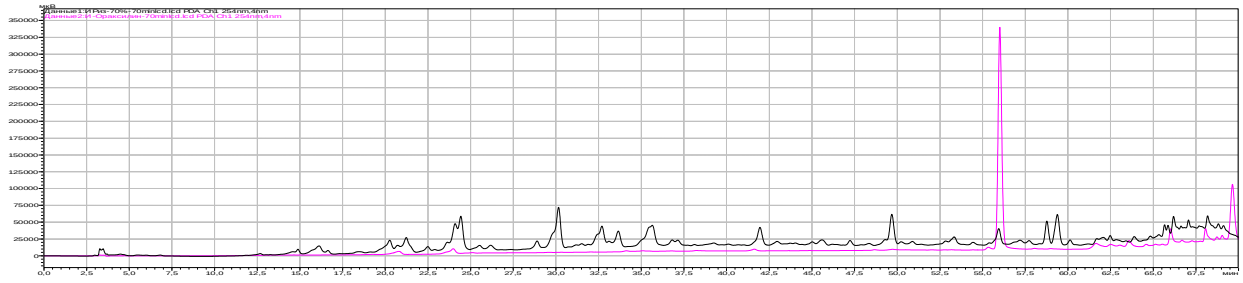
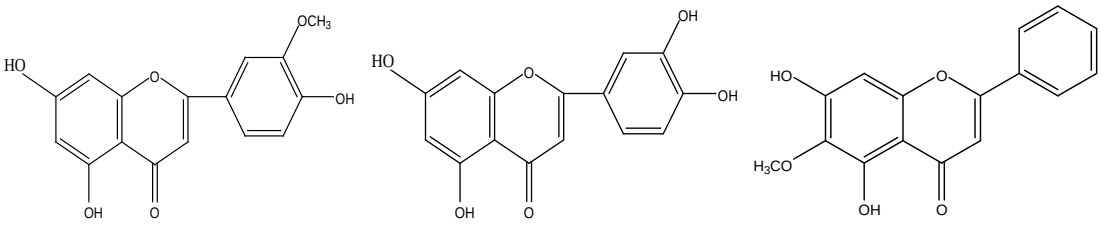


Fig.8. HPLC chromatogram 70% ethanol extract of *Inula rhizocephala*, standard oroxylin.



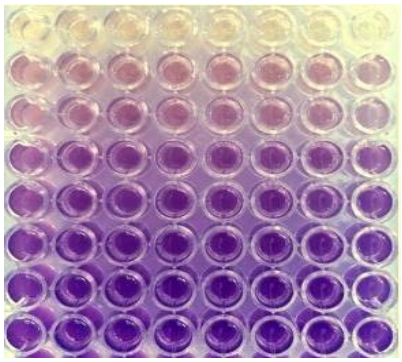
Chrysoeriol **Luteolin** **Oroxylin**

Fig.9. HPLC analysis of individual isolates of *Inula rhizocephala*, flavonoids.

ANTIOXIDANT ACTIVITY OF THE *Inula rhizocephala*

As a result of the conducted studies, it was determined that these flavonoids and medicinal plant raw materials can be recommended as a promising source.

A



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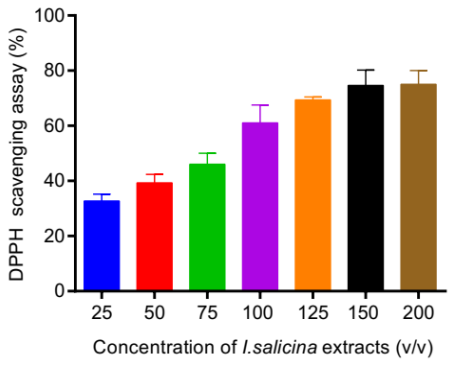

Fig. 10. Antioxidant activity assays of *Inula rhizocephala* extracts for DPPH. Scavenging (A: Values are shows as the mean± SD from three independent experiments; B:visual color change of this method with different concentrations of

CE, from left to right:25 mg/ mL, 50 mg/mL, 75 mg/mL, 100 mg/mL, 125 mg/mL, 150 mg/mL, 200 mg/ mL).

Antioxidant activity. The DPPH was used to determine free radical scavenging activity as previously described by Shimada et al. About 3.94 mg of DPPH was first dissolved in 100 m L of ethanol to a concentration of 0.1 m L. About 1 m L of DPPH solution was added to 3 m L of the samples with different concentrations (250, 125, 62.5, 31.25, and 15.62 μg/m L). For the control test, the same amount of ethanol was added. All the mixture was mixed well by shaking vigorously and left to stand for 30 minutes at a room temperature. After that, the UV-Vis spectrophotometer was used to measure the value of absorbance of each mixture at 517 nm. e calculation for the percentage of inhibition (*I*%) of the DPPH radical is as follows (Tabl. 1).

Table-1

Antioxidant activity of extracts and fractions of the surface of *Inula rhizocephala*

Sample	Sample	DPPH scavenging activity (%)
1	*Inula rhizocephala* 96% ex	78,46±0.25
2	*Inula rhizocephala* 70% ex	57,53±0.74
3	*Inula rhizocephala* hexane ex fraction	47.88±0.72
4	*Inula rhizocephala* Chloroform fraction	71.34±0.84
5	*Inula rhizocephala* Ethylacetate fraction	73.84±0.29
6	*Inula rhizocephala* Butonal fraction	88,07±0.8

According to the results, the fractions and extracts of the *Inula rhizocephala* plant and antioxidant properties of extracts and fractions showed higher activity than the standard.

CONCLUSIONS

Chrysin, oroxylin, apigenin, kaempferol, tamiflazid, astragalin, luteolin, chrysoeriol flavonoids were isolated for the first time from *Inula rhizocephala*.

ACKNOWLEDGMENT

The work was carried out with financial support from the Foundation for the Program for International Cooperation in Science and Technology. Projects of the

Academy of Sciences of the Republic of Uzbekistan (Grants MUK-2021-38 and MUK-2021-39).

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