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SECONDRY METABOLITS OF *INULA CASPICA*

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ABSTRACT

The chemical constituents of the flavonoids, terpenoids, coumarins and sterols of *Inula caspica* (family Asteraceae) aerial parts, growing in Uzbekistan was investigated by column chromatographic methods to isolate eight known compounds. Their structures were established on the basis of physicochemical and spectral parameters (UV, IR and PMR) and were identified as chrysoeriol, luteolin, kaempferol, quercetin, luteolin-7- O-β-D-glucoside, badrakemin, gaillardin and stigmasterol-ß-D-glucopyranoside. Anticancer activity of the gaillardin and chloroform fraction has been studied. Compounds - chrysoeriol, luteolin, kaempferol, quercetin, luteolin-7- O-β-D-glucoside, badrakemin, gaillardin and stigmasterol-ß-D-glucopyranoside were isolated from Inula caspica for the first time.

KEYWORDS: *Inula caspica*, Asteraceae, Uzbekistan, chrysoeriol, luteolin, kaempferol, quercetin, luteolin-7-O-β-D-glucoside, badrakemin, gaillardin, stigmasterol-ß-D-glucopyranoside, NMR-spectrum.

1. INTRODUCTION

Plant of the family Asteraceae are widespread in nature and are rich sources of flavonoids, terpenoids and coumarins in the flora of Uzbekistan. Flavonoids, terpenoids and coumarins - the most numerous classes of natural compounds, are characterized by structural diversity, and versatile high activity and low toxicity. From the plant family Asteraceae isolated natural compounds showed cytotoxic, antioxidant, hypoglycemic, lipid-lowering, hepatoprotective, antibacterial and other types of activities. In Uzbekistan there are more than 596 species of plants from the family Asteraceae, among which, only a few species studied. Therefore, the study of phenolic compounds and terpenoids plant family Asteraceae actually represents a certain theoretical interest, as well as great scientific and fundamental significance. Studies on new structure of flavonoids, coumarins and terpenoids will make some contribution to the chemistry of natural compounds, help address the problems associated with Chemotaxonomy and find new physiologically active compounds. Genus *Inula* (Asteraceae family) – one-, two- or perennial herbs, including 100 species, are widely distributed in the world. A number of plants in this genus are used as traditional herbal medicines throughout the world. The flora of Uzbekistan includes about 9 species and are widely used in the folk medicine as anticancer, antimicrobic, antibacterial and for the treatment of neuroses, epilepsy, gastritis, diabetes, gynecology and others diseases.

It flowers in July and August. It grows in saline, brackish, swampy areas, meadows, riparian forests, sometimes a weed, to the middle mountain zone. Since the roots are used for therapeutic purposes. Carbohydrates found in the roots and inulin derivative, essential oil, alkaloids, in the aerial parts of the plant - rubber alkaloids. A decoction of the roots is used as an expectorant.

2. MATERIALS AND METHODS

UV spectra were measured on a Lambda-16 spectrophotometer (Perkin–Elmer). Melting points were determined on a Boetius heating stage. IR spectra were recorded in KBr on a Perkin–Elmer Model 2000 Fourierspectrometer. PMR and ^{13}C NMR spectra were recorded in $CDCl_3$, Py and DMSO-d6 on a Unity-600 plus spectrometer (Varian) at operating frequency 600 MHz using HMDS as an internal standard for PMR spectra and the DMSO-d6 resonance (39.5 ppm vs. TMS) for ^{13}C NMR spectra. TLC used Silufol UV 254 plates with detection by iodine vapor, ammonia vapor, UV emission at 254 and 365 nm, and vanillin solution (1%) in conc. H_2SO_4. PC was carried out on Filtrak No. 11 paper using n-BuOH: HOAc: H_2O (4:1:5,1) and n-BuOH: Py: H_2O (6:4:3,2). Free monosaccharides were detected in PC by spraying with anilinium phthalate.

2.1 Extraction and isolation of chemical components from the aerial part of *I.caspica*

We have investigated the aerial parts of *Inula caspica* collected in the Namangan region of Uzbekistan. Air-dried ground plant material (1000 g) was extracted at room temperature by EtOH (70%, 6 x 5 L). The combined extracts were vacuum distilled. The extract was condensed to give residue (80 g) diluted with H_2O (1:1). The diluted solution was worked up successively with hydrocarbons, $CHCl_3$, EtOAc and BuOH. Evaporation of the solvents afforded hydrocarbons (12 g), CHCl3 (14 g), EtOAc (17 g), and BuOH (27 g) fractions. The EtOAc extract of flowers of *Inula caspica* (17 g) was chromatography on the ODS, using systems H_2O, EtOH (20%, 30% 50% 70%,100%) to obtain **1-5** fractions. The **5**- fraction (7 g) was rechromatographed on a column (120 x 1.5 cm) of silica gel with a gradient systems of solvents hydrocarbons - EtOAc. Eluting the column with a mixture of hydrocarbons -EtOAc 9:1 isolated 0.08 g badrakemin (**1**), further elution of the column with hydrocarbons -EtOAc 6:1 gave pure 0,11g gaillardin (**2**). The **4**-fraction was rechromatographed on the sephadex LH 20, by elution of CHCl3: MeOH (1:1) and isolated 0.15g chrysieriol (3), 0.07 g luteolin (4) and 0.02 g kaempferol (5). The 3- fraction was rechromatograed, and eluted with MeOH to yield 0.15 g quercetin **(6),** stigmasterol-ß-D-glucopyranoside (**7**) and luteolin-7- O-β-D-glucoside (Cinarosid) (**8**).

3. RESULTS AND DISCUSSION

The structures of isolated compounds were established on the basis of their physical and chemical properties, IR, UV, ^{1}H PMR, ^{13}C NMR and DEPT, HSQC, HMBC, COSY spectral data and also TLC analyses. As a result the compounds were identified to be badrakemin (**1**), (S. E Dzumyrko, 1976), gaillardin (**2**) [K.A.Eshbakova et al., 1996], chrysieriol (**3**) [A. Sultan et.al. 2008], luteolin (**4**) [K.A.Eshbakova et al., 2014], kaempferol (**5**), quercetin **(6)** [K.A.Eshbakova et al., 2011, 2014], stigmasterol-ß-D-glucopyranoside (**7**) [Mahbuba Khatun et.al.2012] and luteolin-7- O-β-D-glucoside (Cinarosid) (**8**) [Muhammadjan Abduwaki et al. 2014].

Badrakemin (**1**) $C_{24}H_{30}0_4$, mp. 199-200 °C. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.24 (1H, d, J = 9.3, H-3), 7.62 (1H, d, J = 9.3, H-4), 7.34 (1H, m, H-5), 6.84 (1H, dd, J = 2.5; 7.3, H-6), 6.82 (1H, s, H-8), 2.33 (1H, м, H-1'), 2.14 and 2.46 (2H, td, J = 4.9; 13.2, H-3'a,b), 3.47 (1H, wt J=2.5, H-6'), 1.66 (2H, м, H-7'a,b), 1.51 (1H, td, J=3.4; 12.7, H-8'a), 1.86 (1H, td, J=2.9; 12.7, , H-8'b), 1.66 (1H, m, H-10'), 0.87 (3H, s, H-11'), 0.99 (3H, s, H-12'), 4.18 (1H, dd, J = 7.8; 9.8, H-13'a), 4.24 (1H, dd, J = 3.9; 9.8, H-13'b), 4.54 (1H, s, H-14'a), 4.90 (1H, s, H-14'b), 0.86 (3H, s, H-15'). ^{13}C NMR (150 МГц, $CDCl_3$, δ, ppm, J/Hz): 161.46 (C-2), 113.14 (C-3), 143.63 (C-4), 128.88 (C-5), 113.41 (C-6), 162.55 (C-7), 101.54 (C-8), 156.14 (C-9), 112.62 (C-10), 54.93 (C-1'), 146.95 (C-2'), 37.74 (C-3'), 23.66 (C-4'), 37.94 (C-5'), 75.95 (C-6'), 25.96 (C-7'), 32.00 (C-8'), 39.01 (C-9'), 48.32 (C-10'), 28.75 (C-11'), 22.50 (C-12'), 65.92 (C-13'), 107.78 (C-14'), 15.48 (C-15').

Gaillardin (**2**), $C_{17}H_{22}0_5$, mp 188-190°C. ^{1}H NMR - spectra (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.16 (1H, д, J=3.0, CH_2-13a) и 5.51 (1H, д, J=3.0, CH_2-13в), 5.87 (1H, д J=1.8, H-9), 5.25 (1H, т, J=3; 6.6, H-2), 4.46 (1H, д, J=9.0, H-8), 2.58 (1H, м, H-7), 2.55 (1H, ддд., J=3.0; 2.4; 13.8, H-6a), и 1.37 (1H, кв., J=13.2; 12.6, H-6в), 2.44 (1H, д, J=12.0, H-1), 2.14 (1H, ддд, J=3.0; 3.6; 12.6, H-5), 2.05 (3H, с, CH_3-OAc), 1.98 (1H, д, J=15.6, H-3), 1.91 (1H, дд, J=4.2; 15.0, H-3'), 1.77 (3H, с, CH_3-14), 1.21 (3H, с, CH_3-15). ^{13}C NMR-spectra(150 MHz, $CDCl_3$, δ, ppm): 51.81 (C-1), 77.23 (C-2), 48.02 (C-3), 79.15 (C-4), 50.42 (C-5), 30.19 (C-6), 45.56 (C-7), 82.70 (C-8), 128.04 (C-9), 136.32 (C-10), 139.36 (C-11), 170.17 (C-12), 119.75 (C-13), 22.49 (C-14), 25.69 (C-15), 170.34 (C-16), 21.66 (C-17).

Chrysoeriol (**3**) yellow crystals, $C_{16}H_{12}O_6$, mp 312-314°C. IR spectrum (KBr, ν_{max}, cm^{-1}): 3420 (OH), 1690 (C=O), 1670, 1601, 1570 (C=C). ^{1}H NMR-spectra (600 MHz, DMSO, δ, ppm, J/Hz): 7.56 (2H, dd, J=2.0; 8.4, H-2′, 6′), 6.93 (1H, d, J=8.4, H-5′), 6.56 (1H, s, H-3), 6.51 (1H, d, J=2.0, H-8), 6.19 (1H, d, J=2.0, 6-H), 3.80 (OCH_3), 8.87 (1H, br.s, 4′-OH), 9.08 (1H, br.s, 7-OH), 10.35 (1H, s, 5-OH). ^{13}C NMR (150 MHz, DMSO, δ, ppm): 161.42 (C-2), 104.05 (C-3), 182.14 (C-4), 163.66 (C-5), 98.85 (C-6), 164.15 (C-7), 94.03 (C-8), 157.32 (C-9), 103.70 (C-10), 121.47 (C-1′), 110.16 (C-2′), 148.02 (C-3′), 150.72 (C-4′), 115.95 (C-5′), 120.35 (C-6′), 55.93 (OCH_3).

Luteolin (4) $C_{15}H_{10}O_6$, т.пл. 328-330°C. ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 7.42 (1H, dd, J=2.4; 8.4, 6′), 7.39 (1H, d, J=2.4, H-2′), 6.89 (1H, d, J=8.4, H-5′), 6.67 (1H, s, H-3), 6.44 (1H,d, J=2.4, H-6), 6.18 (1H, d, J=2.4, H-8), 9.47 (1H, br.s, OH-3′), 9.92 (1H, br.s, OH-4′), 10.91 (1H, br.s, OH-7), 13.00 (1H, s, OH-5). ^{13}C NMR: 164.09 (C-2), 102.85 (C-3), 181.62 (C-4), 161.45 (C-5), 98.80 (C-6), 163.86 (C-7), 93.81 (C-8), 157.26 (C-9), 103.68 (C-10), 121.48 (C-1′), 113.35 (C-2′), 145.71 (C-3′), 149.67 (C-4′), 115.99 (C-5′), 118.96 (C-6′).

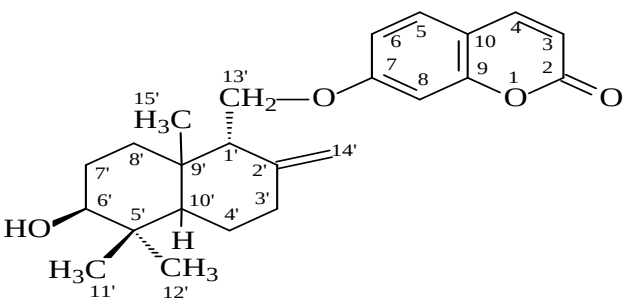
Kaempferol (5), $C_{15}H_{10}O_6$, yellow crystals, mp 276–277°C (EtOAc). ^{1}H NMR-spectra (600 MHz, DMSO, δ, ppm, J/Hz): 6.20 (1H, д, J = 2.0, H-6), 6.46 (1H, д, J = 2.0, H-8), 6.98 (2H, д, J = 8.5, H-3′,5′), 8.10 (2H, д, J = 8.5, H-2′,6′), 9.40 (1H, с, 4′-OH), 10.9 (1H, с, 3-OH).

Quercetin (6), $C_{15}H_{10}O_7$, mp 305–307°C (EtOAc). ^{1}H NMR (600 MHz, DMSO, ppm, J/Hz): 7.71 (1H, д, J=1.8, H-2'), 7.53 (1H, дд, J = 1.8; 9.0, H- 6'), 6.89 (1H, J = 9.0, H-5'), 6.40 (1H, д, J=2.4, H-8), 6.18 (1H,д, J=2.4, H-6), 12.48 (1H, OH-5). ^{13}C NMR (150 MHz, DMSO, δ, ppm): 160.02(C-2), 135.68 (C-3), 176.79 (C-4), 160.68 (C-5), 98.13 (C-6), 163.85 (C-7), 93.30 (C-8), 156.10 (C-9), 102.96 (C-10), 121.90 (C-1'), 115.55 (C-2'), 145.02 (C-3'), 146.76 (C-4'), 115.02 (C-5'), 119.92 (C-6').

Stigmasterol-ß-D-glucopyranoside (7) $C_{35}H_{58}O_6$.
^{1}H NMR –spectra (600 MHz, Py, δ, ppm, J/Hz): 1.03, 1.75 (2H, m;m, H-1), 1.33, 1.66 (2H, m;m, H-2), 3.55 (1H,m, H-3), 1.76 (2H,m,H-4), 5.12 (1H,br.s. J-3.5, H-5), 1.76 (2H, m, H-7), 1.28 (1H,m,H-8), 1.62 (1H, m, H-9), 1.42, 1.58 (2H, mm, H-11), 1.22, 1.49 (2H, mm, H-12), 1.79 (1H,m,H-14), 1.35, 1.46 (2H, mm, H-15), 1.24, 1.64 (2H, mm, H-16), 1.26 (1H, m, H-17), 0.51 (3H,s, H-18), 0.74 (3H,s, H-19), 2.01 (1H, br.q, J=6.6, H-20), 0.99 (3H, d, J=6.6, H-21), 5.16(1H, dd, J=15.2;8.7, H-22), 5.03 (1H, dd, 15.2; 8.8, H-23), 1.52(1H,m,H-24), 1.51 (1H, m, H-25), 0.83 (3H, d,J-6.3, H-26), 0.77 (3H, d, J=6.3, H-27), 1.14 (2H, m, H-28), 0.78 (1H, t, J=7.4, H-29), 4.22 (1H, d, J=7.8, H-1'), 2.88 (1H, dt, J=7.8, H-2'), 3.11 (1H, t, J=8.7, H-3'), 3.01 (1H, t, J=9.0, H-4'), 3.06 (1H, m H-5'), 3.41, 3. 64 (2H, m,d, J=11.4, H-6').

^{13}C NMR: (150 MHz, Py, δ, ppm): 36.54 (C-1), 29.09 (C-2), 76.30 (C-3), 33.94 (C-4), 139.03 (C-5), 117.21 (C-6), 29.21 (C-7), 39.52 (C-8), 48.65 (C-9), 33.91 (C-10), 20.99 (C-11), 38.76 (C-12), 42.82 (C-13), 54.47 (C-14), 22.53 (C-15), 28.11 (C-16), 55.19 (C-17), 12.12 (C-18), 12.78 (C-19), 40.05 (C-20), 21.24 (C-21), 137.91 (C-22), 128.98 (C-23), 50.59 (C-24), 31.32 (C-25), 20.96 (C-26), 18.85 (C-27), 24.87 (C-28), 11.87 (C-29), 100.84 (C-1'), 73.49 (C-2'), 76.75 (C-3'), 70.11 (C-4'), 76.69 (C-5'), 61.12 (C-6').

Luteolin-7- O-β-D-glucoside (8), $C_{21}H_{20}O_{11}$. ^{1}H NMR-spectra (600 MHz, DMSO, δ, ppm, J/Hz):7.45 (1H, dd, J=2.0; 8.4, 6′), 7.42 (1H, d, J=2.0, H-2′), 6.91 (1H, d, J=8.4,H- 5′), 6.79 (1H, d, J=2.0, H-6), 6.75 (1H, s, H-3), 6.44 (1H, d, J=2.0, H-8), 9.47 (1H, br.s, OH-3′), 5.11 (1H, d, J-7.0, H-1″), 3.20-3.70 (protons from sugar), 12.99 (1H, s, OH-5). ^{13}C NMR (150 MHz, DMSO, δ, ppm): 165.12 (C-2), 103.82 (C3), 182.53 (C-4), 161.78 (C-5), 100.19 (C-6), 163.60 (C7), 95.37 (C-8), 157.59 (C-9), 105.99 (C-10), 122.02 (C1′), 114.22 (C-2′), 146.22 (C-3′), 150.59 (C-4′), 116.63 (C-5′), 119.82 (C-6′), 100.55 (C-1″), 73.78 (C-2″), 77.05 (C-3″), 70.21 (C-4″), 77.82 (C-5″), 61.28 (C-6″).



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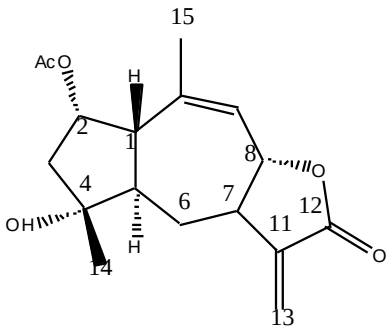

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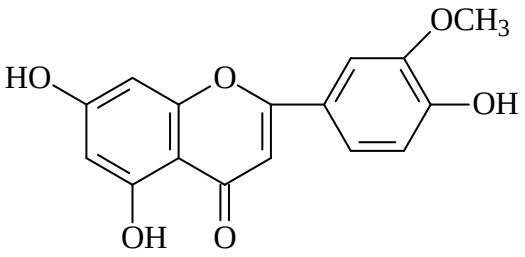

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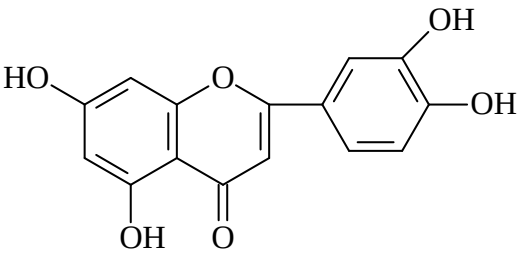

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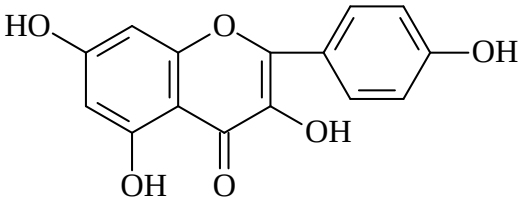

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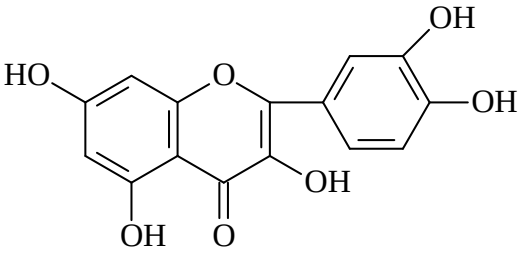

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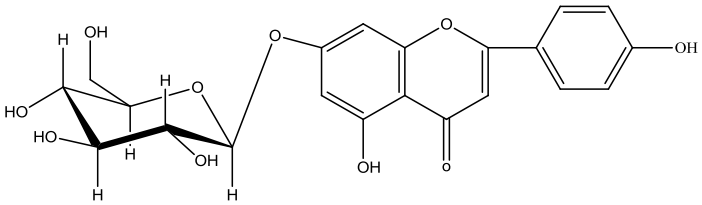

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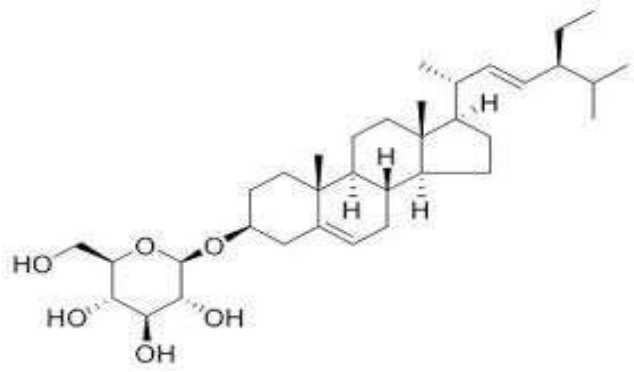

(8)

Table 1: Anti-tumor activity assay.

Sample Name	IC50				
	T47D	Hela	MCF-7	Ishicawa	CHO
Gaillardin (μM)	4.1±0.5	5.0±0.5	22.2±1.4	7.3±0.8	40.1±3.1
$CHCl_3$ fraction (μg/ml)	15.2±1.3	19.4±1.8	43.6±3.5	29.4±1.8	92.76±3.4
DOX (μM)	9.0±1.2	4.1±0.6	6.7±0.9	9.0±1.0	

4. CONCLUSION

Eight known compounds were isolated from aerial parts of *Inula caspica* for the first time. They were coumarins, sesquiterpene lactones, sterols and flavonoids. It was found a new source of antioxidant, hyponitrogenimic, and anti-inflammatory activities Cynaroside. Sesquiterpene lactone Gaillardin and chloroform fraction showed anti-tumor activity. Chrysoeriol and Luteolin inhibit Ca+2 channel activity in the mitochondrial membrane.

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