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Phytochemical studies of the herb, *Tragopogon orientalis* L. (*Asteraceae*). 2. Components of a methanol extract

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Abstract

Vitexin, orientin, isoorientin, luteolin and apigenin were isolated from the herb, *Tragopogon orientalis* L. The occurrence of quercetin and the phenolic acids: chlorogenic, homoprotocatechuic, protocatechuic, caffeic, gentysic, p-hydroxybenzoic, m-hydroxybenzoic o-hydroxyphenylacetic, p-hydroxyphenylacetic, p-cumaric, syringic, vanillic, ferulic and salicylic, and of the sugars: glucose, fructose and sucrose was demonstrated chromatographically.

Key words: Tragopogon orientalis L, flavonoids, phenolic acids, sugars

INTRODUCTION

The results of analysis of the components of a petroleum ether extract of the herb, *Tragopogon orientalis* L. were presented in part 1. This study is on the phenolic components of a methanol extract and sugars in the species of interest.

MATERIAL AND METHODS

Two kilograms of raw material, previously dried and defatted (Krzaczek and Smolarz 1987) were extracted three times for 1 hr with boiling 70° methanol.

The solvent was distilled from the extracts in a vacuum evaporator. The remnants (300 g) were extracted with small portions of hot water. The combined aqueous eluates (approx. 1 dm^3) were left for 24 hr in a cold-room, after which the sediment was filtered off. The filtrate was purified by repeated washing with petroleum ether, then with chloroform. One dm^3 aqueous solution was obtained. Of this, 960 cm^3 (W_1) were used for study of flavonoids and 40 cm^3 (W_2) for assay of free and bound phenolic acids.

The control of the separation and purity of flavonoids and the analysis of the products of their hydrolysis and oxidation was done by means of paper (PC) and thin-layer (TLC) chromatography. Whatman 1 chromatography paper was used for paper chromatography, and the chromatograms were run using the ascending technique and the following solvent phases (Mabry et al. 1970, Harborne 1973, Budzianowski and Skrzypczakowa 1979, Strzelecka et al. 1982):

F_1 : n-butanol-acetic acid-water, 4:1:5 (organic phase);
F_2 : acetic acid-water, 15:85;
F_3 : t-butanol-acetic acid-water, 3:1:1;
F_4 : chloroform-acetic acid-water, 50:45:5;
F_5 : acetic acid-water, 1:1;
F_6 : acetic acid-hydrochloric acid-water, 10:3:30;
F_7 : n-butanol-benzene-pyridine-water, 5:1:3:3;
F_8 : n-butanol-pyridine-water, 6:4:7.

Thin-layer chromatography (Strzelecka and Malinowski 1972, Saito 1976) was done plates coated with a 0.25 mm layer of cellulose powder. MN-300 G, Woelm polyamide or silica gel G. The cellulose-coated plates were run in solvent phases F_1-F_3 and: F_9 – ethyl acetate-methanol-water, 100:6:20 (org. phase); F_{10} – chloroform-acetic acid-methanol-water, 13:3:3:1.

The plates coated with polyamide were run in solvent phase F_{11}: benzene-methanol-methyl ethyl ketone-water, 30:13:7:0.5.

F_{12}, n-propanol-ethyl acetate-water, 7:2:1, was used for plates coated with gel G.

The position of flavonoid spots on the chromatograms was analysed using UV light (about 360 nm) before and after exposure to ammonium vapors and after spraying with the following developers (Mabry et al. 1970, Strzelecka and Malinowski 1972, Saito 1976, Klimek and Królikowska 1984): R_1 – 1% ethanol solution of $AlCl_3$ and R_2 – 5% aqueous solution of $FeCl_3$.

Hydrocarbon spots were visualized with (Stange 1959): R_3 – aniline hydrogen phtalate, R_4 – urea with HCl in ethanol.

The separation of the flavonoid fraction and the isolation of homogenous compounds was carried out by means of preparative paper chromatography and column chromatography. The columns were filled with Woelm polyamide and cellulose powder CM 11 (Whatman). The eluents obtained in preparative chromatography were separated from the paper on 1 × 10 cm column packed with Sephadex LH-20.

UV analysis of flavonoids was done using a Beckman Model 26 Spectrophotometer. The spectra were recorded in the 500-200 nm range for the methanol solutions of the various compounds, and after the addition of the appropriate ionizing and chelating agents (Table 3). The spectrophotometric analyses were done according to Dyer (1967), Olechowicz-Stępień and Rządkowska-Badalska (1968) and Harborne and Swain (1969). The ^1H NMR spectra were recorded using a Jeol SX (90) Q NMR spectrophotometer after dissolving 20 mg of the compound in 0.45 cm^3 DMSO $d_6 + D_2O$ with TMS as the internal standard.

The isolated flavonoids were subjected to hydrolysis in an aqueous solution of 1H HCl with chromatographic control (Budzianowski and Skrzypczałkowa 1979).

The oxidation of C-glycosyl with $FeCl_3$ was done according to Mabry et al. (1970). The sugars were chromatographed on ready-to-use silica gel G plates (Merck) along with standards, using F_{12} in both directions.

ANALYSIS OF FLAVONOIDS

The aqueous W_1 solution was exhaustively washed with ethyl ether. An aqueous fraction, W_3, and an ether fraction were obtained. Acids were removed from the ether fraction with a saturated solution of sodium hydrogen carbonate and the remaining ether fraction E_A was concentrated to a volume of 20 cm^3. From the aqueous W_3 phase, 10 cm^3 (W_4) were set aside for preliminary analysis of flavonoids. The two-dimensional paper chromatography of W_4 (I direction: F_3, II direction: F_2) revelaed the presence of 11 spots, which it was accepted, were flavonoids. Under UV light, 9 of the spots gave a brown fluorescence, the remaining ones – yellow. All of the spots qave a yellow color in the reaction with NH_3 and aluminum chloride (Fig. 1a).

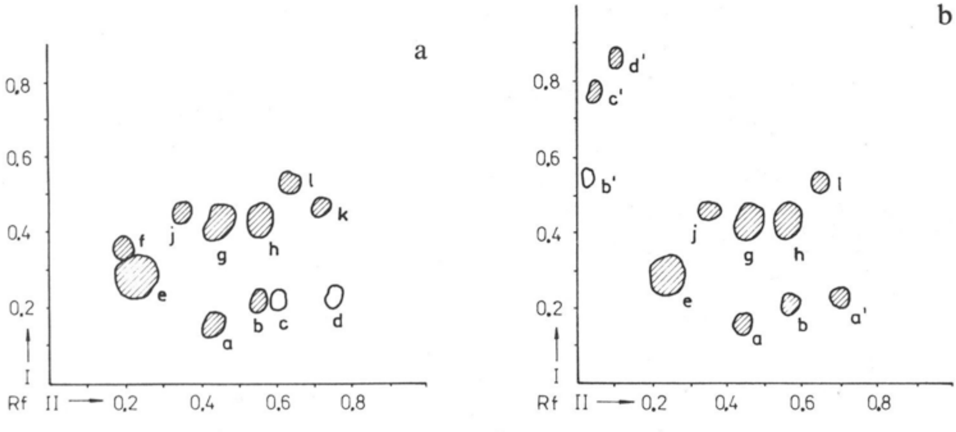

Fig. 1. Two-dimensional chromatogram of flavonoid fractions: a – before hydrolysis, b – after acid hydrolysis. Lined spots – brownfluorescence, unlined – yellow fluorescence in UV. The spots of compounds obtained after hydrolysis are denoted by an apostrophe

In order to obtain an approximate idea about the nature of the glycosidic bond in these compounds, a 4 hour-long acid hydrolysis was conducted. The hydrolysate was chromatographed in two dimensions under the same conditions. The presence of 11 flavonoid spots was found (Fig. 1b). Comparison of the chromatograms before and after hydrolysis showed that the position and color of spots a, b, e, g, h, j and l did not change. Spots c and d, which gave a yellow fluorescence, disappeared after hydrolysis, as did the brown spots f and k, while new spots, a′, b′, c′ and d′ appeared. From the position of spots b′, c′ and d′ on the chromatograms, it can be assumed that they are aglycone spots. Aglycones were isolated from the post-reaction mixture by the known method (Budzianowski and Skrzypczakowa 1979). Chromatography of them with standards (Table 1) showed the presence of 3 aglycone spots (P_1, P_2, P_3) which were taken to be quercetin, luteolin and apigenin.

The presence of aglycone spots was chromatographically (PC, F_1, F_2, F_4, F_5) shown in the ether fraction E_A.

The luteolin and apigenin standards applied simultaneously corresponded to these spots.

The isolation of compounds O_1 and O_2

The ether was evaporated from fraction E_A and the remains dissolved in 15 cm^3 ethanol and separated by preparative paper chromatography using phase F_1, then phase F_4. Chromatographically pure compounds O_1 and O_2 were obtained (Table 1).

Table 1

Results of paper chromatography of aglycones

Aglycones	Color			Rf					
	UV	UV(NH_3)	$AlCl_3$	F_1	F_2	F_3	F_4	F_5	F_6
p_1	yellow	yellow	yellow	0.73	0.03	0.55	0.30	0.25	0.40
p_2	brown	yellow-green	yellow	0.80	0.05	0.77	0.50	0.40	0.63
p_3	brown	yellow-green	yellow	0.88	0.11	0.87	0.85	0.50	0.82
Quercetin	yellow	yellow	yellow	0.73	0.03	0.55	0.30	0.25	0.40
Luteolin	brown	yellow-green	yellow	0.80	0.05	0.77	0.50	0.40	0.63
Apigenin	brown	yellow-green	yellow	0.88	0.11	0.87	0.85	0.50	0.82
Compound O_1	brown	yellow-green	yellow	0.80	0.05	0.77	0.50	0.40	0.63
Compound O_2	brown	yellow-green	yellow	0.88	0.11	0.87	0.85	0.50	0.82

F_1 – n-butanol-acetic acid-water, 4:1:5; F_2 – water-acetic acid, 85:15; F_3 – t-butanol-acetic acid-water, 3:1:1; F_4 – chloroform-acetic acid-water, 50:45:5; F_5 – acetic acid-water, 1:1;

F_6 – acetic acid-hydrochloric acid-water, 10:3:30.

The separation of the flavonoid glycoside fraction and isolation of compounds e, g, h

The aqueous fraction W_3 (950 cm^3) was concentrated under reduced pressure and applied to a column filled with an aqueous suspension of polyamide. The column was eluted with water, water with methanol at the following proportions: 4:1, 3:2, 2:3, 3:1, and methanol. Paper (F_7, F_8, R_3, R_4) and thin-layer (F_{12}, R_3, R_4) chromatography of fractions 11-12, conducted in the presence of standard sugars, showed the presence of 3 sugar spots, C_1, C_2 and C_3, which were identified as fructose, glucose and sucrose (Table 2). Fractions 13-20 contained mainly compounds g and h, with traces of j and l. Fractions 21-39 contained a mixture of 6-7 compounds giving a brown fluorescence under UV light and 2 giving an yellow color. Fractions 40-45 contained compounds a, c or d.

Table 2

Results of chromatography of fraction (11-12) and standard substances

Sugars	Rf		
	PC:F_7	PC:F_8	TLC:F_{12}
C_1	1.09	0.59	0.34
Fructose	1.09	0.59	0.34
C_2	1	0.56	0.44
Glucose	1	0.56	0.44
C_3	0.79	0.24	0.33
Sucrose	0.79	0.24	0.33

F_7 – n-butanol-benzene-pyridine-water, 5:1:3:3; F_8 – n-butanol-pyridine-water, 6:4:7; F_{12} – n-propanol-ethyl acetate-water, 7:2:1.

The separation of the flavonoids contained in fractions 21-39 was done on an identical column eluted with water and rising concentrations of alcohol. Eluates of a similar composition (PC, F_1, R_1) were pooled and condensed.

Two-dimensional paper chromatography was carried out using the individual fractions from both columns (I direction: F_3, II direction: F_2). On the basis of the results of this chromatography, the fractions were combined into 4 groups (G_1, G_2, G_3, G_4), containing from two to six flavonoids.

Group G_2 was applied to a polyamide column which was eluted with F_{11}. On the basis of chromatography (PC, F_{10}), the eluates were combined. Fractions 5-25 contained compounds h and l, fractions 26-30 – a small amount of g, j; fractions 31-40 contained pure compound g (0.095 g). Fractions 5-25 were added to the G_1 group of compounds. In total, 3 g of a mixture of compounds h and i were obtained and separated by preparative paper chromatography in F_2. As a result, 0.113 g of chromatographically pure compound h were obtained. Substance 1 turned out to be unhomogenous.

The solution containing the G_3 group of compounds was evaporated to dryness under reduced pressure. The 3.7 g of sediment obtained were applied to a column filled with cellulose. The column was eluted with: ethyl acetate-methanol-acetone-water, 100:6:6:6. The contents of the eluates were checked chromatographically (PC – F_1, TLC – F_3). Fractions 6-13 contained compounds e and j; fractions 14-17 contained a mixture of all four compounds; fractions 18-43 contained pure compound g (0.078 g).

Fractions 6-13 containing compounds e and j were separated by preparative paper chromatography in F_3. Obtained were 0.12 g of compound e and a small amount of a mixture.
Attempts at isolating the remaining flavonoids were not successful.

ISOLATION OF PHENOLIC ACIDS

The fraction of free phenolic acids was isolated from the aqueous solution (W_2) according to the method of Ibrachim and Towers (1960) as used by Świątek (1977) and Krzaczek (1984). Approximately 0.12 g of free phenolic acids (K_W) were obtained. Hydrolysis of the bound phenolic acids remaining in solution after the isolation of the free acids, was carried out according to Schmidtlein and Herrmann (1975). The procedure included our modification which consisted of separate extraction with ethyl ether followed by the isolation of phenolic acids freed in alkaline hydrolysis (K_E, 0.49 g) and in acid hydrolysis (K_G, 0.3 g). Two-dimensional paper chromatography of phenolic acids was done on 28.5 × 28.5 cm sheets of Whatman 1 chromatography paper, using the appropriate solvent systems (Griffiths 1957, Smith 1958). The chromatograms were viewed under UV light (about 360 nm) before and after exposure to ammonium vapors, and in daylight after spraying with the following solutions (Linskens 1959, Randerath 1962):

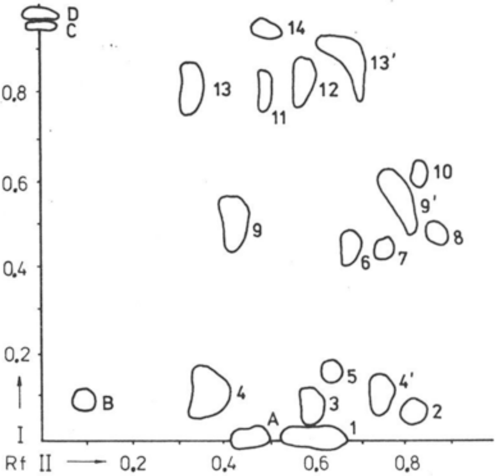

Fig. 2. Two-dimentional paper chromatogram of phenolic acids. Spot numbers correspond to Table 2. Cis-isomers denoted by apostrophe. Direction I: benzene-acetic acid-water, 6:7:3; direction II: sodium formate-formic acid-water, 10:2:200

R_5 – diazotized sulfanilic acid in 10% aqueous Na_2CO_3,
R_6 – diazotized p-nitroaniline in 10% aqueous Na_2CO_3,
R_7 – 2% aqueous $FeCl_3$.

Phenolic acid spots were identified on the basis of the two-dimensional chromatograms made with the appropriate mixture of standards and the agreement of the position of the studied spots with those of the standards (Fig. 2) and on the fluorescence and color reactions with the reagents used (Table 4).

RESULTS

Determination of the structure of the isolated flavonoids: O_1, O_2, e, g, h.

COMPOUND O_1

After crystallization from hydrated methanol, compound O_1 melted at a temperature of 327-330°C. Harborne (1973) reports a mp of 330-331°C for luteolin, Klimek and Królikowska (1984) – 325-328°C. Under UV light it gave a brown fluorescence, after exposure to ammonium vapors – yellow. It gave a yellow color in the reaction with $AlCl_3$. The Rf values of compound O_1 in phases F_1-F_6 (Table 1) were in agreement with those of standard luteolin. The UV spectra agreed with those made for standard luteolin. It was concluded that compound O_1 is 5, 7, 3′, 4′ tetrahydroxyflavone – luteolin.

COMPOUND O_2

After crystallization from hydrated methanol, compound O_2 melted at a temperature of 347°C. El-Moghazi et al. (1980) give a mp. of 348-349°C for apigenin, Klimek and Królikowska (1984) – 335-338°C. It gave a brown fluorescence under UV light, after NH_3 – yellow. With $AlCl_3$ it gave an yellow color. The Rf values of compound O_2 in phases F_1-F_6 (Table 1) were in agreement with the Rf of standard apigenin. The UV spectra of compound O_2 agreed with those of standard apigenin (Table 3).

Compound O_2 was identified as 5, 7, 4′ trihydoxyflavone – apigenin.

COMPOUND e

This compound gave an orange to crimson color in the Shinoda reaction, in the reaction with magnesium acetate – yellow, with aluminum chloride – yellow. It gave a brown fluorescence under UV light, after the action of

Table 3

Results of UV spectroscopic analysis of flavonoid compounds isolated from *Tragopogon orientalis* L.

Compounds	UV	λ_{max}	nm	^{1}H NMR$_{TMS}$	δ ppm
Compound e	MeOH	258,270	351	3.20-3.90	(5H,m, H-2, 3, 4, 5, 6 glucose)
	NaOme	270	408	4.74	(1H,d, J = 10Hz, H-1 glucose)
	NaOAc	278	393	6.30	(1H,s, H-6)
	NaOAc/H_3BO_3	268	378	6.64	(1H,s, H-3)
	$AlCl_3$	277	426	6.84-6.93	(1H,d, Jo = 9Hz, H-5)
	$AlCl_3$/HCl	262	378	7.48-7.56	(2H,d, Jo = 9Hz, H-2, 6)
Compound g	MeOH	272	339	3.22-3.90	(5H,m, H-2, 3, 4, 5, 6 glucose)
	NaOMe	275	405	4.55	(1H,d, J = 10Hz, H-1 glucose)
	NaOAc	277	425	6.52	(1H,s, H-6)
	NaOAc/H_3BO_3	278	382	6.83	(!H,s, H-3)
	$AlCl_3$	275	383	6.86-6.97	(2H,d, Jo = 9Hz, H-3, 5)
	$AlCl_3$/HCl	269	380	7.39-7.46	(2H,d, Jo = 7Hz, H-2, 6)
Compound h	MeOH	255,271	349	3.10-4.20	(5H,m, H-2, 3, 4, 5, 6 glucose)
	NaOMe	270	406	4.66	(1H,d, J = 10Hz, H-1 glucose)
	NaOAc	272	413	6.70	(1H,s, H-3)
	NaOAc/H_3BO_3	265	377	6.80	(1H,s, H-8)
	$AlCl_3$	279,332	428	6.88	(1H,d, Jo = 9Hz, H-5)
	$AlCl_3$/HCl	280	363, 384	7.51	(2H,d, Jo = 9Hz, H-2, 6)
Compound O_1	MeOH	253,267	348		
	NaOMe	270,330p	401		
	NaOAc	270,326p	384		
	NaOAc/H_3BO_3	258	370,430p		
	$AlCl_3$	274,300p	329,423		
	$AlCl_3$/HCl	275	355,384		
Compound O_2	MeOH	267	337		
	NaOMe	275,325	396,402		
	NaOAc	273	383		
	NaOAc/H_3BO_3	268	342		
	$AlCl_3$	276	384		
	$AlCl_3$/HCl	276	383		

ammonium vapors – yellow. As the result of 12 hour controlled hydrolysis in *1N aqueous HCl, the compound presumably underwent partial oxidation with the release of glucose (TLC: F_{12}, R_3) and the disintegration of the aglycone.*

Analysis of the UV spectrum reveals that compound e has an orthodihydroxyl group in its B ring, as well free OH groups at C-5 and C-7.

Analysis of the ^1H NMR spectrum of compound e made possible its final identification. A multiplet from the H^+ at C 2-6 of glucose occurs in the range of 3.2-3.9 nm waves, and at 4.7 nm, a dublet from H^+ at C 1 of glucose. Glucose is seen to bound with the aglycone at position C-6 with a β-linkage (J = 10 Hz).

Compound e was identified as 8-C-β-D-glucopyranosylo-5, 7-3′, 4′ tetrahydroxyflavone – orientin.

COMPOUND g

After crystallization from 50% ethanol, it had a mp. of 289-291°C. In the Shinoda reaction, it gave a yellow color, under UV light it fluorized brown, after NH_3 – yellow. Compound g did not undergo hydrolysis in either 1 N HCl or in Kilian's mixture. As the result of oxidating $FeCl_3$, glucose was split from its molecule. Analysis of the UV spectrum indicates the presence of free OH groups at C-5, C-7 and C-4'.

Analysis of the ^1H NMR spectrum of compound g (Table 3) permits its structure to be definitely determined. It is seen from this that glucose is bound with the aglycone in position 8 by a β-linkage (J = 10 Hz).
Compound g is 8-C-β-D-glucopyranosylo-5, 7, 4′ trihydroxyflavone – vitexin.

COMPOUND h

It gave a yellow Shinoda reaction, brown fluorescence under UV, after NH_3 vapors, yellow. The compound was subjected to a controlled 12 hour long hydrolysis in a 1 N aqueous solution of HCl. The results of control chromatography (PC: F_3, F_5) showed that the compound partially underwent what was presumably oxidation, releasing small amounts of glucose (TLC: F_{12}, R_3). The analysis of its UV spectrums showed that compound h has free OH groups at C-5, C-7, and an orthodihydoxyl group in ring B.

It results from the analysis of the ^1H NMR spectra (Table 3) that glucose (signals in the 3.10-4.20 nm range and 4.55) is bound with the aglycone at position C-6 by a β-linkage (J = 10 Hz).
Compound h was determined to be 5, 7, 3′, 4′ tetrahydroxyflavone – isoorientin.

CHROMATOGRAPHIC ANALYSIS OF PHENOLIC ACIDS

The phenolic acid fractions (K_w, K_E, K_G) were studied by two dimensional paper chromatography (Fig. 2, Table 4).

Table 4

The occurrence in fractions and color reactions after two-dimensional chromatography

Number	Phenolic acids	Fraction			Color with				
		K_W	K_E	K_G	UV	UV/NH_3	R_{13}	R_{14}	R_{15}
1	chlorogenic	+			b	y-gr	y	l	gr
2	homoprotocatechuic	+	+		—	—	p	v	b
3	protocatechuic	+	+	+	—	a	br	p-br	br-b
4	caffeic	+	+	+	b	b	br	br	br-b
5	gentysic			+	b	y	g-gr	g-gr	b
6	p-hydroxybenzoic	+	+	+	—	—	y	p	—
7	m-hydroxybenzoic		+		—	—	y	p	—
8	p-hydroxyphenylacetic	+		+	—	—	p	v	—
9	p-cumaric	+	+	+	—	v	br-p	b	—
10	o-hydroxyphenylacetic	+		+	—	—	y	p	—
11	syringic	+		+	—	v-br	p	b	—
12	vanillic	+		+	—	—	o	v	l-br
13	ferulic	+	+	+	b	b	p-v	b	—
14	salicylic	+		+	v	b	y	o	v
A		+			b	v	o	v	—
B			+		—	v	p	p	—
C			+		—	gr-b	be	be	b
D			+		—	b	p	v	—

K_W – free phenolic acids; K_G – phenolic acids after acid hydrolysis; K_E – phenolic acids after alkaline hydrolysis. R_{13} – diazotized sulfanilic acid in 10% NA_2CO_3; R_{14} – diazotized p-nitroaniline; R_{15} – 2% $FeCl_3$; + – presence of phenolic acid.

Color: b – blue, v – violet, y – yellow, a – absorbing, p – pink, g – grey, br – brown, l-br- – light brown, be – beige, l – lemon, o – orange, gr – green.

The following acids were found in fraction K_W: chlorogenic, homoprotocatechuic, protocatechuic, caffeic, p-hydroxybenzoic, p-hydroxyphenylacetic, p-cumaric, o-hydroxyphenylacetic, syringic, vanillic, ferulic and salicylic as well as the unidentified acid, A.

In fractions K_E and K_G the presence of the following acids was found: homoprotocatechuic, protocatechuic, caffeic, gentysic, p-hydroxybenzoic, m-hydroxybenzoic, p-hydroxyphenylacetic, p-cumaric, o-hydroxyphenylacetic, syringic, vanillic, ferulic, salicylic and three unidentified acids, B, C and D.

DISCUSSION

Fractions of aglycones, flavonoid glycosides and phenolic acids were obtained from a methanol extract of the herb, *Tragopogon orientalis*. Two aglycones were found in the free state, isolated and shown to be luteolin and apigenin. By chromatographic methods it was shown that in addition to luteolin and apigenin, quercetin is found in the bound form.

The major part of the flavonoids were glycosides, which upon chromatography, gave 11 separate spots with a brown or yellow fluorescence under ultrafiolet light, and a yellow color in the reaction with $AlCl_3$. On the basis of a four-hour long acid hydrolysis it can be assumed that the four of them which underwent hydrolysis possess an O-glycosidic linkage in their structure, and the two which gave a yellow fluorescence, can be O-glycosides of quercetin, and the remaining two, giving a brown fluorescence, O-glycosides of luteolin and apigenin. The remaining seven compounds did not undergo acid hydrolysis under these conditions. They are supposedly C-glycosyls. Three of them – the main flavonoids of *Tragopogon orientalis*, were isolated in a chromatographically pure form and identified as orientin, isoorientin and vitexin.

Luteolin, orientin and isoorientin have already been found by Kroschewsky et al. (1969) in *Tragopogon porrifolius*, *T. mirus*, *T. dubius*, *T. miscellus* and *T. pratensis*; apigenin in *T. pratensis*; vitexin in *T. porrifolius* and *T. mirus*. As can be seen from this, flavonoids can be of taxonomical significance within the genus *Tragopogon* L. The finding of apigenin in *T. orientalis* L. brings this species closer to *T. pratensis* L., but the demonstration of vitexin as one of the major flavonoids of *T. orientalis*, allows the supposition that one of the more dependable chemotaxonomic traits has been found which makes possible the chromatographic differentiation of *T. orientalis* from *T. pratensis* L.

The presence of chlorogenic acid (Kuzmanow et al. 1970) was confirmed and the following acids were shown, in the free and bound forms: homoprotocatechuic, protocatechuic, caffeic, gentysic, p-hydroxybenzoic, m-hydroxybenzoic, p-hydroxyphenylacetic, p-cumaric, o-hydroxyphenylacetic, syringic, ferulic, vanillic, salicylic and four unidentified (A, B, C, D) acids. Most of the listed phenolic acids were found in the herb both in the free and bound states. Only genetic, m-hydroxybenzoic and the unidentified acids B, C, D were found exclusively in the state of being bound with other compounds.

The occurrence of flavonoids and phenolic acids in the herb, *Tragopogon orientalis* L., which have an acknowledged inhibitory or stimulatory effect on the growth of plants, would explain the high cytostatic effect of the ethanol extract (Grzycka et al. 1978, Krzaczek et al. 1981).

The results presented in this study are not a full phytochemical characterstic of the plant, but among the identified compounds, none were found which are considered poisonous, which seems to support data about the beneficial properties of the herb, *Tragopogon orientalis* L. (Schlechtendal et al. 1887, Rutkowska 1984, Włodarczyk 1986).

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Badania fitochemiczne ziela Tragopogon orientalis L. (Asteraceae). 2. Składniki ekstraktu metanolowego

Streszczenie

Z ekstraktu metanolowego ziela kozibrodu wschodniego wydzielono frakcje aglikonów, glikozydów flawonoidowych i kwasów fenolowych. W stanie wolnym stwierdzono luteolinę i apigeninę, wykazano ponadto, że w stanie związanym oprócz luteoliny i apigeniny występuje jeszcze kwercetyna. Zasadniczą część związków flawonoidowych stanowią glikozydy, które na chromatogramach dają 11 plam o żółtej lub brunatnej fluorescencji w UV (360 nm) i dają żółte zabarwienie z $AlCl_3$. Można przypuszczać, że 4 z nich, ulegające hydrolizie są O-glikozydami, a pozostałych 7 to C-glikozyle. Trzy z nich — główne flawonoidy kozibrodu wschodniego — wydzielono i zidentyfikowano jako orientynę, izoorientynę i witeksynę. Przy wyodrębnianiu flawonoidów otrzymano uboczną frakcję, która okazała się mieszaniną glukozy, fruktozy i sacharozy. Potwierdzono obecność kwasu chlorogenowego oraz wykryto w stanie wolnym i związanym kwasy fenolowe: homoprotokatechowy, protokatechowy, kawowy, gentyzowy, p-hydroksybenzoesowy, m-hydroksybenzoesowy, p-hydroksyfenylooctowy, p-kumarowy, o-hydroksyfenylooctowy, syryngowy, ferulowy, wanilinowy, salicylowy i niezidentyfikowane (A, B, C, D). Większość wykazanych kwasów fenolowych znajduje się w zielu w stanie wolnym i związanym, jedynie kwasy gentyzowy, m-hydroksybenzoesowy i niezidentyfikowane B, C, D występują wyłącznie w połączeniach z innymi związkami.