UDC 547.972 : 615.32:582.998.1

FUNDAMENTAL RESEARCH INTO CHEMICAL COMPONENTS OF GEORGIAN FLORA

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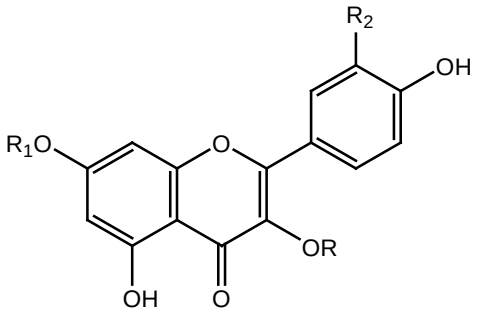
Species of the families Scrophulariaceae, Leguminosae, Urticaceae, Asteraceae and Geraniaceae have been analyzed to identify contents of biologically active substances. Fifty-six phenol compounds have been isolated, and 14 ones found to be structurally new: Flagalosides A-D; Falcosides A-E; kaempferol-3-O-β-D-di-galacto-xyloside; 6‴′-O-[6-O-(4′′-trans-p-methoxy-cinnamoyl-5-hydroxi-aucubigenin-(1→1′)-O-β-D-galactopyranosyl]-6‴′-O-trans-p-methoxy-cinnamoyl-aucubin (laxoside); pelargonidin-3-O-gluco-di-galactoside; pelargonidin-3-O-[(ξ-vanillyl-xylopyranosyl]-ξ-O-xylopyranoside; pelargonidin-3-O-xylopyranoside; 1-O-galloyl-3,6-hexahydroxidiphenyl-β-D-galactopyranoside (Pusilagin); Micranthoside (+) 2R:3R; Neomicranthoside (-) 2S:3S.

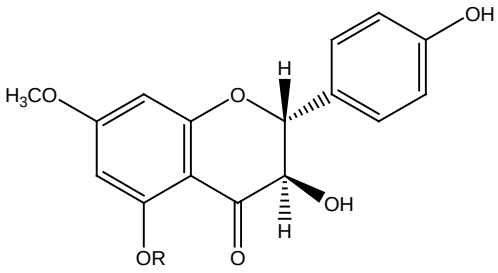
Structures of isolated compounds were established on the basis of their physical, chemical and physical-chemical properties, by studying of products of chemical transformation and by spectral (^{1}H and ^{13}C NMR, HSQC, HMBC, COSY, DEPT, mass-spectroscopy) data.

The new individual compounds displayed interesting biological effect to be promising from medical application standpoint.

Key words: *flavonoids, falcosides, flagalosides, iridoids, laxoside*

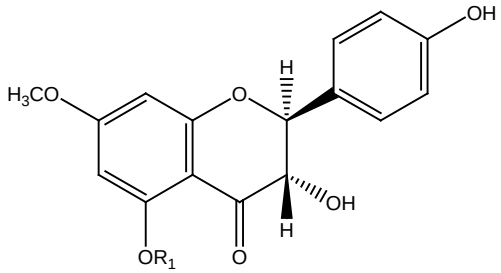
Species of Georgian flora have been notable for their healing properties since ancient times. At present, a special emphasis is laid on analysis of some species of *Scrophulariaceae*, *Leguminosae*, *Urticaceae*, *Asteraceae* and *Geraniaceae families*. Fifty six compounds were isolated by carrying out basic studies into chemical composition of the species. Fourteen compounds of them were structurally new: Flagalosides A-D; Falcosides A-E; kaempferol-3-O-β-D-di-galacto-xyloside; 6‴′-O-[6-O-(4′′-trans-p-methoxy-cinnamoyl-5-hydroxi-aucubigenin-(1→1′)-O-β-D-galactopyranosyl]-6‴′-O-trans-p-methoxy-cinnamoyl-aucubin (laxoside); pelargonidin-3-O-gluco-di-galactoside; pelargonidin-3-O-[(ξ-vanillyl-xylopyranosyl]-ξ-O-xylopyranoside; pelargonidin-3-O-xylopyranoside; 1-O-galloyl-3,6-hexahydroxidiphenyl-β-D-galactopyranoside (Pusilagin); Micranthoside (+) 2*R*:3*R*; Neomicranthoside (-) 2*S*:3*S*. The last two ones are *trans*-type enantiomers of 7-O-methyl-aromadendrin-5-O-β-D-glucopyranoside [1-6]).





Micranthoside

Falcoside C - R = [β-Glcp-(1→ 3)-α–Rhap-(1→ 6)-β–Galp
R_1= Glcp
R_2= OH
Falcoside D - R = [β-Xylp-(1→ 3)-Rhap-(1→ 6)]-Galp
R_1= Rhap
R_2 = OCH_3
Flagaloside C - R= β-Galp-(6→ 1)-α-Rhap-(2→ 1)-Xylp
R_1= H
R_2= OCH_3
Flagaloside D- R= β-Xylp-(2→ 1)-β-Xylp
Pusilaside - R= β–Galp-ξ-β-Galp-ξ-β-Xylp
R_1= R_2= H



Neomicranthoside

R = R_1 = β–D-Glc

Compounds were isolated from dried and crushed raw material through the extraction of 80 % ethanol. The aqueous residue after evaporation was purified with chloroform to get rid of lipophyllic compounds. In some cases, the individual substances were crystallized from the purified aqueous liquid. Micranthoside and Neomicranthoside were isolated in the same manner out of flower extract of Eupatorium Micranthum. After separation of the crystals, an aqueous part was extracted with ethyl acetate. Fractions of phenolic compounds were obtained by evaporating the extragent until a dry residue was obtained. The separation of individual compounds from ethyl acetatic and aqueous fractions were carried out by using silica gel (Kiselgel-60), diaion resin, polyamide and Sephadex LH-20.

Structures of isolated compounds were established on the basis of their physical, chemical and physical-chemical properties, by studying of products of chemical transformation and by spectral (^{1}H and ^{13}C NMR, HSQC, HMBC, COSY, DEPT, mass-spectroscopy) data.

The structure elucidation of the new iridoid glycoside from *Verbascum laxum* is given below. Iridoid glycoside (1) was obtained as light brown crystalls, m.p. 110-114 °C, IR-spectrum (KBr, ν_{max}, cm^{-1}): 3600, 3430 (-OH), 1710, 1708 (C=O), 1645 (C=C), 1604, 1546, 1360 (Ar); UV spectrum (λ_{max}, nm): 206, 218 sh, 225 sh, 290; Mass-spectrum, m/z (I, %): 1011 $[M=H]^+$ (0.8); MS/MS: m/z 691 $[M+H-320]^+$ (0.9) (two molecules of trans-p-methoxy-cinnamic acid), 529 $[M+H-320-162]^+$ (15), 351 $[M+H-320-162-178]^+$ (65).

The molecular ion peak with m/z 1010 $[M+H]^+$. It corresponded to the formule $C_{50}H_{58}O_{22}$. The absorbtion peaks characterising to the enol-ester system of iridoid and aromatic acids at 206, 218 (sh), 225 (sh), 290 nm were visible in the UV-spectrum. The complete assignment of ^{1}H and ^{13}C NMR signals (Tabe 1) and their comparison to the 2D NMR-experiments showed the presence of two iridoid aglycon fragments of trans-*p*-methoxy-cinnamoyl acid. Signals of two anomeric protons at δ_H 5.08 (d, J=7.8 Hz) and δ_H 4.71 (d, J=7.8 Hz), the signals of two couple of methylene at 6′′′′ and 6′ (δ_H 3.71, 3.68; δ_H 3.88, 3.68) also the resonance signals of eight

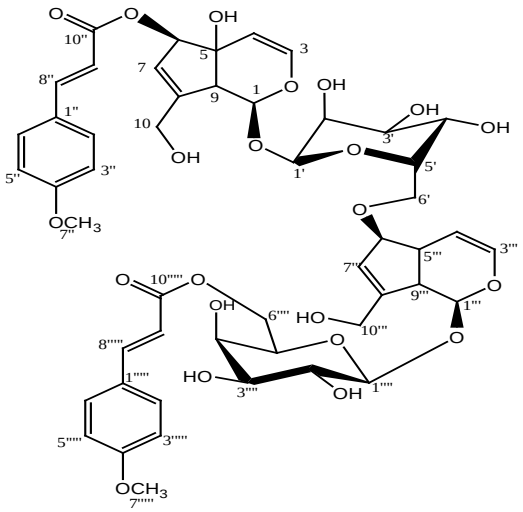
protons at δ_H 3.96 and 3.42 (H-2, 3, 4, 5) indicated the presence of two molecules of carbohydrates. Downfield shift signals of 6′ and 6′′′′ atoms of carbohydrate units suggested the fact of their substitution. The structure of two molecules of trans-*p*-methoxycinnamic acid was established by ^{1}H NMR spectra where there became visible neighboring olefinic protons at δ_H 6.46 and 7.70 (J_{ax}=15.91 Hz), δ_H 6.45 and 7.62; four signals of aromatic protons δ_H 6.99 (2H), 7.60 (2H), 6.81 (2H), 7.30 (2H) and, finally, two signals of methoxyl groups at δ_H 3.86 and 3.83. Signals at 55.6 and 55.9 ppm in the ^{13}C NMR spectrum reaffirmed the presence of methoxyl groups.

One of the two fragments of irodoid glycoside 1 showed characteristic signals δ_H 6.38 (d, J=6.5 Hz, H-3) and δ_H 5.16 (d, J=6.5 Hz, H-4), 5.93 (br. s., H-7) of two ortho- and one olefinic proton in ^{1}H NMR spectrum. Also, proton signals at δ_H 4.96 (d, J=4.2 Hz, H-1), 4.52 (br. s., H-6) indicated the substitution at position 1 and 6. Six signals of tertiary carbon at δ 97.9 (C-1), 140.5 (C-3), 103.9 (C-4), 87.9 (C-6), 125.4 (C-7), 46.7 (C-9) and one signal of secondary carbon at δ 60.7 (C-10) respectively became apparent in the DEPT. A signal of quaternary carbon at δ_C71.9 showed the presence of hydroxyl group in the position C-5 [7]. Acylation at C-6 position was confirmed by the downfield chemical sift (δ_H4.52) of H-6 proton. Proceeding from the NMR spectral data and literature sources, iridoid was specified as 5-hydroxyaucubigenin [8, 9].

Chemical shifts of the second part of molecule slightly differed from the first one (Table 1); there was just a single difference: presence of H-5′′′proton signal at δ_H 2.80.

Interconnection between the identified structures (2 molecules of iridoid, trans-p-methoxycinnamic acid and carbohydrate) was established by HMBC experiment. Correlations were clearly displayed between δ_H4.52 (H-6) and δ_C 168.7 (C-10′′); δ_H4.71 (Gal H-1′) and δ_C 97.9 (C-1); δ_H4.96 (H-1) and δ_C 98.5 (C-1′); δ_H4.50 (H-6′′′) and δ_C 62.7 (C-6′); δ_H4.99 (H-1′′′) and δ_C 100.0 (C-1′′′′); δ_H3.68 (H-6′′′′) and δ_C 172.2 (C-10′′′′′).

It should be noted that the spectral data were corroborated by the results of chemical transformation. Acyd hydrolysis of 1 gave D-galactose, D-glucose, as well as a product with typical reaction for iridoids . Trans–p- methoxy-cinnamic acid and less polar glycoside were formed by alkaline hydrolysis of 1. Acid hydrolysis of less polar glycoside provided D-Glucose and D-Galactose.



Laxoside

Based on the results of spectral analysis and chemical transformation. Comparison of the literature data with the structure 1 made it possible to identify 6′′′′-O-[6-O-(4′′-trans-p-methoxy-cinnamoyl-5-hydroxi-aucubigenin-(1→1′)-O-β-D-galactopyranosyl]-6′′′′-O-trans-p-methoxy-cinnamoyl-aucubin. The substance with the identical structure has not been described in literature: the point is about a new compound termed as laxoside.

Table 1. ^{1}H and ^{13}C NMR spectral data of 1 (500 MHz, CD_3OD, δ, ppm, J/Hz)

C	δ_C	δ_H	C	δ_C	δ_H	Aucubigenin
1	97.9	4.96 (*d*, J=4.2)	1′′′	98.5	4.99 (*d*, J=6.0)	4.82 (*d*, J=1.9)
3	140.5	6.38 (*d*, J=6.5)	3′′′	143.9	6.44 (*d*, J=5.6)	5.84 *br.s.*
4	103.9	5. 16 (*d*, J=6.5)	4′′′	106.9	5. 17 (*d*, J=5.6)	4.92 (*dd*, J=6.0; 3.5)
5	71.9	-	5′′′	46.2	2.80 *m*	2.50 *m*
6	87.9	4.52 *br.s.*	6′′′	89.4	4.50 *m*	4.70 *m*
7	125.4	5.93 *br.s.*	7′′′	126.2	5.90 *br.s.*	5.84 *br.s.*
8	148.5	-	8′′′	149.9	-	-
9	46.7	2.96 (*d*, J=4.2)	9′′′	47.2	2.98 (*dd*, J=6.0; 7.2)	2.80 *m*
10 a	60.7	4.22 (*d*, J=14.0)	10′′′a	61.0	4.25 (*d*, J=14.4)	4.25 *br.s.*
10 b		4.40 (*d*, J=14.0)	10′′′b		4.47 (*d*, J=14.4)	
1′	98.5	4.71 (*d*, J=7.8)	1′′′′	100.0	5.08 (*d*, J=7.8)	
2′	73.3	3.62 (*dd*, J=9.8; 7.8)	2′′′′	74.5	3.68 (*dd*, J=9.0; 7.8)	
3′	76.2	3.42 (*dd*, J=9.8; 3.4)	3′′′′	77.7	3.48 (*dd*, J=9.0; 9.0)	
4′	70.1	3.90 (*dd*, J=3.4; 1.1)	4′′′′	71.7	3.96 (*dd*, J=9.0; 9.0)	
5′	76.7	3.50 m	5′′′′	77.7	3.48 m	
6′α	62.7	3.68 (*dd*, J=11.8; 5.9)	6′′′′α	63.0	3.68 (*dd*, J=12.0; 4.5)	
β		3.88 (*dd*, J=11.8; 7.0)	β		3.71 (*dd*, J=12.0; 2.0)	
1′′	127.6	-	1′′′′′	129.2	-	
2′′	130.0	7.60 (*d*, J=8.4)	2′′′′′	131.5	7.30 (*d*, J=8.4)	
3′′	114.0	6.99 (*d*, J=8.4)	3′′′′′	115.5	6.81 (*d*, J=8.4)	
4′′	162.0	-	4′′′′′	163.4	-	
5′′	130.0	6.99 (*d*, J=8.4)	5′′′′′	115.5	6.81 (*d*, J=8.4)	
6′′	113.9	7.60 (*d*, J=8.4)	6′′′′′	131.3	7.30 (*d*, J=8.4)	
8′′α	145.6	6.46 (*d*, J=16.0)	8′′′′′	115.0	6.45 (d, J=16.0)	
9′′β		7.70 (*d*, J=16.0)	9′′′′′	146.1	7.62 (d, J=16.0)	
10′′	168.7	-	10′′′′′	172.2	-	
7′′-OCH_3	55.9	3.83 *s*	7′′′′′-OCH_3	55.6	3.86 *s*	

The new individual compounds displayed interesting biological activities to be promising from medical point of view.

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ФУНДАМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ ХИМИЧЕСКИХ КОМПОНЕНТОВ РАСТИТЕЛЬНОСТИ ГРУЗИИ

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Виды семейств Scrophulariaceae, Leguminosae, Urticaceae, Asteraceae, Geraniaceae были исследованы на содержание биологически активных веществ. Из изученных объектов выделены и охарактеризованы 56 фенольных соединений; 14 из них оказались структурно новыми: флагалозиды A-D; фалкозиды A-E; кемпферол-3-O-β-D-ди-галакто-ксилозид; 6′′′-O-[6-O-(4′′-trans-p-метокси-циннамоил-5-гидрокси-аукубигенин-(1→1′)-O-β-D-галактопиранозил]-6′′′-O-trans-n-метокси-циннамоил-аукубин (лаксозид); пеларгонидин-3-O-глюко-ди-галактозид; пеларгонидин-3-O-[(ξ-ванилил-ксилопиранозил]-ξ-O-ксилопиранозид; пеларгонидин-3-O-ксилопиранозид; 1-O-галлоил-3,6-гексагидроксидифенил-β-D-галактопиранозид (пусилагин); микрантозид (+) 2R:3R; неомикрантозид (-) 2S:3S. Структуры выделенных веществ установлены на основании их физико-химических свойств, изучением продуктов химического превращения и данными спектров ИК, УФ, ^{1}H и ^{13}C ЯМР, масс, а также методами корреляционной спектроскопии HSQC, HMBC, DEPT, COSY.

Ключевые слова: *флавоноиды, фалкозиды, флагалозиды, иридоиды, лаксозид*.

Поступила в редакцию 19.11.2016.