ANTHRACENE DERIVATIVES IN SOME SPECIES OF *RUMEX* L. GENUS

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ABSTRACT

Eight anthracene derivatives (chrysophanol, physcion, emodin, aloe-emodin, rhein, barbaloin, sennoside A and sennoside B) were signified in six species of *Rumex* L genus: *R. acetosa* L., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. and *R. obtusifolius* L. For the investigations methanolic extracts were prepared from the roots, leaves and fruits of these species. Reverse Phase High Performance Liquid Chromatography was applied for separation, identification and quantitative determination of anthracene derivatives. The identity of these compounds was further confirmed with UV-VIS. Received data were compared.

The roots are the best organs for the accumulation of anthraquinones. The total amount of the detected compounds was the largest in the roots of *R. confertus* (163.42 mg/g), smaller in roots *R. crispus* (25.22 mg/g) and the smallest in roots of *R. hydrolapathum* (1.02 mg/g).

KEY WORDS: *Rumex sp.*, roots, fruits, leaves, anthracene derivatives, RP-HPLC.

INTRODUCTION

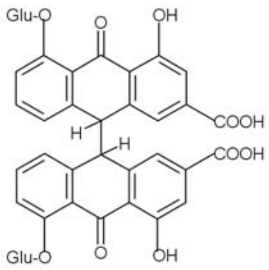
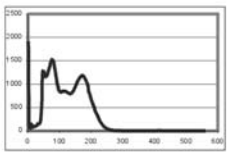
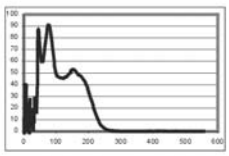
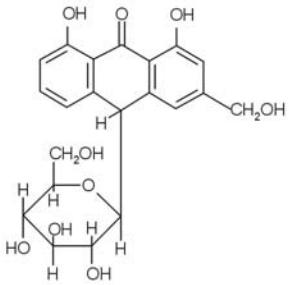
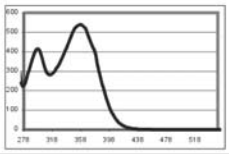
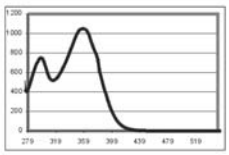
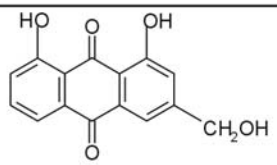
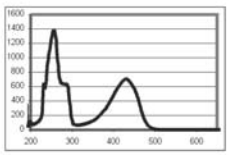
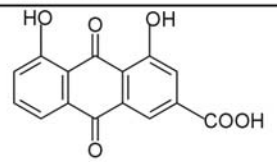
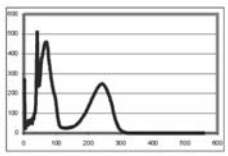
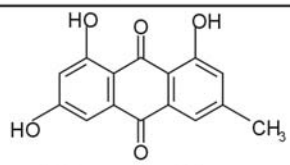
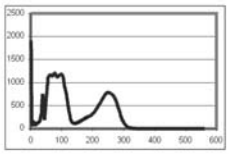
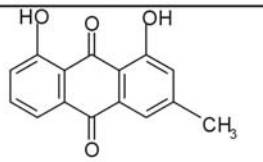
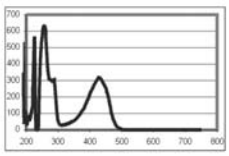
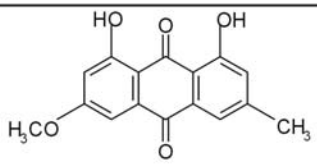
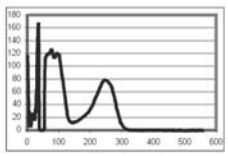
The species belonging to the *Rumex* L. genus are widespread in the world. Twenty five of these species grow in Poland. The roots from five of them: *R. alpinus* L., *R. crispus* L., *R. hydrolapathum* Huds., *R. obtusifolius* L. and *R. patientia* L., which deliver material named „Radix Lapathi", are used in phytotherapy due to their laxative properties. Other two species (*R. acetosa* L. and *R. acetosella* L.) are components of our diet (Miłkowska et al. 1997; Wegiera and Smolarz 2005).

The latest research report on antioxidant, antibacterial, antiviral, antifungal, antimutagenic, antitumor and immunosuppressive activities of some species of *Rumex* L. genus (Lee et al. 2005; Wegiera and Smolarz 2005; Yildirim et al. 2001).

The present study comprises phytochemical investigations on anthracene derivativatives of six species of *Rumex* L.

Derivatives of anthracene as secondary metabolites important for these plants have been chromatographically detected and isolated. There are the following compounds: chrysophanol, emodin, physcion and their 8-O-β-D-glucopyranosides, physcion-1-O-β-D-glucopyranoside, aloe-emodin as aloe-emodin ω-acetate, emodin-anthrone, physcion-anthrone, rhein, nepodin, nepodin-O-β-D-glycoside and 1,8-dihydroxyanthraquinone from *R. acetosa* (Dedio 1973; Demirezer and Kuruuzum 1997; Fairbairn and El-Muhtadi 1972a; He et al. 1981; Hsiao et al. 1980; Kato and Morita 1987; Sharma and Ragaswami 1977; Tamano and Koketsu 1982; Varma et al. 1984); chrysophanol, emodin, aloe-emodin, nepodin, rumicin and rhein from *R. acetosella* (Dedio 1973; Fairbairn and El-Muhtadi 1972a; Hegnauer 1973; Kaczmarek and Urszulak 1964; Martinod et al. 1978; Mowszowicz 1976); chrysophanol, physcion, emodin, aloe-emodin, nepodin, nepodin-1-O-β-glycoside, chrysophanein, frangulin and rhein from *R. confertus* (Bagrii and Kurmaz 1966; Broda and Mowszowicz 1996; Dedio 1973; Fairbairn and El-Muhtadi 1972a; Hegi 1981; Hoppe 1975; Kaczmarek and Urszulak 1964; Sayed et al. 1974; Tétényi 1970); chrysophanol, physcion, emodin and their glycosides, rhein, nepodin, nepodin-1-O-β-D-glycoside, 1.5-dihydroxyanthraquinone, oxymethyloanthraquinone and glucofranguline B from *R. crispus* (Dedio 1973; Demirezer 1994; Fairbairn and El-Muhtadi 1972a; Gunaydin et al. 2002; He et al. 1981; Hegi 1981; Kaczmarek and Urszulak 1964; Leveau and Durand 1969; Midiwo and Rokunga 1985; Sayed et al. 1974; Tétényi 1970); chrysophanol, chrysophanol-anthron, physcion, physcion-anthron,

TABLE 1. Structure, UV-VIS spectra and R_T-values of anthracene derivatives identified in the *Rumex* species.

Name	Chemical name	Chemical structure	Spectra UV-VIS	Retention time
sennoside B	rhein-dianthrone D-glycoside (mezo)			4.0-4.6
sennoside A	rhein-dianthrone D-glycoside (R+)			5.9-6.3
barbaloin (aloin A and B)	aloe-emodin-anthrone C-glycoside			9.3-9.7
				10.1-10.4
aloe-emodin	1,8-dihydroxy-3- -hydroxymethyl anthraquinone			19.6-20.0
rhein	1,8-dihydroxy-3- -carboxyanthraquinone			24.1-24.9
emodin	1,6,8-trihydroxy-3- -methylanthraquinone			30.7-31.2
chrysophanol	1,8-dihydroxy-3- -methylanthraquinone			32.9-33.3
physcion	1,8-dihydroxy-3- -methyl-6- -methoxyanthraquinone			37.0-37.5

aloe-emodin, emodin and chrysophanol-, physcion- and emodin-glycosides from *R. hydrolapathum* (Dedio 1973; Fairbairn and El-Muhtadi 1972a; Hegi 1981; Labadie et al. 1972); emodin, chrysophanol, physcion, aloe-emodin, rhein, nepodin, frangulin-emodin, frangulin-emodin-glycoside and nepodin-8-glycoside from *R. obtusifolius* (Arellano 1998; Brazdova et al. 1969; Dedio 1973; Fairbairn and El-Muhtadi 1972a, b; He et al. 1981; Hegi 1981; Hsiao et al. 1980; Hoppe 1975; Sayed et al. 1974; Kaczmarek and Urszulak 1964).

However, there are no reports on comparative investigations of these therapeutically active compounds in the roots, leaves and fruits of the above mentioned species.

MATERIAL AND METHODS

Plant material

Fruits, leaves and roots of six species from *Rumex* L. genus: *R. acetosa* L., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. and *R. obtusifolius* L. were used in this study.

The plant materials were collected from different places: near Sandomierz and Nisko in July 2003; near Słupia Nadbrzeżna and around Lublin in June 2005.

Chemicals and standards

Methanol (HPLC grade) and glacial acetic acid (analytical-reagent grade), were purchased from Polish Factory of Chemicals (POCh Gliwice, Poland). Water was purified on a Milli-Q system from Millipore (Millipore, USA).

Standards are as follows: chrysophanol (Aldrich), physcion (Roth Karlsruhe), emodin (Carl Roth Gmbh Sigma), aloe-emodin (Roth Karlsruhe), aloin A and aloin B mixture (Carl Roth Gmbh Sigma), rhein (Carl Roth Gmbh Sigma), sennoside A (Roth Karlsruhe), sennoside B (Roth Karlsruhe).

In this investigation we used 0.2-0.002% methanolic solutions of these standards.

Extraction

The air-dried and powdered plant materials (4 g) from fruits, leaves and roots of each of the taxons were extracted with 40 ml 80% aq. methanol and three times with 30 ml 80% aq. methanol (15 minutes of each extraction) in supersonic water bath in room temperature. The combined extracts were evaporated from the solvent under reduced pressure at 40˚C to 4 ml and subjected to further chromatographic investigations. The rest of dried methanol extracts has been stored in the refrigerator.

RP-HPLC analysis of the extracts

RP-HPLC measurements were performed on Dionex liquid chromatograph (Dionex Corp., Sunnyvale, CA, USA) consisting of: chromatography enclosure (LC20) equipped with Rheodyne automated injection valve to which 25 µl sample loop was connected; gradient pump (GP50); absorbance detector (AD25); photodiode array detector (PDA100). All analyses were under control of PeakNet6 data acquisition system.

Chromatographic separations were carried out at 25˚C using Prodigy ODS-3 column (5 µm, 250×4.6 mm I.D.) (Phenomenex, Torrance, CA, USA) and a security guard column of the same factory. The column and the guard column were placed in the oven and the analyses were performed at 30˚C (Column Thermostat, JetStream II Plus, Knauer, Warsaw, Poland).

The chromatographic analyses were performed in gradient elution conditions using as solvent A – aqueous CH_3COOH solution containing 5 ml of glacial acetic acid per litre and as solvent B – methanol. The following gradient of mobile phase (at a flow of 1 ml/min) was applied: linear increase of B from 45 to 85% (0-30 min), next 85% B during 10 min and finally linear decrease of B from 85 to 45% during 2 min. In the end the column was conditioned using the mobile phase containing 45% B (5 min). Chromatograms were monitored by the AD25 detector at 254 nm and by the PDA detector and the absorbance spectra (190-750 nm) were collected continuously during the course of each run.

RESULTS AND DISCUSION

In this study we used Reversed Phase High Performance Liquid Chromatography to separate and identify anthracene derivatives in methanolic extracts from the leaves, roots and fruits from six species belonging to *Rumex* L. genus. The mobile phase used for RP-HPLC separation of the detected compounds in extracts was optimized experimentally. The retention times of the peaks in the samples were compared with authentic reference compounds. The identity of compounds on chromatogram was confirmed by UV-VIS spectra (Table 1).

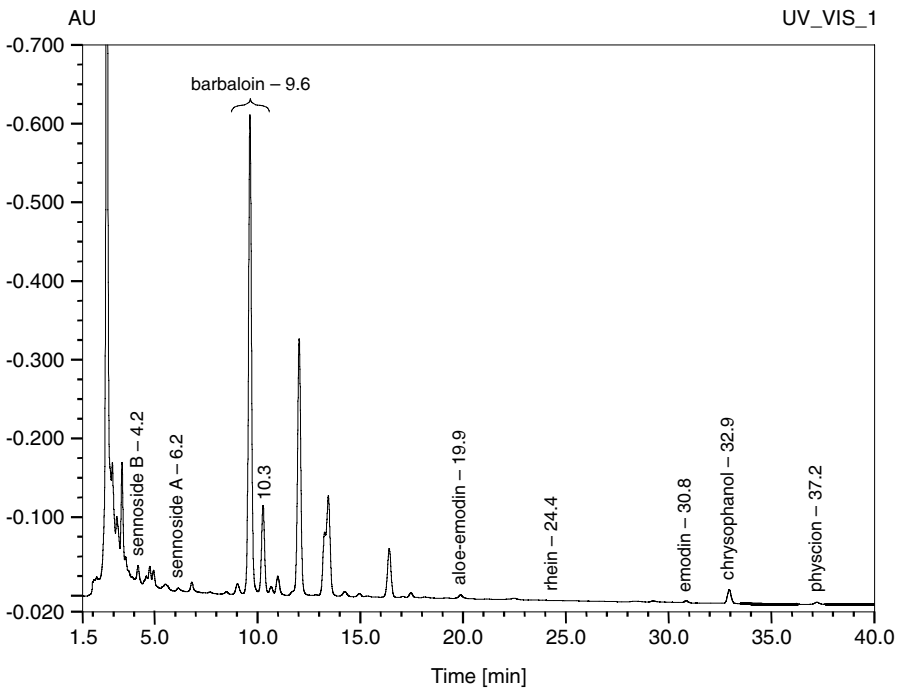


Fig. 1. Chromatogram (RP-HPLC) obtained from methanolic extracts from the leaves of *R. confertus*.

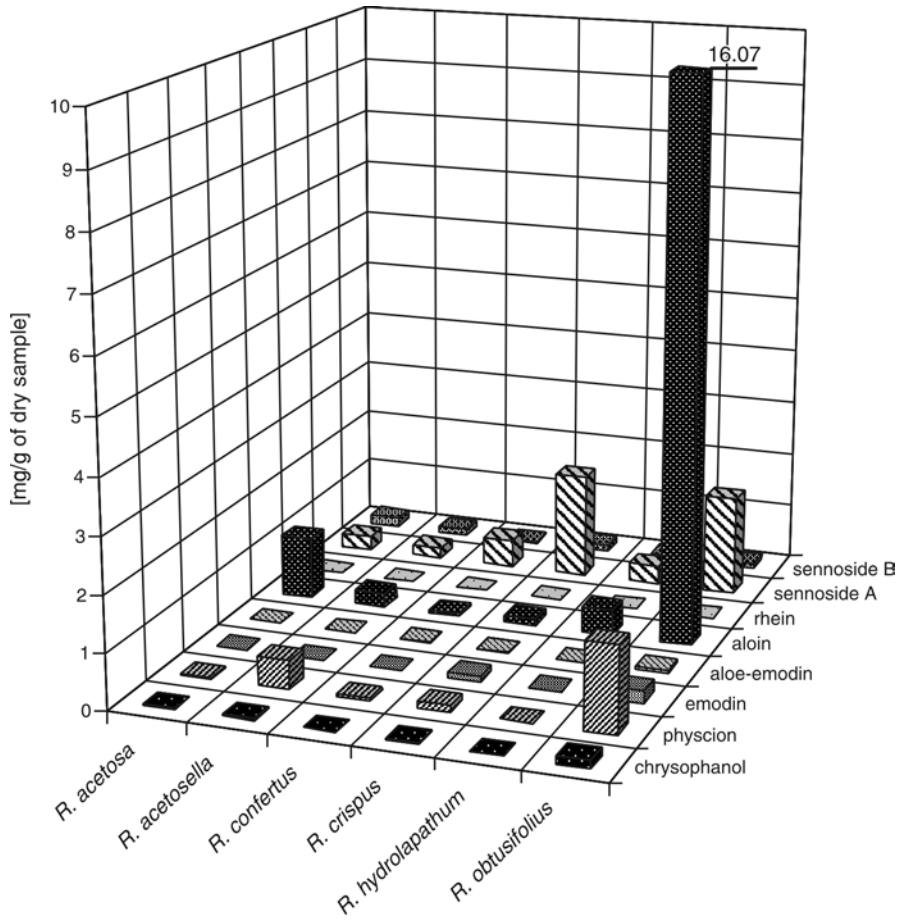


Fig. 2. The content and distribution of anthracene derivatives obtained from the leaves of different species of *Rumex* genus (in mg/g of dry sample).

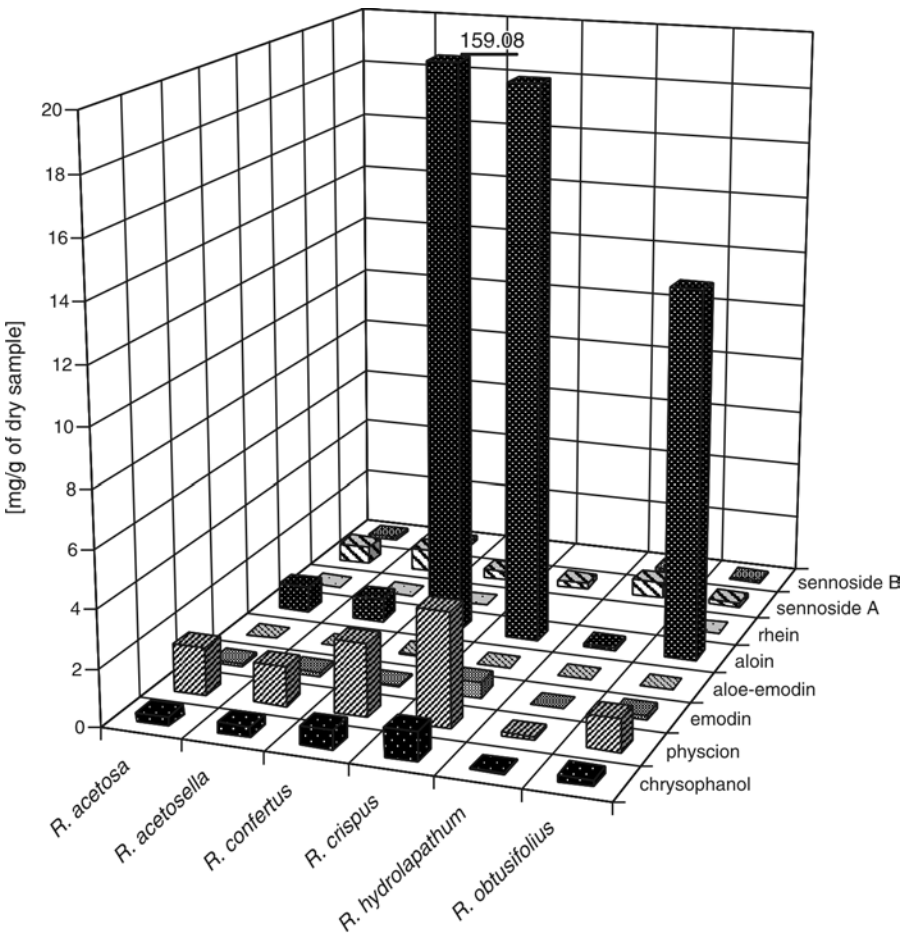


Fig. 3. The content and distribution of anthracene derivatives obtained from the roots of different species of *Rumex* genus (in mg/g of dry sample).

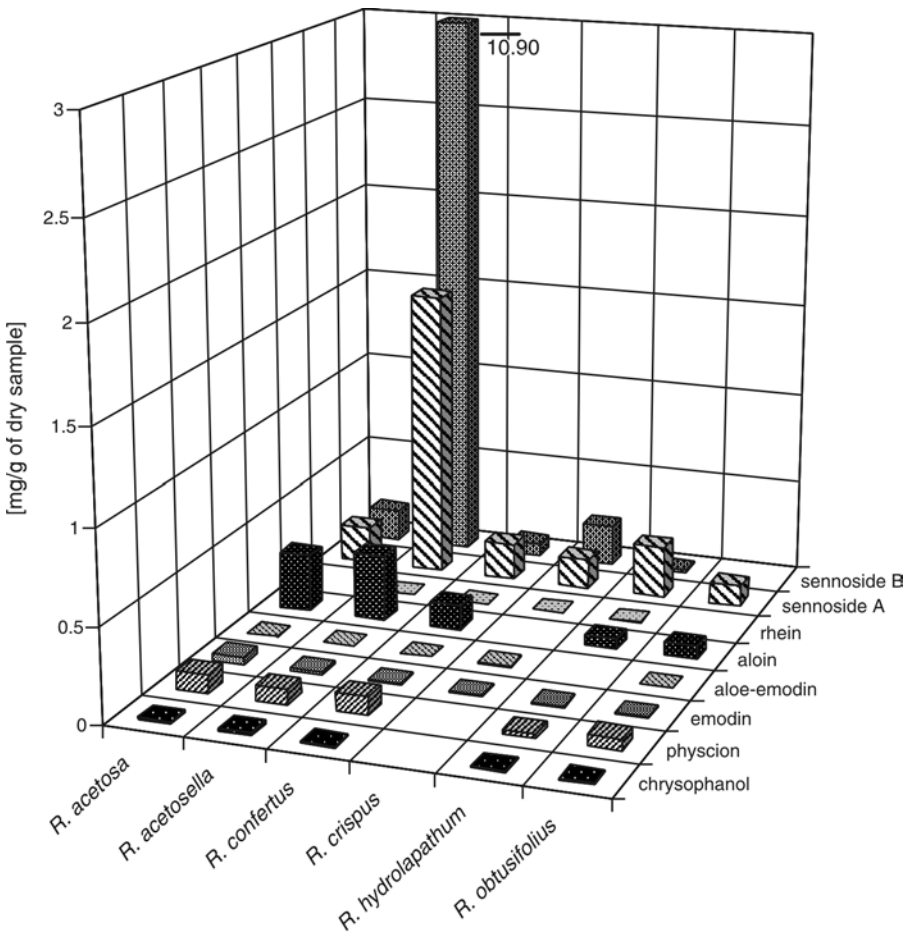


Fig. 4. The content and distribution of anthracene derivatives obtained from the fruits of different species of *Rumex* genus (in mg/g of dry sample).

The qualitative investigation showed, that the detected compounds are widespread in genus *Rumex* L. The leaves of the investigated species contain a complete set of the identified compounds (Fig. 1 and 2), whereas sennoside B is missing in the roots of *R. crispus* and rhein is absent from the roots of *R. hydrolapathum* and *R. crispus* (Fig. 3). As is shown in Figure 4, only chrysophanol, physcion and barbaloin were not detected in the fruits of *R. crispus*, rhein and sennoside B are not present in fruits of *R. obtusifolius* and aloe-emodin has not been found in fruits of *R. hydrolpathum*. Results from quantitative analysis of anthracene derivatives are summarised in Table 2. The total content of the detected compounds ranged from 0.26 to 12.93 mg/g in fruits; from 0.67 to 19.91 mg/g in leaves and from 1.02 to 163.42 mg/g in dry roots.

This data show that in most cases the roots are the richest in anthracene derivatives whereas fruits (withouth *R. acetosella*) are the poorest.

The major identified compound in the roots of *R. confertus*, *R. crispus* and *R. obtusifolius* is barbaloin, whose contents in the mentioned species are: 159.08 mg/g, 19.44 mg/g, 12.94 mg/g respectivelly. In this paper we designate barbaloin the sum amount of two isomers: aloin A and aloin B. Large concentration of barbaloin was detected in the leaves of *R. obtusifolius*, too. The fruits of *R. acetosella* are characterized by high concentrations of sennoside A and sennoside B. Rhein is present in the investigated raw materials in trace amounts. Very small amounts of chrysophanol, aloe-emodin and emodin were observed in all parts of the investigated species, whereas the concentration of physcion ranges between 0.002 mg/g in leaves of *R. hydrolapathum* and 3.98 mg/g in roots of *R. crispus*.

TABLE 2. Distribution of investigated anthracene derivatives in different taxons of *Rumex* L. (mg/g of dry samples).

No.	Species	Fruits	Leaves	Roots	Total content of investigated compounds [mg/g]
1	*R. acetosa*	0.83	1.59	3.76	6.18
2	*R. acetosella*	12.93	1.10	3.66	17.69
3	*R. confertus*	0.52	0.67	163.42	164.61
4	*R. crispus*	0.39	2.40	25.22	28.01
5	*R. hydrolapathum*	0.39	0.89	1.02	2.30
6	*R. obtusifolius*	0.26	19.91	14.71	34.88

The anthraquinones that we identified for the first time are as follows: chrysophanol from fruits and leaves of *R. acetosella*; physcion from fruits and leaves of *R. acetosella*; emodin from fruits of *R. crispus*, fruits and leaves of *R. acetosella*; aloe-emodin from leaves of *R. acetosella*, fruits and leaves of *R. confertus*, *R. hydrolapathum*, *R. obtusifolius* and all parts of *R. crispus*; leaves and roots of *R. crispus* and all parts of *R. acetosa*, *R. acetosella*, *R. confertus*, *R. hydrolapthum* and *R. obtusifolius*; rhein from leaves of *R. crispus* and roots of *R. hydrolapathum*; sennoside A from leaves and roots of *R. crispus*, fruits and roots of *R. acetosella*, *R. hydrolapathum* and all part of *R. acetosa*, *R. confertus* and *R. obtusifolius*; sennoside B from roots of *R. acetosa* and *R. acetosella*, fruits and roots of *R. aquaticus*, leaves and roots of *R. confertus* and *R. obtusifolius*.

CONCLUSIONS

From the investigation carried out by RP-HPLC method it follows that the investigated species from Rumex L. genus contain aglicones and glucosides anthraquinones and some other anthracene derivatives. The richest in the detected compounds is *R. confertus*.

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