Phenolic compounds of *Rumex* L: aerial part fractions and essential oil results of in vitro screening for antibacterial and antifungal activity

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Abstract: Continuing studies on the chemical composition of *Rumex pamiricus* Rech. f. led to the isolation of anthraquinones: chrysophanol, emodin and rhein from the plant root extract using column chromatography on KSK silica gel. The structure of chrysophanol, emodin and rhein (Figure 2) was established on the basis of the analysis of the data of MS, 1H, and 13C NMR spectra, and of the DEPT, HSQC and HMBC experiments. The 60% acetone extracts of *Rumex conglomeratus* Murray was successively separated by MCI gel CHP 20P, and Toyopearl HW 40F column chromatography to yield two compounds. Their structures were elucidated by spectroscopic analyses as: rosmarinic acid and caffeic acid (Figure 2). All compounds were separated from *Rumex pamiricus* and *Rumex conglomeratus* plants for the first time. The ester oil from the aerial parts of *Rumex confertus* Willd. and *Rumex pamiricus* were tested in vitro for antibacterial and fungal activity in the fractions of gasoline, chloroform, ethyl acetate and butanol. As a result, it was found that the aerial part of the *Rumex pamiricus* plant, chloroform and ethyl acetate fractions have antibacterial activity against fungi and gram-positive bacteria.

Keywords: *Polygonaceae* L., *Rumex pamiricus* Rech. f., *Rumex conglomeratus* Murray., *Rumex confertus* Willd., sorrel, dock, phenolic compounds, in vitro, antibacterial, antifungal, extract, fraction

Introduction

Herbal remedies play an important role in modern medicine and it appears feasible that the compounds from herbs can be helpful in prevention or treatment of different diseases. The interest of natural drugs as adjunctive therapy for acute and chronic diseases has grown significantly in the recent years. The phenolic compounds are of great importance in terms of various biological activities in the research work in this area. Phenolic compounds are probably the most explored natural compounds due to their potential health benefits as demonstrated in a number of studies. Continuing these studies, we began to study the phenols of the plants *Rumex pamiricus*, *Rumex confertus* and *Rumex conglomeratus* in order to isolate natural compounds from local plant raw materials and study their biological activity (Figure-1).

The Genus *Rumex*: The name *Rumex* derived from the Latin word for dart, alluding to the shape of the leaves. It is the largest genus of family *Polygonaceae* [1]. Plants of the genus *Rumex* L. (sorrel, dock) are widely distributed in North America, Central and Eastern Europe, Kazakhstan, the Far East, and partly in the Caucasia, Russia, and East Asia [2-5]. This genus includes more than 250 species distributed worldwide. 16 species grow in Uzbekistan. *Rumex pamiricus*, *Rumex Confertus*, and *Rumex conglomeratus* are the most common species among them [6-8]. Since ancient times *Rumex L.* species have been well known for their use in

Comment [PB2]: Only write, the genus Rumex derived from…

Comment [PB3]: Family name should not be italic traditional medicine, due to therapeutic efficacy and various biological activities [9]. The herb *Rumex pamiricus* belongs to the family of *Polygonaceae* which is widespread in Central Asia (Pamir-Alay,Tian Shan, Dzungarian Alatau), Kashgaria. One of the most common types of *Rumex* in Uzbekistan (Samarkand and Kashkadarya regions). It grows along wet mountain meadows, along the banks of mountain rivers and lakes. Perennial herbaceous plant reaching 60–100 cm in height [10]. Since ancient times, concoction or tea from various parts of this herb has been used in folk medicine to treat diarrhea, dysentery, stercoral ulcer, as apetizer, analeptic medicine for lever, heart, as antihaemorrhagic, to treat hepatitis, fever and other diseases [4,11]. The consumption of wild edible plants has been an integral part of human nutrition and traditional medicine since ancient times [12,13]. Thus, researchers began to pay more attention to wild flora. Plants of the genus *Rumex* are no exception. In addition, this plant genus is known as a super-producer of secondary phenolic compounds [14]. Wild plants are known to be a good source of primary nutritional compounds (proteins, fats, sugars, vitamins, and minerals) [15]. Wild plants contain various biologically active components that demonstrated health benefits effects (flavonoids, phenolic acids, anthocyanins, tannins, terpenoids, steroidal saponins, glucosinolates, and so on) [13]. This shows their potential as nutritional supplements, feed additives, and medicinal agents [13,16]. Among wild plants, *Rumex* plants have a great potential. They are already widely used as food, fodder, melliferous, and medicinal plants [5,17,18]. In some countries, the leaves of *Rumex* plants (such as R. *vesicarius*, R. *acetosella*, R. *abyssinicus*, R. *crispus*, R. *induratus*, R. *sanguineus*, R. *obtusifolius*, R. *tuberosus*, R. *thyrsiflorus*, and R. *acetosa*) are used for food, mainly as salads [19,20]. According to the literature informations, several *Rumex* species are included in the pharmacopoeias of various countries. For example, *Rumex crispus* is listed in the American Herbal Pharmacopoeia as a general detoxifier and an agent for skin treatment [21]. The State Pharmacopoeia of the Russian Federation includes the roots of *Rumex confertus* Willd. as a herbal medicine, which is used in the treatment of liver diseases, dysentery, pulmonary, and uterine bleeding, as well as a laxative [22,23]. Plants of the genus *Rumex* have traditionally been used as edible or medicinal plants in various regions of the world. However, today, their biotechnological potential is becoming evident, and these species can act as a resource of biologically active substances. The *Rumex* plants are abundant, undemanding, gain phytomass easily, and have a short vegetative cycle (and, as a consequence, can reproduce frequently throughout the year), thus they have a real advantage among wild plants of the temperate zone. It should also be noted that *Rumex* species have a high potential for regrowth after injury [23-25].

1 2 3

Comment [PB4]: Check spacing

Comment [PB5]: Complete sentence…one of the most common type of Rumex is present in Uzbekistan

Comment [PB6]: Check spelling 'appetizer'

Comment [PB7]: To correct spelling 'antihemorrhagic'

Comment [PB8]: Add and source of secondary metabolites such as flavonoids, phenolic acids,…….

Comment [PB9]: information

Comment [PB10]: make sentence more effective like 'Rumex plants have….

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Figure 1. *Rumex pamiricus* Rech. f.(1), *Rumex confertus* Willd (2), *Rumex conglomeratus* Murray (3).

Experimental part

Plant material: The aerial parts (during the flowering period on May 2020) and roots (on August 2020) of the plants were collected from Botanic Garden, Tashkent, Uzbekistan.

Extraction and methods: The roots of the herb ***Rumex Pamiricus*** dried at room temperature, in shade. The pounded herb roots were first subjected to extraction in chloroform, then three times in 70% acetone hydrous solution. The acetone extract was distilled under vacuum, the remaining water solution was subjected to extraction with ethyl acetate. Ethyl acetate extracts were collected and were dehydrated by adding anhydrous salt Na2SO4. The dehydrated extract was filtered, its concentartion increased under vacuum, the total phenols were precipitated by adding pure hexane to the condensed extract. The created precipitate was washed, and filtered and the extracted total phenols of chloroform and ethyl acetate fractions constituted 3.4% of the herb dry weight.

Results: The roots are the best organs for the accumulation of anthraquinones [26]**.** The chloroform fraction subjected with column chromatography on KSK silica gel, eluted with a mixture of extraction benzene – ethyl acetate: (50:1, 40:1, 30:1, 20:1 and 10:1). The structure of chrysophanol, emodin and rhein (Figure 2) was established on the basis of the analysis of the data of MS, 1H, and 13C NMR spectra, and of the DEPT, HSQC and HMBC experiments.

Discussion: The anthraquinones that we identified for the first timeare chrysophanol, emodin and rhein have been isolated from other types of *Rumex* in studies before us. According to the literature: chrysophanol from fruits and leaves of *R. acetosella*, emodin from fruits of *R. crispus*, fruits and leaves of *R. acetosella*, rhein from leaves of *R. crispus* and roots of *R. hydrolapathum* [26], chrysophanol, emodin, rhein from *R. acetosa* [27-29], chrysophanol, emodin, rhein from *R. crispus* [30] and et all . The novelty of our work is that we first isolated these compounds from the plant *Rumex pamiricus*.

Comment [PB12]: Write willd as 'Willd.'

Comment [PB13]: Add picture of the collected sample

Comment [PB14]: Na_2SO4

Comment [PB15]: Concentration

Comment [PB16]: ^{1H} and ^{13}C

Comment [PB17]: Write full name of these methods

Comment [PB18]: First person pronouns should be avoid

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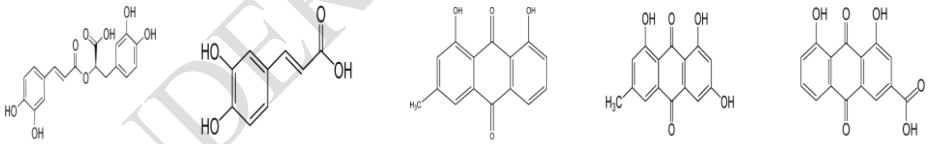
Comment [PB20]: Read sentence carefully and make it complete

Comment [PB21]: What…et all already reported from these plants part

Extraction and methods: The ***Rumex conglomeratus*** (20 kg) were extracted with 60% acetone four times at room temperature, each one time one week. After filtration and condensed to little volume by Rotary evaporation machine, the concentrated liquid (most are water) was fractionated by EtOAc (1/4 volume of water) five times, to give two parts, water layer and EtOAc layer. After filtration, the EtOAc layer was dried by Rotary evaporation machine to give 72.4 g of the EtOAc sample.

Results: The EtOAc fraction was then subjected to Diaion HP 20SS column chromatography with MeOH containing increasing proportions of water (6 cm i.d.- 90 cm, 10-100%, 10% stepwise elution, each 3L), to afford seven fractions E1-E7. Fraction E-1 was further fractionated by Sephadex LH-20 column chromatography (7 cm i.d.-110 cm) with 10-100% MeOH(10% stepwice elution, each 3-L) and give to six fractions E1-1-E1-6. And the fraction E1-2 were separated by column chromatography using the MCI gel CHP 20P (3 cm i.d.- 40 cm) with 10-100% MeOH (5% stepwise elution, each 300 ml) to yield caffeic acid (Figure 2) (0.092 g). Fraction E1-5 was successively applied to a Toyopearl HW 40F column chromatography (2 cm i.d.- 28 cm) with 5-100% MeOH (5 stepwise elution, each 150 ml) to give rosmarinic acid (Figure 2) (0.252 g).

Discussion: The novelty of our work is that we first isolated caffeic acid and rosmarinic acid from the plant *Rumex conglomeratus.* Rosmarinic acid and caffeic acid have been isolated from other species and families in previous studies and their properties have been studied. Rosmarinic acid was first isolated in 1958 by Scarpati and Oriente from *R. officinalis* [31], from *Melissa officinalis* L.[32], from *Hyptis atrorubens* Poit [33,34], [35], caffeic acid [36,37,38,39] and et all.



1 2 3 4 5

Figure 2. Rosmarinic acid (1), caffeic acid (2), chrysophanol (3), emodin (4), rhein (5).

All compounds were separated from *Rumex pamiricus* and *Rumex conglomeratus* plants for the first time.

Comment [PB22]: Add spectral data for confirmation that was isolated first time

Antibacterial and antifungal activity

We studied the leaves and roots of *Rumex confertus* Willd. in vitro for antibacterial and fungal activity in the fractions of gasoline, chloroform, ethyl acetate and butanol in our previous work. As a result, it was found that the leaves of the *Rumex confertus* plant, chloroform and ethyl acetate fractions of the root part have antibacterial activity against fungi and gram-positive

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bacteria [1]. Continuing these studies, the ester oil from the aerial parts of *Rumex confertus* Willd. and *Rumex pamiricus* were tested in vitro for antibacterial and fungal activity in the fractions of gasoline, chloroform, ethyl acetate and butanol (Table-1).

Table-1. In vitro screening results for antibacterial and antifungal activity

Name of substances	Inhibition zone diameter (mm)				
	Gram-positive microorganisms		Gram-negative strains		Conditionally pathogenic fungus
	Bacillus subtilis	*Staphylococcus aureus*	*E.coli*	*Pseudomonas aeruginosa*	*Candida albicans*
Ester oil of *Rumex pamiricus* Rech. f. aerial part	6	7	na	na	na
Ester oil of *Rumex confertus* Willd. aerial part	6	6	na	na	na
Alcohol extract of *Rumex pamiricus* Rech. f. aerial part	10	8	na	na	na
Gasoline extract of *Rumex pamiricus* Rech. f. aerial part	6	6	na	na	na
Ethyl acetate extract of *Rumex pamiricus* Rech. f. aerial part	10	12	na	na	14
Alcohol extract of *Rumex pamiricus* Rech. f. root	na	na	na	na	9
Butanol extract of *Rumex pamiricus* Rech. f. aerial part	9	9	na	na	Na
Chloroform extract of *Rumex pamiricus* Rech. f. aerial part	9	11	na	na	12
Ampicillin (10 µg/disc)	27	26	nt	nt	Nt
Ceftriaxone (30 µg/disc)	nt	nt	26	25	Nt
Flucanazole (25 µg/disc)	nt	nt	nt	nt	28

na- not active; nt – not tested
Inhibition zones ≤ **6-8 mm;**

Comment [PB25]: Write in the same manner like 'gram-positive strains and gram-negative strains'

Appreciable: **8-14 мм**;
Pronounced: **14-20 мм**;
Strong: ≤ **20 мм**

Results: As a result, it was found that the aerial part of the *Rumex pamiricus* plant, chloroform and ethyl acetate fractions have antibacterial activity against fungi and gram-positive bacteria.

Comment [PB26]: Read it carefully
How is it possible that antibacterial activity against fungi also

Discussion

Nowadays, the role of secondary metabolites as regulatory and adaptogenic is not questioned. For instance, the wide geographical distribution of the *Rumex* plants can be partly associated with the flexible system of secondary metabolism. In this study, wild plants with relatively uniform growing conditions were used. The collection sites were located in similar climatic and landscape conditions, with a low anthropogenic load. In addition, the plants were analyzed within the same ontogenetic phase- the flowering phase. This point is fundamental, as the level of regulatory secondary compounds can differ significantly at different stages of growth [14, 27].

Conclusion

1. Continuous studies on the chemical composition of *Rumex pamiricus* Rech. f. led to the isolation of anthraquinones: chrysophanol, emodin and rhein from the plant root extract using column chromatography on KSK silica gel. The structure of chrysophanol, emodin and rhein was established on the basis of the analysis of the data of MS, 1H, and 13C NMR spectra, and of the DEPT, HSQC and HMBC experiments.
2. The 60% acetone extracts of *Rumex conglomeratus* Murray was successively separated by MCI gel CHP 20P, and Toyopearl HW 40F column chromatography to yield two compounds. Their structures were elucidated by spectroscopic analyses as: rosmarinic acid and caffeic acid.
3. All compounds were separated from *Rumex pamiricus* and *Rumex conglomeratus* plants for the first time.
4. The ester oil from the aerial parts of *Rumex confertus* Willd. and *Rumex pamiricus* were tested in vitro for antibacterial and fungal activity in the fractions of gasoline, chloroform, ethyl acetate and butanol. As a result, it was found that the aerial part of the *Rumex pamiricus* plant, chloroform and ethyl acetate fractions have antibacterial activity against fungi and gram-positive bacteria.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of

the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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