

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

Abstract: The aim of the present study to clarify the phytochemical constituents of *Rumex* plants, extraction, separation and structural identification of chemical components, provide theoretical support and scientific basis for its traditional use, further expand the application scope of traditional medicinal plant resources in Uzbekistan, and develop its potential value. Three known anthraquinone derivatives has been isolated from the chloroform fraction extract of *Rumex pamiricus* roots and two known compounds were separated from the ethyl acetate fraction extract of *Rumex conglomeratus* roots. Their structures were elucidated by extensive spectroscopic evidence and chemical methods. Qualitative analyses of major phenolics by TLC analysis were also evaluated. The ester oil from the aerial parts of *Rumex confertus* and *Rumex pamiricus*, the gasoline, chloroform, ethyl acetate and butanol extracts of *Rumex pamiricus* aerial part and alcohol extract of *Rumex pamiricus* roots were tested *in vitro* for antimicrobial activity. This study aimed to determine the *in vitro* antimicrobial activity of the medicinal plants traditionally used in Uzbekistan against the microbial strains associated with infectious diseases.

Keywords: *Polygonaceae* L., *Rumex pamiricus* Rech. f., *Rumex conglomeratus* Murray., *Rumex confertus* Willd., sorrel, dock, phenol, phenolic acid, flavonoid, anthraquinone, *in vitro*, antimicrobial, extract, fraction.

1. Introduction

In the field of medicinal chemistry natural products research, Uzbekistan is a latter-coming and less active player even though Uzbekistan has a very long history to use local medicinal plants to treat various diseases. In recent years, Uzbekistan government has launched several projects, to support scientists carrying out natural products research. As a result, in the course of our continued searching for bioactive compounds from Uzbekistan and in the territory of Central Asia plants we did find that Uzbekistan possesses a great biodiversity and a great chemical diversity as well.

1.1. Importance of medicinal plants

“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” [1]. “The interest of natural drugs as adjunctive therapy for acute and chronic diseases has grown significantly in the recent years” [2]. 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 priority is this research to study the discovery of biological active substances constituents basis of genus *Rumex* plants in Uzbekistan and study chemical constituents of phenolic compounds.

1.2. Role of Phenolic Compounds

“Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants. Major polyphenolic compounds found in plants are flavonoids, catechins, epigallocatechin-3-gallate (EGCG), flavonones, iso-flavones, flavanols, anthocyanins, phenolic acids, stilbenes, flavonoids, chalcones, lignans etc. These compounds are secondary plant metabolites and possess antimicrobial, antiviral and anti-inflammatory properties along with high antioxidative activity. These properties make polyphenols interesting for the treatment of various diseases like inflammation, cancer and also used for anti-ageing purposes in cosmetic formulations as well as have nutraceutical applications”. [3]. Phenolic compounds are found in different organs and tissues of plants differ from each other not only in quantity but also in quality. Because their participation in various biochemical processes is determined by the structure and degree of polymerization. Most simple phenolic compounds are easily oxidized and are actively involved in metabolism. This is why they are concentrated in the tissues of leaves, flowers, and growth points, where most biochemical processes are most active [4].

1.3. The genus *Rumex* and distribution

“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” [5-7]. “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” [2,8,9]. “Since ancient times, concoctions and tea from leaves and roots of *Rumex* L. species have been used to treat various intestinal inflammations” [2,10]. “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 type of *Rumex* is present 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” [11]. “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 appetizer, analeptic medicine for liver, heart, as antihemorrhagic, to treat hepatitis, fever and other diseases” [2]. “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].

1.4. Source of secondary metabolites

“Higher plants synthesize several thousand known different phenolic compounds and the number of those fully characterized is continually increasing” [16]. “Phenolic compounds are known to have strong antioxidant as well as cardioprotective, immune system promoting, antibacterial, anti-cancer, and anti-inflammatory effects” [14]. “Plants of the *Rumex* genus are rich in secondary metabolites, in particular flavonoids, phenolic acids and anthraquinones, which are likely to be responsible for the medicinal properties attributed to these species” [17]. “The list of **anthraquinones** particularly common in *Rumex* plants includes chrysophanol, physcion, emodin and their glycosides, rhein, nepodin, and so on” [14]. “These compounds also show anticarcinogenic, anti-inflammatory, antiarthritic, antifungal, antibacterial, antioxidant and diuretic activity” [18,19]. “**Flavonoids** are another important class of compounds that determine the therapeutic effect of *Rumex* plants. Derivatives of kaempferol, quercetin, apigenin, luteolin, and catechins, as well as derivatives of benzoic and cinnamic acids, lignans, coumarins, and proanthocyanidins, have been isolated from various *Rumex* species” [20]. “Flavonoids constitute the largest class of phenolic compounds with more than 3,000 structures. These consist of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. For many years there has been much interest in flavonoids because of their beneficial effects on human health: e.g. antioxidant activity, free radical scavenging capacity, digestive stimulation action, anti-inflammatory, antimicrobial, antiviral, hypolipidemic, antimutagenic effects and anticarcinogenic potential” [21]. “**Phenolic acids** are further divided into hydroxycinnamic acid (HCA) and hydroxybenzoic acid (HBA). Phenolic acids account for about a third of the polyphenolic compounds in our diet and are found in all plant materials, but are particularly abundant in acidic fruits. Caffeic acid, gallic acid, ferulic acid are some common phenolic acids” [22]. “Fruits, vegetables, beverages such as tea, chocolate and wine are the chief sources of

phenolic compounds in the human diet” [3, 23]. “This shows their potential as nutritional supplements, feed additives, and medicinal agents” [13, 24].

1.5. Nutritional aspects and traditional uses of *Rumex* L.

“Among wild plants, *Rumex* plants have a great potential. They are already widely used as food, fodder, melliferous, and medicinal plants” [7, 25, 26]. “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” [18,19]. “According to the literature information, several *Rumex* species are included in the pharmacopoeias of various countries. For example, *R. crispus* is listed in the American Herbal Pharmacopoeia as a general detoxifier and an agent for skin treatment” [27]. “The State Pharmacopoeia of the Russian Federation includes the roots of *R. confertus* as a herbal medicine, which is used in the treatment of liver diseases, dysentery, pulmonary and uterine bleeding, as well as a laxative” [28, 29]. “*Rumex* plants have traditionally been used as edible or medicinal plants in various regions of the world” [14,29]. “Several *Rumex* species have been used in traditional Chinese medicine (TCM) for the therapy of different diseases. *R. dentatus*, found almost everywhere in China, has been employed traditionally for the treatment of many kinds of bacterial and fungal infections, e.g. dysentery, enteritis and acariasis” [30]. “*R. crispus* has a long history of domestic herbal use in India and Pakistan. It is a gentle and safe laxative and useful for treating a wide range of skin problems (sores, ulcers and wounds). The root of the plant is alterative, mildly tonic, antiscorbutic, cholagogue and astringent, while the seeds effective in the treatment of diarrhea” [31,32]. In Australia *Rumex* species are used for the treatment of stings [33]. The extracts of some *Rumex* species (*R. hymenosepalus* and *R. maderensis*) are used as a “blood depurative” or “blood purifier” [34,35]. “Literature demonstrates that *R. hastatus* is traditionally used in the treatment of sexually transmitted diseases, including AIDS” [36]. “*R. nepalensis* is applied to treat stomachache in Ethiopian regions. *R. vesicarius* is a wild edible Egyptian herb. In folk medicine, it is used as a tonic and analgesic and for the treatment of hepatic diseases, constipation, poor digestion, spleen disorders, flatulence, asthma, bronchitis, dyspepsia, vomiting and piles, among others” [37]. “The roots of *R. confertus* are used for liver diseases, dysentery, pulmonary and uterine bleeding, as a laxative, for hemorrhoids and anal fissures, externally for burns, wounds, stomatitis, gingivitis, and skin diseases in Russian Federation” [7]. “However, today, their biotechnological potential is becoming evident, and these species can act as a source 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” [14].



Figure 1. *R. pamiricus* Rech. f. (1); *R. confertus* Willd. (2); *R. conglomeratus* Murray (3). Location: Beldersay, Chimgan mountains (Ugam Chatkal National Park), Tashkent region. (Pictures author: G.D.Shermatova).

2. Experimental part

2.1. 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.

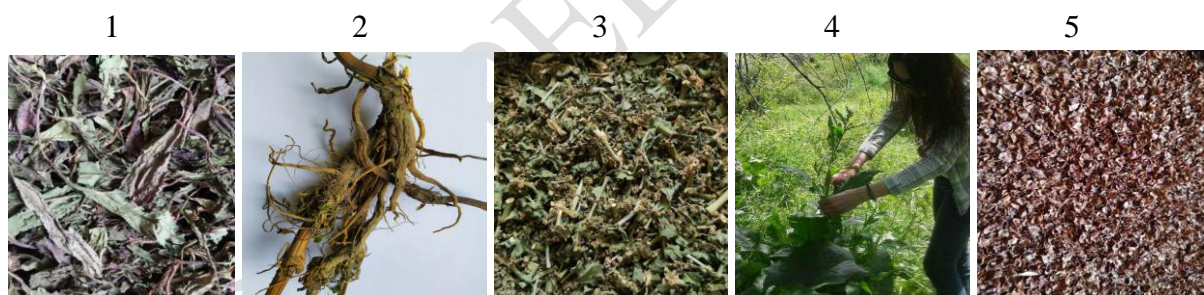


Figure 2. Some pictures of the collected samples. The leaves of *Rumex pamiricus* Rech. f. (1); the roots of *Rumex conglomeratus* (2); the aerial part of *Rumex confertus* Willd. (3); The process of collecting *R. Pamiricus* Rech. f. (4); the seeds of *Rumex confertus* Willd. (5). Location: Tashkent Botanical Garden named after F. N. Rusanov. (Pictures author: G.D.Shermatova).

2.2. 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 Na_2SO_4 . The dehydrated extract was filtered, its concentration 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.

2.3. Isolation and results: The roots are the best organs for the accumulation of anthraquinones [2]. 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 3) was established on the basis of the analysis of the data of MS (Mass spectrometry), ^1H and ^{13}C NMR spectra (Nuclear Magnetic Resonance), and of the DEPT (Distortionless Enhancement of Polarization Transfer), HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation) experiments. Qualitative analyses of major phenolics by TLC (Thin Layer Chromatography) analysis were also evaluated.

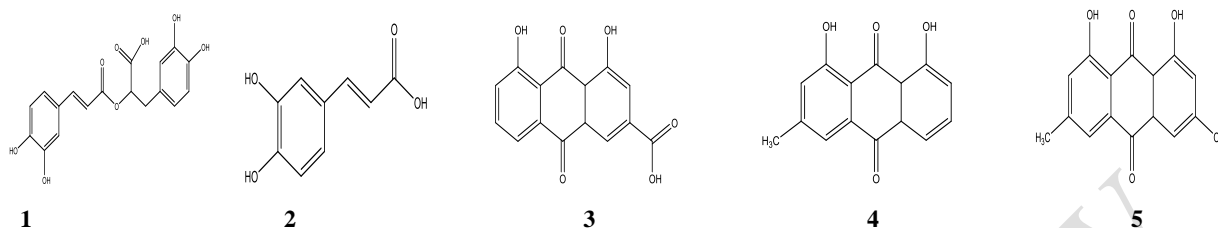
2.4. Discussion: The anthraquinones, chrysophanol, emodin and rhein have been isolated from other types of *Rumex* in studies before us. According to the literature: chrysophanol from roots of *R. dentatus* [30], emodin from roots of *R. abssinicus* [38], from leaves of *R. chalepensis* [39], rhein from *R. acetosa* [38], chrysophanol and emodin from roots of *R. crispus* [38], *R. abyssinica*, *R. patientia* [39], *R. nepalensis* [40], and from the aerial parts of *R. acetosa* [41].

2.5. 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.

2.6. Isolation and 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% stepwise 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 3) (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 3) (0.252 g).

2.7. Discussion: 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* [42], from *Melissa officinalis* L.[43],

from *Hyptis atrorubens* Poit [44,45], [46], [47,48,49,50], caffeic acid from the aerial parts of *R. aquatica* [39], rosmarinic acid and caffeic acid from leaves of *R. acetosa*, *R. acetosella*, *R. confertus*, *R. crispus*, *R. maritimus*, *R. obtusifolius* and *R. sanguineus* [14].



2.8. Figure 3. Chemical structures of isolated compounds 1–5: rosmarinic acid (1), caffeic acid (2), chrysophanol (3), emodin (4), rhein (5). All known pure compounds were isolated in our laboratory from *Rumex pamiricus* and *Rumex conglomeratus* plants for the first time.

2.9. Data of isolated compounds:

- 1. Rosmarinic acid (1)** [44]. $C_{18}H_{16}O_8$. Yellow amorphous powder. ESI-MS (negative ion) m/z 359 $[M-H]^-$; UV λ_{max} (CH_3OH): 221, 330 nm; IR (KBr) (cm^{-1}) ν_{max} : 3382, 1697, 1606, 1522; 1H NMR (CD_3OD): δ 7.52 (1H, d, $J = 15.5$ Hz, H-7'), 7.04 (1H, d, $J = 2.0$ Hz, H-2'), 6.91 (1H, dd, $J = 8.0, 2.0$ Hz, H-6'), 6.75 (1H, d, $J = 8.0$ Hz, H-5'), 6.70 (1H, d, $J = 2.0$ Hz, H-2), 6.69 (1H, d, $J = 8.0$ Hz, H-5), 6.57 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 6.26 (1H, d, $J = 15.5$ Hz, H-8'), 5.19 (1H, dd, $J = 10.0, 3.5$ Hz, H-8), 3.06 (1H, dd, $J = 14.5, 5.5$ Hz, H-7a), 3.00 (1H, dd, $J = 14.5, 5.5$ Hz, H-7b); ^{13}C -NMR (CD_3OD): δ 174.3 (C-9), 169.4 (C-9'), 149.5 (C-4'), 145.7 (C-7'), 145.0 (C-3), 144.9 (C-4), 143.2 (C-3'), 130.4 (C-1), 128.2 (C-1'), 122.2 (C-6), 121.4 (C-6'), 117.6 (C-2), 116.3 (C-5), 116.1 (C-5'), 115.8 (C-8'), 115.3 (C-2'), 77.8 (C-8), 38.9 (C-7).
- 2. Caffeic acid (2)** [51], colorless acicular crystals, mp 222.1–225.8°C. HR- ESI-MS m/z 179.0378 $[M-H]^-$ (calcd for $C_9H_7O_4$, 179.0344). 1H NMR (400 MHz, DMSO- d_6 , δ , ppm, J/Hz): 7.39 (1H, d, $J=16.0$, H-7), 7.02 (1H, d, $J=2.0$, H-2), 6.94 (1H, dd, $J=8.0, 2.0$, H-6), 6.75 (1H, d, $J=8.0$, H-5), 6.17 (1H, d, $J=16.0$, H-8). ^{13}C NMR (125 MHz, DMSO- d_6 , δ , ppm): 125.8 (C-1), 115.6 (C-2), 144.3 (C-3), 148.1 (C-4), 115.8 (C-5), 121.1 (C-6), 145.6 (C-7), 114.6 (C-8), 168.2 (C-9).
- 3. Chrysophanol (3)** [52]. 1H NMR (600 MHz, $CDCl_3$, δ , ppm, J/Hz): 12.14 (1H, s), 12.03 (1H, s), 7.83 (1H, d, $J = 6.8$, H-5), 7.67 (2H, s, H-6, 4), 7.31 (1H, d, $J = 6.8$, H-7), 7.12 (1H, s, H-2), 2.48 (3H, s, 3-Me). ESI-MS m/z 255.0 $[M + H]^+$.
- 4. Emodin (4)** [53]. Orange needles, $C_{15}H_{10}O_5$, mp 254–256°C. 1H NMR (600 MHz, acetone- d_6 , δ , ppm, J/Hz): 7.54 (1H, s, H-4), 7.23 (1H, d, $J = 2.3$, H-5), 7.12 (1H, s, H-2), 6.63 (1H, d, $J = 2.3$, H-7), 2.44 (3H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ , ppm): 190.9 (C-9), 181.4 (C-10), 165.5 (C-3), 165.2 (C-1), 162.4 (C-8), 148.8 (C-6), 135.8 (C-14), 135.8 (C-11), 124.2 (C-5), 124.1 (C-7), 120.7 (C-13), 113.7 (C-12), 108.8 (C-4), 108.0 (C-2), 21.2 (CH_3).
- 5. Rhein (5)** [54]. $C_{15}H_8O_6$, yellowish powder. ESI-MS m/z 307.2 $[M + Na]^+$. 1H NMR (600 MHz, DMSO- d_6 , δ , ppm, J/Hz): 8.15 (1H, d, $J = 1.7$, H-4), 7.78 (1H, d, $J = 1.7$, H-2), 7.75 (1H, dd, $J = 7.8, 1.0$, H-5), 7.85 (1H, t, $J = 8.0, 7.8$, H-6), 7.42 (1H, dd, $J = 8.0, 1.0$,

H-7). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 161.6 (C-1), 124.3 (C-2), 138.2 (C-3), 118.9 (C-4), 119.6 (C-5), 137.7 (C-6), 124.7 (C-7), 161.2 (C-8), 191.5 (C-9), 181.2 (C-10), 133.4 (4a), 116.4 (8a), 118.4 (9a), 134.1 (10a), 165.6 (COOH).

3. Antimicrobial activity

Medicinal plants are widely used for the treatment of different infectious diseases. Infectious diseases caused by bacteria have a large impact on public health [55]. 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 positive bacteria [1]. Continuing these studies, the ester oil from the aerial parts of *Rumex confertus* and *Rumex pamiricus*, the gasoline, chloroform, ethyl acetate and butanol extracts of *Rumex pamiricus* aerial part and alcohol extract of *Rumex pamiricus* roots were tested *in vitro* for antimicrobial activity (Table-1). It was studied at the Institute of the Chemistry of Plant Substances named after Acad. S.Yu. Yunusov AS of Uzbekistan, Laboratory of Molecular Genetics by Dr. Sasmakov S.A.

3.1. Table-1. *In vitro* screening results for antimicrobial activity

Name of substances	Inhibition zone diameter (mm)				
	Gram-positive strains		Gram-negative strains		Conditionally pathogenic fungus
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
Ester oil of <i>Rumex pamiricus</i> Rech. f. aerial part	6	7	na	na	na
Ester oil of <i>Rumex confertus</i> Willd. aerial part	6	6	na	na	na
Alcohol extract of <i>Rumex pamiricus</i> Rech. f. aerial part	10	8	na	na	na
Gasoline extract of <i>Rumex pamiricus</i> Rech. f. aerial part	6	6	na	na	na
Ethyl acetate extract of <i>Rumex pamiricus</i> Rech. f. aerial part	10	12	na	na	na
Alcohol extract of <i>Rumex pamiricus</i> Rech. f. root	na	na	na	na	na
Butanol extract of <i>Rumex pamiricus</i> Rech. f. aerial part	9	9	na	na	na
Chloroform extract of <i>Rumex pamiricus</i> Rech. f. aerial part	9	11	na	na	na

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;

Appreciable: 8-14 mm;

Pronounced: 14-20 mm;

Strong: ≤ 20 mm

Results: As a result, the aerial part of the *Rumex pamiricus* plant, chloroform and ethyl acetate fractions showed moderate antimicrobial activity against gram-positive bacteria.

4. 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].

During the study process, it was observed that the difference in the color of *R. pamiricus*, a plant that grows in mountainous areas, and *R. pamiricus*, which grows in urban areas. That is to say, it was found that the redness characteristic of the genus *R. pamiricus* is not clearly visible in plants growing in the mountainous region (Figure 1), but is dark red in the city (Figure 2).

5. 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, TLC, 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. The ester oil from the aerial parts of *Rumex confertus* and *Rumex pamiricus*, the gasoline, chloroform, ethyl acetate and butanol extracts of *Rumex pamiricus* aerial part and alcohol extract of *Rumex pamiricus* root were tested for antimicrobial activity. As a result, the aerial part of the

Rumex pamiricus plant, chloroform and ethyl acetate fractions showed moderate antimicrobial activity against gram-positive bacteria.

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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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