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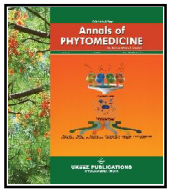

Original Article : Open Access

Total phenol and flavonoid content, antibacterial and antioxidant activity of extract and fractions of medicinal plants of the *Rumex* (Polygonaceae) family in the flora of Uzbekistan

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Abstract

In this study, we have determined the antimicrobial, antioxidant activity, the content of phenols and flavonoid in extracts and fractions of plants belonging to the genus *Rumex* (Polygonaceae), widely distributed in the flora of Uzbekistan. To determine the antimicrobial activity of extracts and fractions, test strains of *E. coli*, *B. subtilis*, *P. aureginosa*, *S. aureus*, *C. albicanc* were used. To determine the antioxidant activity, standard solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used. The best results for the total content of phenols and flavonoids in the extracts were as follows: ethanol extract of *R. tianschanicus* (TPC) 41.23 ± 4.10 mg GAE/g, (TFC) 58.41 ± 2.18 mg CE/g. With regard to antimicrobial activity, the following results were obtained. The *R. confertus* EtOH fraction showed higher antimicrobial properties against *E. coli* (17 ± 0.12), *B. subtilis* (14 ± 0.17), *P. aureginosa* (13 ± 0.10), *C. albicans* (17 ± 0.10) compared to other samples. The *Rumex* pomiricus CHC_{l3} fraction showed high antimicrobial properties against *E. coli* (15 ± 1.21), *B. subtilis* (13 ± 1.01), *P. aureginosa* (20 ± 0.21), *C. albicans* (25 ± 0.13). The results of the minimum inhibitory concentration were as follows: extracts of *R. pomiricus* MIC (6.25 ± 0.02 µg/ml); MIC of *R. pomiricus* CHC_{l3} fraction (6.25 ± 0.107 µg/ml). In terms of antioxidant activity (DPPH), IC_{50} values (p<0.01) for ascorbic acid 24.53 µg/ml, *R. confertus* EtOH fraction 134.9 µg/ml significant changes were noted. *R. syriacus* leaf extract 70% showed IC_{50} values (p<0.01) of 48.54 µg/ml.

1. Introduction

Plants belonging to the genus, *Rumex* make up the Polygonaceae family with over 200 species. It is mainly distributed in the Northern regions of the temperate zone. They are mostly perennial herbs with strong taproots, lanceolate inflorescences and enlarged spiral triangular fruits.The name "*Rumex*" comes from the Greek word for "spear" and refers to the shape of its leaves (Kambhar, 2014; Jingfu *et. al*., 2015; Jain and Parkhe, 2018, Berillo *et. al*., 2022). Species belonging to the *Rumex* species occupy a valuable place in world folk medicine. For example, in countries such as Asia, South Africa, America, India, China and Turkey, it has been used to treat headaches (migraines), fevers and gynecological diseases. The roots of plants belonging to the genus, *Rumex*, are reported to have therapeutic properties against bacterial infections, inflammation, tumors and cardiovascular disease (Mostafa *et al*., 2011; Elzaawely and Tawata, 2012; Sichani *et al*., 2013; Quradha *et al*., 2019; Bektašević *et al*., 2022; Li *et al*., 2022). Pharmacological studies have shown that *Rumex* species have antibacterial and antifungal properties and are used anti-inflammatory agent (Alotibi *et al*., 2020). Plants belonging to *Rumex* species exhibited different chemical characteristics and biological activities (Tsamo *et al*., 2021; Shifa *et al*., 2021; Pelzer *et al*., 2022). To date, more than 260 biologically active compounds have been identified in plants belonging to the *Rumex* species. These compounds include anthraquinones, flavonoids, tannins, naphthalenes, diterpene alkaloids, terpenes and lignans (Vasas *et al*., 2015; Shaikh *et al*., 2018). These compounds are chemical components with anti-inflammatory, antioxidant, antibacterial, antitumor and antidiabetic properties (Baig *et al*., 2011; Idris *et al*., 2017; Shifa *et al*., 2021; Noshad, 2021). In folk medicine, plants belonging to the *Rumex* family are widely used as a potentially effective remedy for many diseases. This article discusses comprehensive information on the biological activity of plant extracts and fractions related to *Rumex* family common in the flora of Uzbekistan.

2. Materials and Methods

2.1 Geographical distributions, local names, parts used and traditional uses

In the flora of Uzbekistan, plants belonging to the *Rumex* family are distributed in different geographical zones. In this article, we will get acquainted in detail with some species belonging to the *Rumex* family which are common on the territory of Uzbekistan.

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2.1.1 *Rumex confertus* Willd.

Polygonaceae *R. confertus* is a perennial herbaceous plant with a powerful root reaching a height of 60-150 cm. The stem is erect, the leaves are oblong-triangular-oval, 15-25 cm long, 6-12 cm wide. The tip of the leaves is blunt, heart-shaped, the back side is covered veins and coarse hairs. The leaves on the stem are smaller, pointed, oval and spear-shaped. The flowers are cylindrical in shape, densely located in place of the flower. The fruit is triangular, 3-5 mm long, 1.5-2.5 mm wide, Flowering and fruiting in May-June, reproduction by seeds and vegetatively. The root contains tannins (ellagic acid, phloroglucinum and caffeic acid), flavonoids (nepodin, chrysophanoicacid, emodin), resins, essential oils and calcium oxalate. The leaves contain flavone glycosides (hyperoside and rutin), carotene, vitamin C and calcium oxalate (Eisenman *et al*., 2012). Preparations prepared from the plant *R. confertus*, have an astringent effect in small doses, and a cleansing effect in large doses. Currently, they are recommended to improve bowel function. It is also used with simultaneous violation of the function of the gastrointestinal tract, colitis, hemorrhagic enterocolitis, hemorrhagic colitis and childhood diarrhea. The plant extract showed a cytotoxic effect on human lymphoblastoid cells *in vitro* (Feduraev *et. al*., 2019; Eom *et. al*., 2020).

2.1.2 *Rumex tianschanicus* Losinsk

Polygonaceae *R. tianschanicus* is an upright, hollow and branched perennial plant up to 2 meters high. Basal leaves are broadly ovate, 17-25 cm long, 15 cm wide, leaves at the top of the stem are heart-shaped with sharp edges. The leaves are smaller at the root. The fruits are trihedral, light brown, 2 mm long, flowering and fruiting in May-June. It occurs in the Tashkent region and the foothills of the Chotkal mountains. The entire body of the plant contains biologically active compounds such as phenolic acids, flavonoids and catechins. The seeds contain fatty acids. The roots contain sugar, inulin, organic acids, tannins, anthroquinones and leucoanthocyanidin. The leaves contain compounds such as vitamins C, P, K, carotenoids and tannins. At the same time, plants belonging to the genus, *Rumex* are widely used for medicinal purposes in folk medicine (Eisenman *et al*., 2012). The total extracts and fractions of these plants rich in biologically active secondary metabolites contain quinones, flavonoids, tannins, terpenes, diterpene alkaloids, lignans and other components below shows plants belonging to the *Rumex* species common in the flora of Uzbekistan (Figure 1).

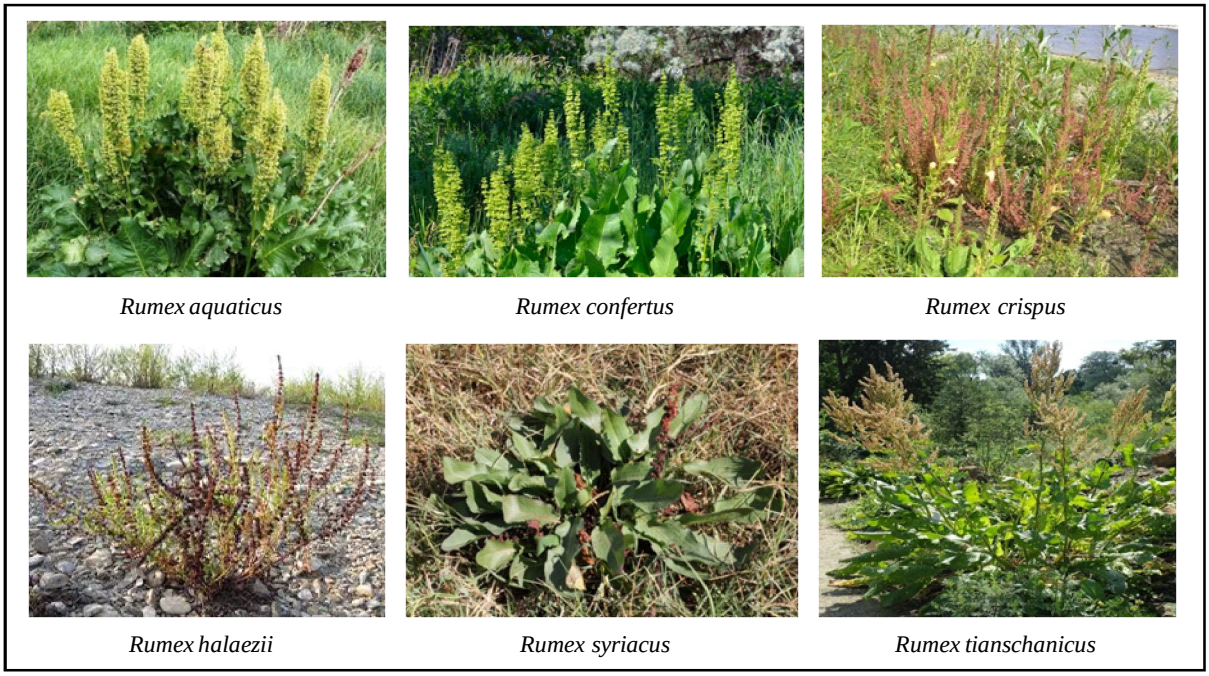

Figure 1: Distribution of *Rumex* species in the flora of Uzbekistan.

2.2 Plant material

Selected plants belonging to the *Rumex* species were collected in natural conditions mainly from the territory of Tashkent and Kashkadarya regions. Then, the plant was divided into parts (root, stem, leaf) and dried in a cool dry place protected from sunlight. Dried plants were prepared for the extraction process.

2.3 Preparation of crude extracts

Dried plant parts were crushed and extracted for five days with 70-80% ethanol until the color disappeared. Then, the entire content of the extract was dissolved in 80% ethanol to remove other impurities and mixed with hot water in a ratio of 1:1 kept at room temperature for a day. At the next stage, the organic phase was evaporated on a rotary evaporator at a temperature of 40°C (Al-Farhan *et al*., 2022). After that, the concentrated extract was diluted with water 1:1 and the organic phase was separated using a separating funnel. Then, the solvents were separated into fractions in order of increasing polarity (Figure 2). The extraction liquid was fractionated in gasoline, chloroform, ethyl acetate and n-butanol. The resulting fractions were dried for 2 days in an oven at a temperature of 50°C.

Individual separation of substances in dried fractions was performed using silica gel column chromatography (pore size 60 Å, particle size 230-400 mesh, particle size 40-63 mm) (Babu-Kasimala *et al*., 2014; Mhalla *et al*., 2017).

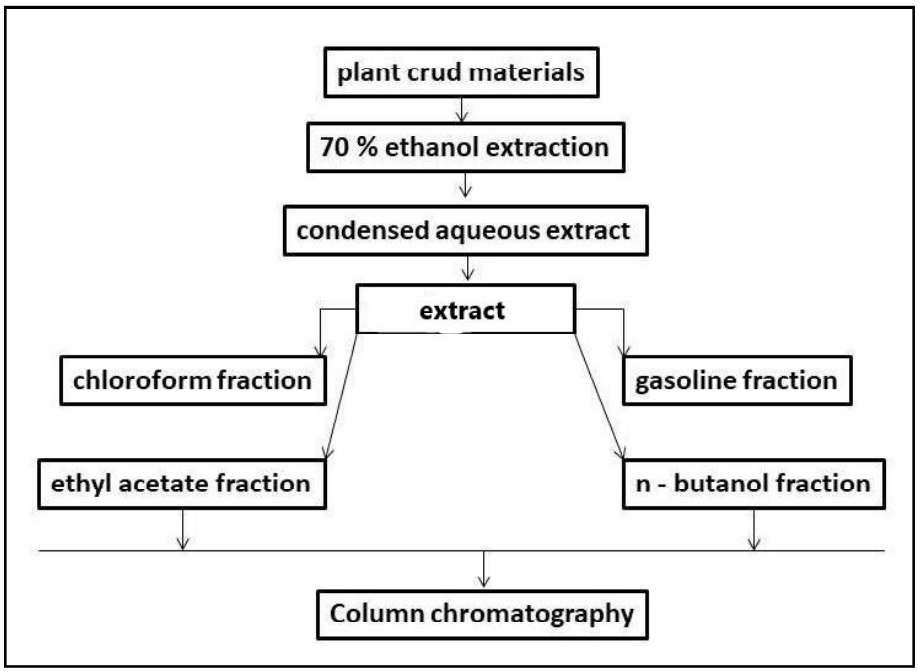

Figure 2: Diagram of extraction and fractionation of the plants.

2.4 Determination of phenol content

2.4.1 Total phenol content

The amount of phenol in plant extracts was determined using the Folin-Ciocalteu reagent. The extract of each plant (0.5 ml) was mixed with 2.5 ml of the Folin-Ciocalteu reagent (ratio 1:10) diluted with water. In the next step, 4 ml of 7.5% Na_2CO_3 (w/v) was added to the sample and kept in a dark room at 45°C for 30 min. Then, the samples were measured on a spectrophotometer at a wavelength of 760 nm. The total content of phenolic compounds is expressed as gallic acid equivalent (GAE) per gram of extract (mg GAE/g E) (Unal *et. al*., 2022).

2.4.2 Total flavonoid content

The amount of flavonoids in the extract was determined by colorimetric method using 10% aluminum chloride solution. The extract of each plant sample (0.25 ml) was diluted with 1.25 ml of distilled water mixed with 0.075 ml of sodium nitrate solution $NaNO_3$ (5%) and kept for 5 min. At the next stage, 0.15 ml of aluminum chloride (10%) was added to the sample and held for 10 min. Then, 0.5 ml of 1M sodium hydroxide was added. The mixture was then diluted with 0.275 ml of distilled water. The final solutions were incubated at room temperature in the dark for 30 min and measured at a wavelength of 510 nm. Flavonoid content is expressed as quercetin equivalent (ÑÅ) (mg ÑE/g E) per gram of extract (Jimoh *et. al*., 2008).

2.5 Determination of the antibacterial activity of raw extracts of *Rumex* plants

2.5.1 Fundamentals of selection of microorganisms

The bacteria and fungi selected for this work were chosen to confirm their role in human and animal pathogenicity including gastrointestinal diseases associated with dysentery and many other diseases and primarily to confirm the pharmacological properties of plants belonging to the species *Rumex*.

2.5.2 Tested microorganisms

Test microorganisms used to determine antimicrobial activity are bacteria that are conditionally pathogenic for humans (*Staphylococcus aureus*-91, *Bacillus subtilis*-5, gram-negative bacteria, *Escherichia coli*-221 and *Pseudomonas aeruginosa*-225, microscopic fungi, *Candida albicans*-247). All used test cultures are registered and stored at the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.

2.5.3 Disc diffusion method

The antimicrobial activity of the crud extracts of plants against strains of pathogenic bacteria was determined by the disk diffusion method (Humeera *et al*., 2013; Feduraev *et al*., 2022; Pelzer *et al*., 2022). Selected test strains (*Staphylococcus aureus*-91, *Bacillus subtilis*-5, gram-negative bacteria *Escherichia coli*- 221 Ba. *Pseudomonas aeruginosa*-225, microscopic fungi, *Candida albicans*-247) were grown on nutrient broth medium at 37 ± 2°C 24 h. The cultured test strains were diluted in 20 ml sterilized distilled water. The turbidity level of the strains used for the test was adjusted with a McFarland 0.5 standard (0.5 × 108 CFU / ml). Pathogen test bacterial strains were then inoculated on a lawn using a pre-prepared nutrient agar medium (Hi Media Lab. Ltd., Mumbai, India) sterilized L-shaped glass rod. Crud extracts of plants was soaked in 6-8 mm sterile Whatman 1 paper. The nutrient was then placed in a petri dish containing pathogenic bacterial strains grown in a medium. A antibiotic canamycin was used as a positive control and dimethyl sulfoxide (DMSO) solution was used as a negative control. Petri dishes were kept in a thermostat at a temperature of 37 ± 2°C (Memmert Made in Germany Schutzart DIN EN 60529 - IP20) for 18-24 h. At the end of the incubation period, the zones of inhibition around the disks impregnated with extracts were measured and compared with the values of the zone of inhibition of the antibiotic canamycin.

2.6 Minimum inhibitory concentration (MIC)

2.6.1 Determination of minimum inhibitory concentration

The minimum inhibitory concentration of plant samples was carried out in sterilized round-bottom 96-well microplates. Separate microplates were used for each pathogenic strain. 200 µl of nutrient broth (*S. aureus*, *E. coli*, *P. aureginose*, *B. sibtilis*) and sabouraud broth (*C. albicans*) were poured into each well of the plate.

In the first well of the microplate (lane A) (columns 1 and 10) was added 100 µl of plant extracts. As a positive control, the antibiotic ceftriaxone (an antibiotic against pathogenic test strains) was added to the eleventh column. The next step was serial dilutions. At the next stage, 100 µl was taken from column A and sequentially poured into column B and other wells in the same order. The result is the following dilution ratio : 1:2 (A), 1:4 (B), 1:8 (C), 1:16 (D), 1:32 (D), 1:64 (E), 1: 128 (G) 1:256 (H) gave. Then, 20 µl of pathogenic test strain inoculum (0.5 McFarland 10^8 CFU/ml) was added to each well. Nutrient broth was added to the twelfth column used as a control. After that, the microplate was incubated at 37 ± 2°C for 24 h. After incubation, 20 µl of 2,3,5-triphenyltetrazolium chloride solution was added to each well and incubated for another 2 h. Color development was then observed in each well. If, the wells turn red, the test indicates that strains have grown. If, the color does not develop it can be concluded that the extracts inhibited the growth of the strains. The area of no color in each column is the minimum inhibitory concentration of the extracts (Rodríguez-Melcón *et al*., 2022).

2.7 DPPH radical scavenging activity assay

The radical scavenging activity of the crude extracts was determined using the standard reagent 2,2-dephenyl-1-picrylhydrosil (DPPH) at concentrations of 100 µl, 150 µl, 200 µl. First, a solution of the DPPH reagent (0.1 mM) in methanol was prepared. Then, 1 ml of the extract (1-100 µg/ml) was dissolved in 2 ml of methanol and 2 ml of 0.1 mM DPPH solution was added. Then, it was vortexed and stored in the dark for 30 min at room temperature. After the incubation time, the mixture was measured on a spectrophotometer at 517 nm (Coruh *et al*., 2008; Ahmad *et al*., 2015; Nengroo *et al*., 2021). The percentage of DPPH• scavenging (RSA %) was estimated using the equation:

$$\% \text{ scavenging of DPPH•} = [(A_0 - A_1)/A_0] \times 100$$

2.8 Statistical analysis

The data are expressed as mean values ± standard deviation for each measurement and analyzed by means of analysis of variance (one-way ANOVA), followed by Tukey posttests. The statistical analysis was performed using excel. The correlation between phenolic compounds and antioxidants was conducted using SPSS.

3. Results

3.1 Total phenols and flavonoids content

The was determined amount of phenol contained in the total extracts of plants, *R. confertus*, *R. crispus*, *R. halaezii*, *R. syriacus*, *R. tianschanicus*, *R. aquaticus* belonging to the *Rumex* species (Table 1). The content of phenolic substances (TPC) in the ethanol extract of the *R. confertus* plant was 21.38 ±1.43 mg GAE/g, and in the methanol extract, it was 26.14 ± 1.63 mg GAE/g. It was also found that the amount of flavonoids in the extract of *R. confertus* in ethanol is 44.12 ± 2.74 mg CE/g, and in the extract in methanol 46.65 ± 2.41 mg CE/g. The content of phenolic substances (TPC) in the ethanol extract of the plant, *R. crispus* was 19.56 ± 1.32 mg GAE/g and in the methanol extract 27.32 ± 1.61 mg GAE/g. The determination amount of flavonoids was in the ethanol extract of the plant, *R. confertus* is 34.84 ± 2.31 mg CE/g and in the methanol extract 42.67 ± 1.47 mg CE/g. The results of the total phenols and flavonoid content of are presented in Table 2. Good results in terms of the amount of phenolic and flavonoid substances in the extract were as follows: extract of *R. tianschanicus* in ethanol (TFC) 41.23 ± 4.10 mg GAE/g, (TFC) 58, 41 ± 2.18 mg CE/g.

Table 1. Total content of phenols and flavonoids of *R. confertus, R. crispus, R. halaezii, R. syriacus, R. tianschanicus, R. aquaticus* ethanol and methanolic extracts

	Rumex confertus		*Rumex crispus*		*Rumex halaezii*	
	EE	ME	EE	ME	EE	ME
TPC	21.38 ± 1.43	26.14 ± 1.63	19.56 ± 1.32	27.32 ± 1.61	22.36 ± 1.47	25.74 ± 2.10
TFC	44.12 ± 2.74	46.65 ± 2.41	34.84 ± 2.31	42.67 ± 1.47	42.78 ± 1.74	51.65 ± 2.10
	Rumex syriacus		*Rumex tianschanicus*		*Rumex aquaticus*	
	EE	ME	EE	ME	EE	ME
TPC	31.21 ± 1.32	34.41 ± 2.32	41.23 ± 4.10	40.12 ± 3.10	33.41 ± 2.04	36.14 ± 2.10
TFC	54.12 ± 1.01	46.10 ± 3.14	57.12 ± 4.10	58.41 ± 2.18	41.23 ± 2.04	56.10 ± 2.47

TPC : Total phenol content; TFC : Total flavonoid content; EE: Ethanolic Extract; ME: Methanolic extract.

Unal *et al*. (2022) reported results on the amount of phenolic and flavonoid compounds in the plant extract of *R.scutatus* more consistent with our studies. Changes in the amount of phenolic and flavonoid compounds in plant extracts can be explained by a change in the method of extraction and storage, drying of the plant, and climatic conditions.

3.2 Antibacterial activity

Ethyl acetate and chloroform fractions were prepared from the total extracts of plants *R. confertus* and *R. pomiricus*. When studying the activity of the prepared fractions against pathogenic microbes, the following results were obtained. Extracts and fractions of *R. confertus* and *R. pomiricus* showed positive antimicrobial properties (Table 2).

Table 2: Antibacterial activity of extracts of *R. confertus* and *R. pomiricus* fraction and extracts using well diffusion method

Pathogen test strains	*Rumex confertus*		*Rumex pomiricus*		Positive control canamycin	Negative control DMSO
	EtOH fraction	CHCl fraction	EtOH fraction	CHCl fraction		
E. coli	17 ± 0.12	0 ± 00	0 ± 00	15 ± 1.21	24 ± 1.32	00 ± 00
B. subtilis	14 ± 0.17	17 ± 0.23	7 ± 0.14	13 ± 1.01	21 ± 0.46	6.1 ± 1.21
P. aureginosa	13 ± 0.10	0 ± 00	0 ± 00	20 ± 0.21	28 ± 0.38	00 ± 00
S. aureus	0 ± 00	0 ± 00	0 ± 00	0 ± 00	21 ± 1.32	00 ± 00
C. albicans	17 ± 0.24	15 ± 0.11	14 ± 0.41	25 ± 0.13	21 ± 1.32	00 ± 00

The test strains used in the experiment did not have the same sensitivity to extracts and fractions. The ethyl acetate fraction of *R. confertus* showed an inhibition zone of *E. coli* 17 ± 0.12 mm, *B. subtilis* 14 ± 0.17 mm, *P. aureginosa* 13 ± 0.10 mm, *C. albicans* 17 ± 0.24 mm. However, the ethyl acetate fraction of *R. confertus* could not inhibit the growth of the pathogenic strain *S. aureus*. Also, the plant chloroform fraction of *R. confertus* inhibited the growth of *B. subtilis* 17 ± 0.23 mm and *C. albicans* 15 ± 0.11 mm. However, it was found that *E. coli*, *P. aureginosa* and *S. aureus* could not inhibit the growth of the test strains. At the same time, it was found that a 70% extract of the *R. confertus* plant inhibited the growth of test strains *E. coli* 10 ± 0.13 mm, *B. subtilis* 15 ± 1.10 mm and *C. albicans* 15 ± 0.14 mm (Figure 3).

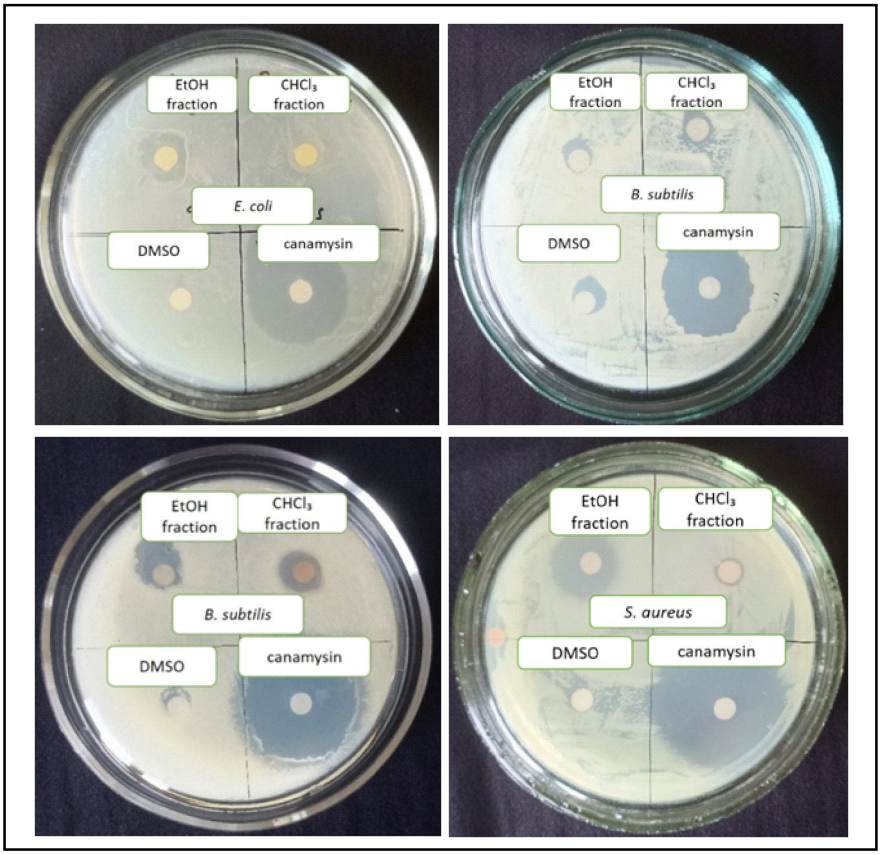

Figure 3: Antimicrobial activity of *R. confertus and R. pomiricus* plants in EtOH fraction, $CHCl_3$ fraction and 70% extracts.

Also, the chloroform fraction of *R. pomiricus* inhibited the growth of test strains *E. coli* 15 ± 1.21 mm, *B. subtilis* 13 ± 1.01 mm, *P. aureginosa* 20 ± 0.21 mm and *C. albicans* 25 ± 0.13 mm . The *R. pomiricus* extract inhibited the growth of test strains *B. subtilis* 13 ± 0.16 mm, *P. aureginosa* 9 ± 0.18 mm, *S. aureus* 14 ± 0.32 mm and *C. albicans* 18 ± 0.35 mm.The antimicrobial activity of root and leaf extracts of *R. confertus*, *R. holaezii*, and *R. syriacus* against test strains was determined (Table 3 and Figure 4). The antimicrobial activity of root and leaf extracts of *R. confertus*, *R. holaezii* and *R. syriacus* was determined. The *R. confertus* root extract inhibited the growth of pathogenic test strains *B. subtilis* (12 ± 0.17), *P. aureginosa* (7 ± 0.17) and *S. aureus* (4 ± 0.01). The extract prepared from plant leaves inhibited the growth of test strains *B. subtilis* (5 ± 0.27), *P. aureginosa* (7 ± 1.20), *S. aureus* (7 ± 0.13) and *C. albicans* (4 ± 1.01). *R. holaezii* root extract inhibited growth of the pathogenic test

strains *B. subtilis* (6 ± 1.02), *S. aureus* (6 ± 1.42), and *C. albicans* (5 ± 0.17).The extract obtained from plant leaves inhibited growth of the test strains *P. aureginosa* (4 ± 1.10), *B. subtilis* (6 ± 0.13), *S. aureus* (4 ± 1.32) and *C. albicans* (4 ± 0.21). The root and leaf extract of *R. syriacus* inhibited the growth of pathogenic test strains *B. subtilis* (8 ± 0.16) and *C. albicans* (6 ± 0.04). The root extract inhibited growth of the pathogenic test strains *B. subtilis* (6 ± 1.21), *S. aureus* (16 ± 0.14) and *C. albicans* (4 ± 0.11).

Table 3: Antibacterial activity of extracts of *R. confertus*, *R. holaezii* and *R. syriacus*rootand leaf extracts using well diffusion method

Pathogen test strains	*R. confertus*		*R. holaezii*		*R. syriacus*		Positive control canamycin	Negative control DMSO
	root extract 70% ethanol	root extract 70% ethanol	root extract 70% ethanol	root extract 70% ethanol	root extract 70% ethanol	root extract 70% ethanol		
E. coli	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00	0 ± 00	21 ± 0.13	0 ± 00
B. subtilis	12 ± 0.17	5 ± 0.27	6 ± 1.02	6 ± 0.13	6 ± 1.21	8 ± 0.16	24 ± 1.43	4 ± 0.32
P. aureginosa	8 ± 0.17	10 ± 1.20	8 ± 00	6 ± 1.10	10 ± 00	12 ± 00	26 ± 1.47	5 ± 0.36
S. aureus	8 ± 0.01	7 ± 0.13	6 ± 1.42	11 ± 1.32	16 ± 0.14	19 ± 00	22 ± 1.38	4 ± 0.27
C. albicans	8 ± 00	11 ± 1.01	14 ± 0.17	8 ± 0.21	12 ± 0.11	13 ± 0.04	21 ± 1.36	6 ± 0.14

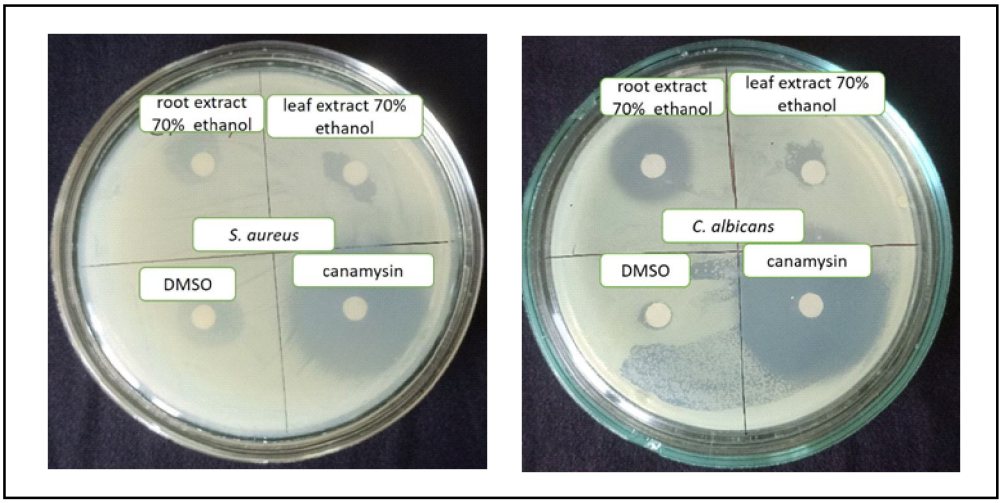

Figure 4: Antimicrobial activity of *R. confertus*, *R. holaezii*, *R. syriacus* plants in root and leaf extracts.

3.3 Determination of minimum inhibitory concentration

Determination of the minimum inhibitory concentration of extracts and fractions was carried out on the basis of the following test strains of *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*. The minimum inhibitory concentration of extracts and fractions with high antimicrobial activity was determined. The following results were obtained during the experiment: *R. confertus* EtOH fraction inhibited the growth of the *E. coli* test strain in a ratio of 1:4 at a concentration of 25 µg/ml. *R.confertus* root extract in 70% alcohol inhibited the growth of the *E.coli* test strain at a concentration of 50 µg/ml in a ratio of 1:2. *R. pomiricus* extract and chloroform fraction inhibited the growth of the *E. coli* test strain in a ratio of 1:8 at a concentration of 12.5 µg/ml. The root extract of *R. holaezii* in 70% ethanol at a ratio of 1:2 inhibited the growth of the *E. coli* strain at concentration of 50 µg/ml. It was observed that the leaf extract inhibited the growth of the *E. coli* strain at concentration of 25 µg/ml in a ratio of 1:4. The ethyl acetate fraction of the plant *R. confertus* inhibited the growth of the *B. subtilis* strain at a concentration of 50 µg/ml in a ratio of 1:2. The plant *R. confertus* leaf extract in 70% ethanol inhibited the growth of the *B. subtilis* strain at a concentration of 50 µg/ml in a ratio of 1:2. The plant *R. pomiricus* extract and the chloroform fraction inhibited the growth of the *B subtilis* strain in a ratio of 1:16 at a concentration of 6.25 µg/ml. The extract of the roots and leaves of plant *R. holaezii* in 70% ethanol inhibited the growth of the test strain *B. subtilis* at 50 µg/ml in a ratio of 1:2 and 12.5 µg/ml in a ratio of 1:8. Also, of the plant, *R. pomiricus* extract and the chloroform fraction inhibited the growth of the *P. aureginosa* strain at a concentration of 50 µg/ml in a ratio of 1:2. Root and leaf extracts of the plant *R. holaezii* in ethanol at a ratio of 1:2 and 1:4 inhibited the growth of the strain *P. aureginosa* concentrations of 50 µg/ml and 25 µg/ml. The ethyl acetate fraction of *R. confertus* inhibited the growth of the test strain *S. aureus* in a ratio of 1:2 at a concentration of 50 µg/ml. Also, extracts of roots and leaves in ethanol inhibited the growth of the test strain *S. aureus* at a concentration of 25 µg/ml in a ratio of 1:4. At the same time, the leaf extract in ethanol at a ratio of 1:2 inhibited the growth of the *S. aureus* test strain at a concentration of 50 µg/ml. Also, the plant *R. pomiricus* extract and the chloroform fraction inhibited the growth of the *S. aureus* test strain in a ratio of 1:2 at a concentration of 50 µg/ml. Root and leaf extracts of *R. holaezii* in ethanol at a ratio of 1:2 inhibited the growth of the *S. aureus* test strain at a concentration of 50 µg/ml (Table 4).

Table 4: Minimal inhibitory concentration of methanol crude extracts of the the genus, *Rumex* (*Polygonaceae*)

Plant samples	Pathogen test strains			
	E. coli	*B. subtilis*	*P. aureginosa*	*S. aureus*
	concentration (µg/ml)			
	MIC	MIC	MIC	MIC
Rumex confertus EtOH fraction	25 ± 0.011	50 ± 0.017	0.0 ± 0.0	50 ± 0.002
*Rumex confertus*root extract 70% ethanol	50 ± 0.011	50 ± 0.014	0.0 ± 0.0	25 ± 0.102
Rumex confertus leaf extract 70% ethanol	0.0 ± 0.0	50 ± 0.001	0.0 ± 0.0	50 ± 0.021
*Rumex pomiricus*70 % extracts	12.5 ± 0.103	6.25 ± 0.02	50 ± 0.032	50 ± 0.011
Rumex pomiricus $CHCl_3$ fraction	12.5 ± 0.010	6.25 ± 0.107	50 ± 0.041	50 ± 0.032
Rumex holaezii leaf extract 70% ethanol	50 ± 0.016	50 ± 0.108	50 ± 0.105	50 ± 0.036
Rumex holaezii root extract 70% ethanol	25 ± 0.018	6.25 ± 0.013	25 ± 0.032	50 ± 0.014
Ceftriaxone	6.25 ± 0.02	3.125 ± 0.02	6.25 ± 0.02	3.125 ± 0.02

3.4 Antioxidant activity

In this study, we have determined of the radical scavenging activity DPPH of the medicinal plant *R. confertus* EtOH, $CHCl_3$ fraction and 70% ethanol extract *R. pomiricus* EtOH fraction, $CHCl_3$ fraction, 70% extracts, *R. holaezii* and *R. syriacus* root and leaf extract (Figure 5 and Figure 6). The IC_{50} value of the extract and fractions was calculated using the average percentage of DPPH radical scavenging and concentration. Ascorbic acid used as a control showed higher activity than plant extracts and fractions at all concentrations. The medicinal plant *R. confertus* EtOH fraction showed antioxidant activity of 19.72 µg/ml at 50 µg/ml, 36.78 µg/ml at 100 µg/ml, and 56.82 µg/ml at 150 µg/ml.In addition, the *R. confertus* $CHCl_3$ fraction showed antioxidant activity of 25.65 µg/ml at 50 µg/ml, 39.47 µg/ml at 100 µg/ml and 61.42 µg/ml at 150 µg/ml. *R. confertus* extract showed antioxidant properties: 32.46 µg/ml at 50 µg/ml, 46.32 µg/ml at 100 µg/ml, and 68.23 µg/ml at 150 µg/ml. At the same time, the ethanol fraction of the plant *Rumex pomiricus* showed antioxidant activity of 19.98 µg/ml at a concentration of 50 µg/ml, 32.38 µg/ml at a concentration of 100 µg/ml and 59.94 µg/ml at a concentration of 150 µg/ml. The *R. pomiricus* $CHCl_3$ fraction showed the ability to radical scavenge activity at 29.54 µg/ml at 50 µg/ml, 47.83 µg/ml at 100 µg/ml, and 65.23 µg/ml at 150 µg/ml. The *R. pomiricus* extract showed antioxidant activity of 27.36 µg/ml at 50 µg/ml, 51.24 µg/ml at 100 µg/ml and 67.83 µg/ml at 150 µg/ml.

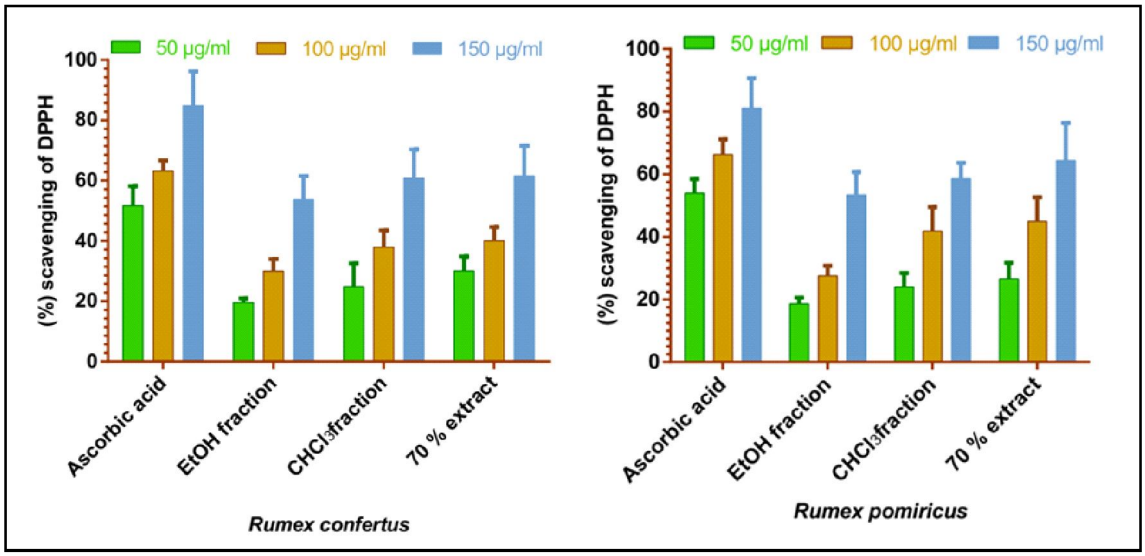

Figure 5: DPPH radical scavenging àctivity of *Rumex confertus* and *Rumex pomiricus* fractions and extracts.

At the next stage, the following results were obtained in determining the antioxidant activity of root and leaf extracts of plants *R. holaezii* and *R. syriacus*. Ascorbic acid used as a control showed higher activity than plant extracts. *R. holaezii* root extract in 70% ethanol showed antioxidant activity of 39.76 µg/ml at 50 µg/ml, 57.84 µg/ml at 100 µg/ml, and 64.52 µg/ml at 150 µg/ml. Plant leaf extract in 70% ethanol showed radical scavenging activity of 54.12 µg/ml at 50 µg/ml, 64.73 µg/ml at 100 µg/ml, and 74.68 µg/ml at 150 µg/ml. *R. syriacus* root extract showed antioxidant activity of 39.75 µg/ml at 50 µg/ml, 58.27 µg/ml at 100 µg/ml, 71.16 µg/ml at 150 µg/ml.The plant leaf extract showed DPPH radical scavenging activity of 45.84 µg/ml at 50 µg/ml, 67.19 µg/ml at 100 µg/ml, and 79.83 µg/ml at 150 µg/ml.

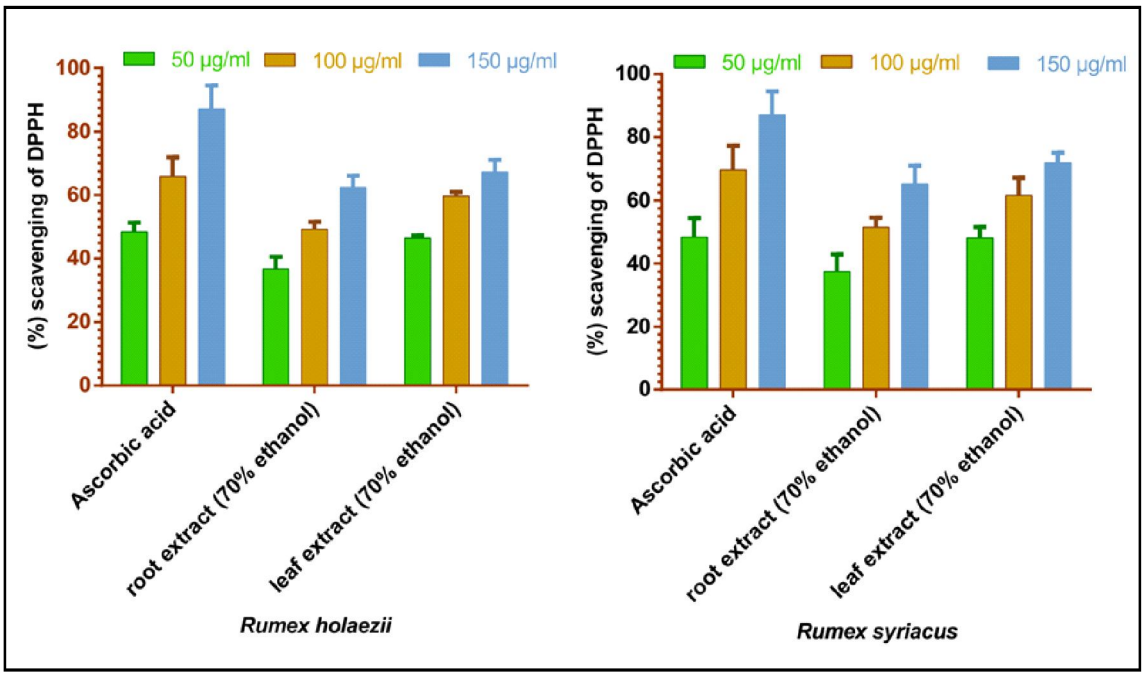

Figure 6: DPPH radical scavenging activity of *R. holaezii* and *R. syriacus* plant fractions and extracts.

IC_{50} values (p<0.01) for antioxidant activity were 24.53 µg/ml for ascorbic acid and 134.9 µg/ml for the EtOH fraction of *R. confertus*. It is known that the lower the IC_{50} value, the higher the antioxidant property of the plant extract. As can be seen from the table below, the *R. syriacus* leaf fraction in 70% ethanol has high antioxidant properties (Table 5).

Table 5: Percentage inhibition of DPPH free radical scavenging activity and IC_{50} of ascorbic acid and plants extract common bean

Plant samples		DPPH scavenging (%)			
		50 µg/ml	100 µg/ml	150 µg/ml	IC_{50}value(µg/ml)
R. confertus	EtOH fraction	19.72 ± 0.37^{c}	36.78 ± 1.48^{b}	56.82 ± 0.24^{a}	134.9
CHCl fraction	25.65 ± 1.34^{c}	39.47 ± 0.27^{b}	61.42 ± 0.41^{a}	116.7	
70% extracts	32.46 ± 1.36^{c}	46.32 ± 1.21^{b}	68.23 ± 0.18^{a}	111.3	
R. pomiricus	EtOH fraction	19.48 ± 1.41^{c}	32.38 ± 0.54^{b}	59.94 ± 1.32^{a}	121.4
CHCl fraction	29.54 ± 1.74^{c}	47.83 ± 0.31^{b}	65.23 ± 1.12^{a}	101.2	
70%extracts	27.36 ± 0.13^{c}	51.24 ± 0.41^{b}	67.83 ± 1.10^{a}	101.5	
R. holaezii	70% root extract	30.76 ± 1.42^{c}	57.84 ± 0.17^{b}	64.52 ± 1.17^{a}	109.1
70% leaf extract	54.12 ± 1.44^{c}	64.73 ± 0.12^{b}	74.68 ± 0.15^{a}	84.12	
R. syriacus	70% root extract	39.75 ± 2.51^{c}	58.27 ± 1.14^{b}	71.16 ± 1.02^{a}	64.32
70% leaf extract	45.84 ± 0.19^{c}	67.19 ± 1.26^{b}	79.83 ± 0.13^{a}	48.54	
Ascorbic acid (vitamin C)	57.82 ± 1.00^{c}	72.46 ± 1.78^{b}	97.83 ± 0.02^{a}	24.53	

All data are the mean standard deviation of a triplicate analysis (n = 3). The values in this column differ significantly (p<0.05).

4. Discussion

Plants belonging to the genus *Rumex* are widely used in traditional medicine and modern pharmaceuticals to combat many diseases such as cardiovascular disease, obesity, diabetes and cancer (Harshaw *et al*., 2010; Korpelainen and Pietiläinen, 2020; Khalil *et. al*., 2022). Biologically active compounds found widely in plants of the genus *Rumex* provide antimicrobial and antioxidant properties. Also, phenolic and flavonoid compounds contained in the plant destroy free radicals and prevent the initiation of oxidation processes (Jiang *et. al*., 2007; Laouini and Ouahrani, 2017). In this article, the antimicrobial and antioxidant activity, the of total phenols and flavonoids content in extracts and fractions of plants belonging to the genus *Rumex* were determined. The methanolic extract of *R. crispus* showed the highest result (27.32 ± 1.61 µg/ml) in terms of total phenol content. The methanol extract of *R. halaezii* showed a high result (51.65 ± 2.10 µg/ml) in terms of the total flavonoid content. The flavonoid content in the methanol extract of *R. tianschanicus* showed the pozitive result (58.41 ± 2.18 µg/ml). In articles published by Elzaawely *et al.* (2005) regarding the amount

of phenolic and flavonoid compounds, the hexane extract obtained from the aerial parts of the *R. japonicus* plant was 5.04 ± 1.01 µg/ml, the chloroform extract was 14.4 ± 1 .01 µg/ml, and the aqueous extract is 20.8 ± 1.01 µg/ml. In the data published by Sumaira *et al.* (2011) on the amount of flavonoid compounds, the n-hexane fraction of the plant *R. hastatus* showed results of 6.4 ± 1.13 µg/ml and the aqueous fraction showed results of 6.54 ± 1.84 µg/ml.

Changes in the amount of phenolic and flavonoid compounds in a plant can also vary depending on geographical distribution, environment, climatic conditions and processing methods. Medicines made from *Rumex* species have been used for centuries to treat a variety of ailments. *Rumex* species are reported to have good activity against pathogenic microbes. The activity of plant extracts against pathogenic microbes depends on factors such as plant species, plant organs (underground and aboveground), extraction methods and organic solvents (acetone, hexane, ethyl acetate, ethanol, methanol). Wegiera *et al.* (2011) reported that extracts and fractions obtained from plants *R. confertus*, *R. crispus*, *R. hydrolapathum* and *R. obtusifolius* have a good inhibitory effect against pathogenic strains of *S. aureus*and *S. epidermidis*.But, some gram-negative bacteria *E. coli*, *P. vulgaris* had a weak effect. It also had an antifungal effect on strains of the yeast *C. albicans.*

The best results obtained at the minimum inhibitory concentration are as follows: *R. pomiricus* 70 % extract MIC (6.25 ± 0.02 µg/ml); *R. pomiricus* $CHCl_3$ fraction MIC (6.25 ± 0.107 µg/ml).The results obtained in our study are consistent with the data presented by Wegiera *et al.* (2011). The values of antioxidant activity obtained in our studies were (p<0.01) IC_{50} for ascorbic acid - 24.53 µg/ml, *R. confertus* EtOH fraction - 134.9 µg/ml, significant changes were noted in the results obtained. The values of antioxidant activity obtained in our studies were IC_{50} for ascorbic acid ($p<0.01$) - 24.53 µg/ml. The IC_{50} value of the *R. confertus* EtOH fraction was 134.9 µg/ml, and significant changes were noted in the results obtained. The IC_{50} value of the *R. syriacus* leaf extract ($p<0.01$) was 48.54 µg/ml. Plants of the *Rumex* family contain many biologically active compounds that are a potential source of antibacterial, antifungal and antioxidant substances. The mechanism of antibacterial activity of these bioactive compounds is associated with a violation of the cytoplasmic membrane and inhibition of the activity of the dehydrogenase enzyme of the respiratory chain of microbes.The results obtained allow us to consider extracts and fractions of plants of the *Rumex* family as antibacterial and antioxidant agents. mL

5. Conclusion

With over 200 species worldwide, the genus *Rumex* has a long history of use in both food and medicine. These plants, rich in biologically active secondary metabolites, contain quinones, flavonoids, tannins, naphthalenes, terpenes, diterpene alkaloids, lignans and other components that exhibit antimicrobial, antioxidant, anti-inflammatory, antiviral and various pharmacological activities. In particular, many scientific articles have reported that quinones, which are the main components of *Rumex*, have a stronger antibacterial effect. The results obtained allow us to consider extracts and fractions of plants of the *Rumex* family as antibacterial and antioxidant agents. It is concluded that plants of the genus *Rumex* synthesize alternative compounds in the treatment of diseases caused by related pathogenic strains. These results provide a solid scientific basis for the discovery of new compounds of importance to the food and pharmaceutical industries. In addition, the chemical characterization of compounds from these plants requires chromatographic analysis using GC-MS and HPLC. At the same time, bioactive compounds derived from plants belonging to the *Rumex* genus require preliminary clinical trials to ensure that they do not cause toxic damage to human health when used in the medical and pharmaceutical industries.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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