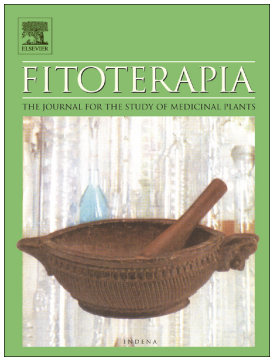

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Antibacterial screening of *Rumex* species native to the Carpathian Basin and bioactivity-guided isolation of compounds from *Rumex aquaticus*

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ABSTRACT

Plants belonging to the genus *Rumex* (family Polygonaceae) are used worldwide in traditional medicine for the treatment of various diseases caused by different microorganisms (e.g. bacteria-related dermatologic conditions, dysentery and enteritis). The present study focused on the antibacterial screening of *Rumex* species native to the Carpathian Basin, and isolation of compounds from one of the most efficient species, *Rumex aquaticus*. The antibacterial effects of *n*-hexane, chloroform and aqueous fractions of methanol extracts prepared from different parts of 14 *Rumex* species (*R. acetosella*, *R. acetosa*, *R. alpinus*, *R. aquaticus*, *R. conglomeratus*, *R. crispus*, *R. hydrolapathum*, *R. obtusifolius* subsp. *obtusifolius*, *R. obtusifolius* subsp. *subalpinus*, *R. patientia*, *R. pulcher*, *R. scutatus*, *R. stenophyllus* and *R. thyrsiflorus*) were investigated against *Staphylococcus epidermidis*, *S. aureus*, MRSA, *Bacillus subtilis*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, *S. pneumoniae*, *S. agalactiae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* using the disc diffusion method. Mainly the *n*-hexane and chloroform extracts prepared from the roots of the plants displayed high antibacterial activity (inhibition zones > 15 mm) against one or more bacterial strains. The highly active extracts of the aerial part and root of *R. aquaticus* were subjected to a multistep separation procedure. 19 Compounds, among them naphthalenes (musizin, and its glucoside, torachrysone-glucoside, 2-methoxystypandrone), anthraquinones (emodin, chrysophanol, physcion, citreorosein, chrysophanol-8-*O*-glucoside), flavonoids (quercetin, quercetin-3,3'-dimethylether, isokaempferide, quercetin 3-*O*-arabinoside, quercetin 3-*O*-galactoside, catechin), stilbenes (resveratrol, piceid), and 1-stearoylglycerol were isolated from the plant. The antibacterial activities of isolated compounds were determined, and it was observed that especially naphthalenes exerted remarkable antibacterial effects against several bacterial strains.

Keywords: Polygonaceae, *Rumex* species, *Rumex aquaticus*, antibacterial activity, 2-methoxystypandrone, musizin

1. Introduction

Despite the wide spectra of antibacterial pharmaceutics, the hospital-acquired infections – also known as nosocomial infections – are still one of the major problems of modern medicine. The uncontrolled usage of antibiotics may increase the selection pressure of resistant strains. According to the assessment of the World Health Organization, 5-10% of all patients in hospitals suffer from nosocomial infection, often caused by methicillin-resistant *Staphylococcus aureus* (MRSA). This bacterium is resistant to penicillin and cephalosporin and sensitive only to vancomycin and teicoplanin, however vancomycin-resistant *S. aureus* strains (VRSA) have also been reported [1]. MRSA can cause wound, lower respiratory and urinary infections or septicaemia. Severe infections are more common in intensive care units and in older population, which can elongate their hospital stays and increase the therapeutic costs [2]. Besides MRSA, several bacterial strains, including *Staphylococcus epidermidis*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*, can cause nosocomial infections [3–6].

The increasing number of resistant strains of microorganisms motivates the development of new synthetic and plant-derived antibacterial agents. Plants can be effective sources of antimicrobials as they have been traditionally used for centuries to treat different microbial infections. More than 70% of pharmacologically active plant derived compounds were discovered after ethnomedicinal evaluations of plant species. In case of plant derived compounds with antimicrobial activity, large chemical diversity can be observed. This rich diversity has partly arisen because of the evolutionary selection for improved defensive mechanisms against a broad array of microorganisms [7].

Plants belonging to the genus *Rumex* (family Polygonaceae) are used traditionally for the treatment of different diseases related to microbial infections, e.g. dermatologic conditions, dysentery, enteritis and ascariasis [8–10]. The antibacterial capacity of several

extracts of *Rumex* species with different polarity have been published in the literature. Wegiera et al. tested the inhibitory effects of extracts prepared from *R. confertus, R. crispus, R. hydrolapathum* and *R. obtusifolius* on the growth of Gram-positive (Staphylococci) and Gram-negative (*E. coli, P. mirabilis* and *P. aeruginosa*) bacterial strains and different effects were detected [11]. The EtOH extract of aerial parts of *R. obtusifolius* inhibited the growth of *S. pyogenes* [12]. Among the *n*-hexane, CH_2Cl_2 and MeOH extracts of the leaves of the same plant, the CH_2Cl_2 extract was active only against *E. coli*, while the MeOH extract showed significant antibacterial effect against all tested bacterial strains (*B. cereus, B. subtilis, E. coli*, ampicillin-resistant *E. coli, S. aureus* and *Salmonella typhii*) [13].

The MeOH, $CHCl_3$ and ether extracts of different parts of *R. vesicarius* (collected at different vegetative stages) were found to be effective against *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. pneumoniae* and and *S. pyogenes* [14]. Remarkable antibacterial activity of the EtOH extract of *in vitro* germinated seedlings of the same plant was detected against *P. aeruginosa, E. coli* and *S. pneumoniae* [15]. The MeOH extract of the leaves of *R. nepalensis* had moderate antimicrobial activity against *Bacillus cereus, B. subtilis, E. coli* and *P. aeruginosa* [16]. In another investigation, antibacterial activities of different parts of the plant were also determined. The highest inhibition was recorded in case of leavesH_2O extract against *E. coli* [17]. The benzene and EtOAc extracts prepared from the roots of the same plant showed significant activity against *S. aureus, S. mutans, E. coli* and *P. aeruginosa* [18].

Evaluation of the antibacterial activity of various extracts gained from aerial parts of *R. japonicus* revealed that the EtOAc extract possessed the strongest antibacterial activity against *B. subtilis, B. cereus* and *E. coli*. It was correlated with the high amount of phenolic compounds presented in the extract [19]. 70% MeOH extract of *R. dentatus* showed antibacterial activity against *B. megaterium, B. subtilis, Enterobacter cloacea* and *P. aeruginosa* [20]. *R. nervosus* and *R. abyssinicus* exhibited activity against *S. pyogenes* and

Corynebacterium diphtheriae. Neither of the plants were active against the Gram negative *P. aeruginosa* and *E. coli* [21]. The EtOAc extract of *R. hastatus* was highly active against *S. aureus* and *E.coli* [22]. The EtOH and MeOH extracts of the leaves of *R. alveolatus* exhibited dose-dependent antibacterial activity against *S. aureus* and *P. aeruginosa* [23]. The EtOH extracts of leaves of *R. chalepensis* showed high activity against multi-resistant *E. coli*, *S. aureus* and *P. aeruginosa* [24].

R. patientia subsp. *pamiricus*, which is used to treat inflammatory ailments, pain, fever and infections in the Pamir Mountains in North-East Afghanistan, had weak inhibitory effect against *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa* [25]. The aerial parts of *R. alpinus* and *R. caucasicus*, extracted with H_2O and MeOH, were screened for antibacterial activity against 52 bacterial strains. It was concluded that methanol is a better solvent for extracting antimicrobial substances compared to water. The plant extracts did not show any selectivity against Gram-negative or Gram-positive bacteria [26].

This study aimed to screen the antibacterial activity of *Rumex* species, collected in the Carpathian Basin, against standard bacterial strains. The further objective of this work was the isolation of the pharmacologically active components of the most active species, *R. aquaticus*. From the investigated species (n = 14), only *R. crispus* and *R. hydrolapathum* were tested previously for antibacterial activity [11].

2. Experimental

2.1. General procedures

NMR spectra were recorded in DMSO (dimethyl sulfoxide), on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) or 125 MHz (^{13}C); the signals of the deuterated solvent

were taken as reference. Two-dimensional (2D) experiments ($^{1}H–^{1}H$ COSY, HSQC, HMBC and NOESY) were set up, performed and processed with the standard Bruker protocol. ESIMS was performed on an API 2000 instrument in APCI positive mode. Separations with medium pressure liquid chromatography (MPLC) was carried out on a Büchi MPLC (Pump Manager C615, Pump Module C605) using silica gel (Kieselgel 60, 40-63 μm, Merck, 1.09385.1000) or prepacked RP-cartridge (RP18ec sorbent, 40-63 μm, Büchi, 054863). HPLC was carried out on a Wufeng LC-100 HPLC, using normal (LiChrospher Si60 (5 μm) LiChroCART 125-4) and reverse phase [Phenomenex, Kinetex 5u C18 100A; LiChrospher LiChroCART 250-4 RP-18e (5 μm)] columns. For vacuum liquid chromatography (VLC), silica gel (60G, 15 μm, Merck 11677) was applied. Silica gel plates were applied for analytical and preparative TLC (Merck 5717 and 5715). Polyamide (MP Biomedicals) and Sephadex LH-20 were used for column chromatography (CC and GFC). Reverse phase column chromatography was carried out on reverse phase silica gel (LiChroprep RP-18, 40-63 μm, Merck). Separation was monitored at UV 254 nm.

2.2. Plant material

Plants were collected mainly in the flowering period between June and September 2010 (*R. aquaticus* was collected in July 2013), in several regions of the Carpathian Basin (Hungary and Romania). Botanical identification of the plant material was performed by Dr. Gusztáv Jakab (Institute of Environmental Sciences, Szent István University, Szarvas, Hungary) and voucher specimens (No. 790-803 and 816) have been deposited at the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

2.3. Extraction of plant samples

For the antimicrobial-screening assay, extracts were prepared from 10 g of air-dried plant materials [root, herb (aerial parts of small-stature plants with small leaves or flowers), leaves or flowers] with 3 × 100 mL of MeOH, then the solutions were evaporated to dryness under vacuum. The residues were dissolved in 50 mL of 50% aqueous MeOH and then solvent–solvent partitions were performed between *n*-hexane (3 × 50 mL) (extracts A) and chloroform (3 × 50 mL) (extracts B), and the residues gave extracts C. The yields (w/w) of the extracts are shown in Table 1.

2.4. Isolation of compounds from R.aquaticus

2.4.1. Isolation of compounds from the aerial parts of R. aquaticus

The dried aerial part of *R. aquaticus* (550 g) was percolated with MeOH (15 L) at room temperature. The crude extract was concentrated to 200 mL under reduced pressure, the residue was dissolved in 200 mL 50% aqueous MeOH and solvent–solvent partition was performed with *n*-hexane (3 × 500 mL) and $CHCl_3$ (3 × 500 mL). In order to remove water soluble compounds, the remaining aqueous fraction was partitioned with EtOAc (3 × 500 mL). The $CHCl_3$ fraction (5 g) was separated first on polyamide CC, using the gradient system of MeOH–H_2O (1:4, 2:3, 3:2 and 4:1). The fraction eluted with 40% MeOH was separated by RP-VLC (MeOH–H_2O from 3:7 to 7:3) to yield five subfractions. The separation of subfraction 4 by VLC (CH_2Cl_2–MeOH from 99:1 to 8:2) resulted in five fractions. From fraction 4, compound **3** (10 mg) was isolated by preparative TLC (MeOH–H_2O 6:4). From fraction 5, compound **10** (7.6 mg) was crystallized. The fraction eluted with 80% MeOH from

polyamide column was further separated by RP-VLC (MeOH–H_2O from 1:1 to 9:1) to afford 15 subfractions. From subfraction 4, compounds **14** (2.5 mg) and **15** (1.5 mg) were isolated by preparative TLC, using CH_2Cl_2–MeOH 9:1 as eluent. Further separation of subfraction 14 by VLC (*n*-hexane–EtOAc–MeOH from 8:2:0 to 5:5:1) resulted compound **19** (55 mg).

After evaporation, the EtOAc fraction (30 g) was separated by VLC on silica gel (60 GF_{254} 15 μm) with gradient mixtures of $CHCl_3$–MeOH (from 99:1 to 1:1). Fractions with similar composition were combined to afford fractions F1–F16. From F5, compound **8** (3.4 mg) was isolated by gel filtration chromatography (GFC), using using CH_2Cl_2–MeOH (1:1) as eluent.

After GFC fractionation of F7, nine subfractions were obtained. Separation of subfraction 6 by RP-VLC (using gradient mixtures of MeOH–H_2O from 1:1 to 8:2) resulted in four fractions. From fractions 2 and 3, compound **11** (8.1 mg) was isolated by RP-HPLC (MeOH–H_2O 3:2, with the flow rate of 1 mL/min, t_R = 3.3 min).

Fractionation of F8 by GFC afforded eight subfractions. From subfraction 6, compound **13** (4.5 mg) was isolated using RP-HPLC (MeOH–H_2O 3:2, with a flow rate of 1 ml/min, t_R = 6 min). Fractionation of F9 by reverse phase medium pressure liquid chromatography (RP-MPLC), using gradient system of MeOH–H_2O, resulted in nine subfractions. Further separation of subfraction 1 by GFC, yielded four fractions. Compound **18** (6.3 mg) was isolated from fraction 3 by preparative RP-TLC (MeOH–H_2O 7:3). From subfraction 4, compound **16** (150.6 mg) was crystallized. Further fractionation of subfraction 6, using GFC, six fractions were obtained. From fraction 2, compound **2** (5.3 mg) was isolated by preparative TLC (EtOAc–MeOH–H_2O 100:16:12), while compound **9** (6.4 mg) was crystallized from fraction 6. Finally, compound **17** (139.7 mg) was crystallized from F11.

2.4.2. Isolation of compounds from the roots of R. aquaticus

The dried roots (800 g) were percolated with MeOH (35 L) at room temperature. The crude extract was concentrated to 400 mL under reduced pressure, the residue was dissolved in 200 mL 50% aqueous MeOH and solvent–solvent partition was performed with *n*-hexane (3 × 500 mL), $CHCl_3$ (3 × 500 mL) and EtOAc (3 × 500 mL). After evaporation, the *n*-hexane fraction (14 g) was separated by VLC on silica gel (60 GF_{254}, 15 μm) with gradient mixtures of $CHCl_3$–MeOH (from 99:1 to 1:9), to afford fractions 1-14. From fraction 2, compound **6** (15.6 mg) was crystallized. Further separation of fraction 3 by VLC, using cyclohexane–EtOAc gradient systems (from 99:1 to 6:4), six subfractions were obtained. From subfraction 3, compound **1** (14.2 mg) was isolated by preparative TLC (cyclohexane–EtOAc 8:2).

Separation of fraction 5 with RP-VLC (MeOH–H_2O gradient, from 6:4 to 99:1) resulted in eight subfractions. From subfraction 2, compound **4** (10.2 mg) was isolated by preparative TLC (cyclohexane–EtOAc–MeOH 7:3:1).

After evaporation, the $CHCl_3$ fraction (7 g) was subjected to MPLC on silica gel with gradient mixtures of $CHCl_3$–MeOH (from 99:1 to 6:4), to afford eleven subfractions. From subfraction 8, compound **10** (4.4 mg) was obtained by RP-VLC (MeOH–H_2O from 1:1 to 99:1).

After evaporation, the EtOAc fraction (100 g) was separated by CC on polyamide, using the gradient system of MeOH–H_2O (2:3, 1:1, 3:2 and 4:1). Further separation of the fraction eluted with MeOH–H_2O 2:3 by VLC (CH_2Cl_2–MeOH from 95:5 to 8:2) resulted in nine subfractions. From subfraction 8, compound **12** (20.7 mg) was isolated using preparative TLC (CH_2Cl_2–MeOH 8:2). Separation of the fraction eluted with MeOH–H_2O 1:1 by VLC (CH_2Cl_2–MeOH from 99:1 to 8:2) nine subfractions were obtained. From subfraction 1 compound **6** (6.5 mg) and **7** (4.4 mg) were isolated by HPLC (cyclohexane–EtOAc 95:5, flow

rate = 1.5 mL/min, t_R = 4.46 and 6.36 min). From subfraction 3, compound **5** (5.2 mg) was isolated by RP-HPLC (MeOH–H_2O 8:2, flow rate = 1 mL/min, t_R = 3.6 min). Finally, from subfraction 4, compounds **2** (7.5 mg) and **3** (10.3 mg) were yielded by RP-HPLC (MeOH–H_2O 1:1, flow rate = 1 mL/ml, t_R = 1.6 and 2.6 min).

2.5. Antimicrobial assay

Antimicrobial activity of the plant extracts was tested against 11 bacterial strains (*Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300, *Staphylococcus epidermidis* ATCC 1228, *Bacillus subtilis* ATCC 6633, *Moraxella catarrhalis* ATCC 43617, *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 49619, *Streptococcus agalactiae* ATCC 13813, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603). The antibacterial screening assay was performed by disc-diffusion method. The test organisms (*S. aureus*, methicillin-resistant *S. aureus*, *S. epidermidis*, *B. subtilis*, *P. aeruginosa*, *E. coli* and *K. pneumoniae*) were cultured at Mueller-Hinton agar plates (bio-Mérieux) at 37 °C. Columbia agar + 5% sheep blood (COS) plates (bio-Mérieux) were used for growing of *M. catarrhalis*, *S. pyogenes*, *S. pneumoniae*, and *S. agalactiae*. The bacteria isolates were picked from overnight cultures and suspensions were prepared in sterile saline solution by adjusting the turbidity to match 0.5 McFarland standards to give a resultant concentration of 1-2 × 10^8 cfu/mL. The sterile filter paper discs (6 mm diameter) impregnated with the extracts (10 µL of dried extracts redissolved in DMSO at 50 mg/mL) or the isolated compounds (10 µL, dissolved in DMSO at 10 mg/mL) were placed on the agar plate seeded with the respective bacteria. The solvent (DMSO) was served as negative control. The plates were then incubated at 37 °C for 24 h under aerobic conditions. The entire diameters of inhibition zone (including

the disc) produced by the plant extracts was measured and recorded. It was observed that DMSO did not inhibit the growth of microorganisms in this used concentration. Erythromycin and vancomycin served as positive controls at 15 μg/disc.

The active extracts and compounds (purity > 96%) were further subjected to determine their minimal inhibitory concentration (MICs) by microdilution method. Briefly, in the 96-well plates the stock solutions of extracts (50 mg/mL in DMSO) were serially diluted with Mueller-Hinton broth to arrive at final concentration between 2.5 mg/mL and 4.9 μg/mL. 100 μL of inoculum (0.5 McFarland, 1-2 × 10^8 CFU/mL) were then added to the wells. A sterility check (medium and DMSO in amount corresponding to the highest concentration), negative control (medium, DMSO and inoculum) and positive control (medium, DMSO, inoculum and vancomycin) were included for each experiment. The plates were then incubated at 37 °C for 24 hours under aerobic environment. The MIC of preparation was the lowest concentration that completely inhibited the visible bacterial growth.

3. Results and discussion

In the course of our screening study, the antibacterial activities of 14 species of the *Rumex* genus (*R. acetosella* L., *R. acetosa* L., *R. alpinus* L., *R. aquaticus* L., *R. conglomeratus* Murr., *R. crispus* L., *R. hydrolapathum* Huds., *R. obtusifolius* subsp. *obtusifolius* L., *R. obtusifolius* subsp. *subalpinus* (Schur) Čelak., *R. patientia* L., *R. pulcher* L., *R. scutatus* L., *R. stenophyllus* Ledeb. and *R. thyrsiflorus* Fingerh.) occurring in the Carpathian Basin were evaluated. The results of the assays are listed in Table 1. The extracts were prepared with methanol from selected plant organs and then solvent–solvent partitions were made with *n*-hexane (A) and $CHCl_3$ (B). The remaining aqueous fractions were signed as (C) (altogether 84 extracts). According to the size of the inhibition zone (mm), antibacterial effects causing

<10 mm inhibition were considered weak, 10-15 mm inhibition were considered moderate, while15< mm were considered strongly active. At 50 mg/mL (500 µg/disc), a total of 42 extracts demonstrated antimicrobial activity against at least one of the tested microbial strains.

Among the fractions with different polarities, fractions B (containing $CHCl_3$-soluble lipophilic constituents) and fractions C (remaining aqueous fractions) proved to be active. The *n*-hexane extracts (fractions A) showed pronounced antimicrobial effects in only a few cases (*R. alpinus* roots, *R. aquaticus* roots and *R. patientia* roots).

From the active fractions, three *n*-hexane extracts [*R. alpinus* roots (A) (26.5 ± 1.5 mm), *R. aquaticus* roots (A) (18.7 ± 0.6 mm) and *R. patientia* roots (A) (21.4 ± 1.2 mm) against *S. aureus* and *R. alpinus* roots (A) on MRSA (16.8 ± 1.2 mm)]; four $CHCl_3$-soluble fractions [*R. acetosa* roots (B) on *S. epidermidis* (18.5 ± 1.5 mm) and *S. aureus* (16.0 ± 1.0 mm); *R. conglomeratus* herbs (B) on *M. catarrhalis* (18.4 ± 0.8 mm); *R. crispus* roots (B) against *S. pneumoniae* (16.6 ± 0.6 mm); *R. pulcher* whole plant (B) on *B. subtilis* (16.5 ± 0.6 mm)] and two aqueous extracts [*R. crispus* herb (C) (15.5 ± 0.5 mm) and *R. patientia* flowers (C) (16.3 ± 0.2 mm) against *S. epidermidis*] exerted strong antibacterial activity against at least one bacterial strains.

Concerning the bacterial strains, *P. aeruginosa, E. coli, K. pneumoniae, S. pyogenes* and *S. agalactiae* showed resistance against most of the extracts and only the chloroform fractions prepared from the roots of *R. acetosa, R. alpinus, R. aquaticus* and *R. crispus* proved to be active against the last two strains listed above. The *n*-hexane extract (A) of *R. alpinus* roots had high inhibitory activity against MRSA, while 28 of the total extracts showed weak or moderate activity against this bacterium.

Table 1.

Antibacterial activity of *Rumex* species on different bacterial strains

Species	Plant parts	Fraction	Yield (w/w%)	Antibacterial activity (inhibition zone in mm)							
				S. epidermidis	*S. aureus*	MRSA	*B. subtilis*	*M. catarrhalis*	*S. pyogenes*	*S. pneumoniae*	*S. agalactiae*
Rumex acetosella L.	whole plant	A	2.9	-	-	-	-	-	-	-	-
		B	2.9	-	-	-	-	-	-	-	-
		C	3.9	-	-	-	-	-	-	-	-
R. acetosa L.	herbs	A	5.0	-	-	-	-	-	-	-	-
		B	3.4	-	-	-	-	-	-	7.5 ± 0.5	-
		C	8.2	-	-	-	-	-	-	-	-
	roots	A	1.8	-	-	-	-	-	-	7.0 ± 0	-
		B	3.3	18.5 ±1.5	16.0 ± 1	12.4 ± 0.6	12.2 ± 0.8	10.2 ± 0.4	8.0 ± 0	8.5 ± 1	-
		C	13.6	9.0 ± 0	8.0 ± 0	9.0 ± 0	-	7.5 ± 0.5	-	-	-
R. alpinus L.	flowers/ fruits	A	1.9	-	-	-	-	-	-	-	-
		B	2.4	-	11.5 ± 0.5	-	-	7.4 ± 0.6	-	-	-
		C	12.6	8.0 ± 1.0	8.4 ± 0.4	8.0 ± 0	7.5 ± 1.5	7.0 ± 0	-	-	-
	leaves	A	3.7	-	-	-	-	-	-	-	-
		B	2.2	-	-	-	-	-	-	-	-
		C	13.4	-	-	-	-	-	-	-	-
	roots	A	2.4	14.4 ± 1.2	26.5 ± 1.5	16.8 ± 1.2	12.0 ± 1.0	10.3 ± 0.6	-	10.6 ± 0.6	-
		B	3.9	12.2 ± 0.6	8.0 ± 0	-	7.5 ± 0.5	-	-	8.0 ± 0	10.4 ± 0.8
		C	21.7	8.0 ± 0	7.4 ± 0.6	7.4 ± 0.6	-	-	-	-	-
R. aquaticus L.	herbs	A	3.3	-	-	-	-	-	-	-	-
		B	1.4	-	-	-	-	-	-	-	-
		C	6.3	10.0 ± 0	10.3 ± 0.6	11.3 ± 0.6	9.6 ± 0.6	12.7 ± 0.6	-	-	-
	roots	A	1.4	12.3 ± 0.6	18.7 ± 0.6	9.0 ± 0	10.3 ± 1.5	12.0 ± 0.5	-	10.5 ± 0.5	-
		B	2.1	13.3 ± 1.5	9.0 ± 0.5	-	9.7 ± 0.6	8.0 ± 0	11.3 ± 0.6	11.0 ± 1.0	-
		C	18.4	12.0 ± 0	13.0 ± 1.0	10.7 ± 0.6	10.3 ± 0.6	12.7 ± 0.6	-	-	-
R. conglomeratus Murr.	herbs	A	3.3	-	-	-	-	-	-	-	-
		B	6.3	11.4 ± 0.6	8.4 ± 0.4	8.0 ± 0	8.5 ± 0.5	18.4 ± 0.8	-	10.5 ± 0.5	-

Species	Plant parts	Fraction	Yield (w/w%)	Antibacterial activity (inhibition zone in mm)							
				S. epidermidis	*S. aureus*	MRSA	*B. subtilis*	*M. catarrhalis*	*S. pyogenes*	*S. pneumoniae*	*S. agalactiae*
		C	10.6	10.0 ± 1.0	10.8 ± 0.6	9.2 ± 0.4	9.5 ± 1.0	7.0 ± 0	-	-	-
R. crispus L.	herbs	A	2.0	-	-	-	-	-	-	-	-
		B	3.1	-	-	-	-	-	-	-	-
		C	7.8	15.5 ± 0.5	12.4 ± 0.6	12.0 ± 0.5	10.2 ± 0.6	10.6 ± 0.6	-	-	-
	leaves	A	3.3	-	-	-	-	-	-	-	-
		B	2.7	-	-	-	-	-	-	-	-
		C	5.6	-	-	-	-	-	-	-	-
	roots	A	2.4	-	-	-	-	-	-	-	-
		B	4.4	-	-	-	-	-	12.4 ± 0.8	16.6 ± 0.6	-
		C	16.6	8.0± 0.5	8.5 ± 1.0	8.5 ± 0.5	-	-	-	-	-
R. hydrolapathum Huds.	leaves	A	2.1	-	-	-	-	-	-	-	-
		B	2.9	-	-	-	-	-	-	-	-
		C	14.5	10.5 ± 0.5	10.4 ± 0.8	8.0 ± 0	10.8 ± 0.6	8.5 ± 1.0	-	-	-
	roots	A	1.0	-	-	-	-	-	-	-	-
		B	4.5	-	-	-	-	-	-	-	-
		C	25.0	12.4 ± 0.8	12.5 ± 0.5	12.3 ± 0.6	13.1 ± 0.4	12.4 ± 0.7	-	-	-
R. obtusifolius subsp. *obtusifolius* L.	herbs	A	7.5	-	-	-	-	-	-	-	-
		B	3.8	-	-	-	-	-	-	-	-
		C	7.1	8.4 ± 0.6	8.0 ± 0.5	8.5 ± 1.5	-	-	-	-	-
	roots	A	3.0	-	-	-	-	-	-	-	-
		B	3.3	-	-	-	-	-	-	-	-
		C	14.3	12.1 ± 0.4	11.4 ± 0.8	10.0 ± 0.5	12.6 ± 0.6	10.5 ± 1.5	-	-	-
R. obtusifolius subsp. *subalpinus* (Schur) Čelak.	herbs	A	3.8	-	-	-	-	-	-	-	-
		B	1.2	8.4 ± 0.4	9.5 ± 1.0	-	-	-	-	8.0 ± 0	-
		C	6.2	12.5 ± 0.5	10.3 ± 0.6	10.8 ± 0.8	12.6 ± 1.2	7.0 ± 0.5	-	-	-
	roots	A	0.9	-	-	-	-	-	-	7.5 ± 1.5	-
		B	1.4	12.8 ± 1.4	7.5 ± 0.5	10.4 ± 0.8	-	-	-	7.4 ± 0.4	-
		C	31.7	-	-	7.3 ± 0.6	-	-	-	-	-
R. patientia L.	flowers	A	3.9	-	-	-	-	-	-	-	-
		B	4.0	-	-	-	-	-	-	-	-

Species	Plant parts	Fraction	Yield (w/w%)	Antibacterial activity (inhibition zone in mm)							
				S. epidermidis	*S. aureus*	MRSA	*B. subtilis*	*M. catarrhalis*	*S. pyogenes*	*S. pneumoniae*	*S. agalactiae*
		C	6.0	16.3 ± 0.2	13.7 ± 0.4	12.6 ± 0.3	10.8 ± 0.7	12.5 ± 0.5	-	-	-
	leaves	A	3.5	-	-	-	-	-	-	-	-
		B	0.7	-	-	-	-	-	-	-	-
		C	3.6	-	-	-	-	-	-	-	-
	roots	A	2.4	13.3 ± 0.6	21.4 ± 1.2	8.4 ± 0.4	14.6 ± 1.2	11.6 ± 0.8	-	14.5 ± 1.0	-
		B	3.0	10.0 ± 0.5	10.0 ± 0.5	10.6 ± 0.6	8.3 ± 0.6	10.0 ± 1.0	10.4 ± 0.6	12.8 ± 0.6	-
		C	22.4	8.5 ± 1.0	8.0 ± 0	8.4 ± 0.4	-	-	-	-	-
R. pulcher L.	whole	A	3.5	-	-	-	-	-	-	-	-
	plant	B	1.6	-	-	-	16.5 ± 0.6	-	-	-	-
		C	8.8	9.2 ± 0.4	-	8.8 ± 0.4	-	7.2 ± 0.3	-	-	-
R. scutatus L.	whole	A	4.7	-	-	-	-	-	-	-	-
	plant	B	3.4	-	-	-	10.4 ± 0.3	-	-	-	-
		C	7.0	-	-	-	-	-	-	-	-
R. stenophyllus Ledeb.	flowers/	A	3.7	-	-	-	-	-	-	-	-
	fruits	B	5.5	-	-	-	-	-	-	-	-
		C	12.6	14.3 ± 0.7	12.2 ± 0.6	11.0 ± 0.5	10.6 ± 0.7	10.3 ± 0.4	-	-	-
	leaves	A	2.0	-	-	-	-	-	-	-	-
		B	0.6	-	-	-	-	7.0 ± 0.5	-	-	-
		C	8.8	-	-	-	-	-	-	-	-
	roots	A	2.4	-	10.5 ± 0.5	-	-	-	-	-	-
		B	5.4	10.4 ± 0.7	12.7 ± 0.3	-	-	-	-	10.5 ± 0.5	-
		C	17.3	12.1 ± 0.8	9.0 ± 1.5	10.6 ± 0.8	-	8.7 ± 0.6	-	-	-
R. thyrsiflorus Fingerh.	herbs	A	3.6	-	-	-	-	-	-	-	-
		B	2.6	-	7.5 ± 0.5	-	-	7.3 ± 0.7	-	7.3 ± 0.6	-
		C	7.1	-	7.8 ± 0.6	8.7 ± 0.3	-	7.6 ± 0.6	-	-	-
	roots	A	1.3	-	-	-	-	-	-	-	-
		B	2.2	-	-	-	-	-	-	-	-
		C	6.3	11.3 ± 0.6	12.3 ± 0.6	9.7 ± 0.6	10.3 ± 0.6	10.7 ± 1.2	-	-	-
Erythromycin (15				32.1 ± 0.7	27.0 ± 0.5	-	30.4 ± 0.2	32.2 ± 0.8	24.4 ± 0.6	32.1 ± 0.3	30±0.4

Species	Plant parts	Fraction	Yield (w/w%)	Antibacterial activity (inhibition zone in mm)							
				S. epidermidis	*S. aureus*	MRSA	*B. subtilis*	*M. catarrhalis*	*S. pyogenes*	*S. pneumoniae*	*S. agalactiae*
µg/disc)											
vancomycin (5 µg/disc)				-	-	15.5 ± 0.6	-	-	-	-	17±0.2

A: *n*-hexane fraction, B: chloroform fraction, C: remaining aqueous fraction, -: diameter of inhibition zone <5 mm

Regarding the different plant parts of *Rumex* species, usually the roots proved to have significant effects against multiple bacterial strains. For example, in case of *R. patientia*, the leaf extract did not possess any antibacterial activity; in case of its flowers only the extract C was active, while the root extract of the plant showed activity against almost all bacterial strains.

The aqueous fraction (C) of the aerial parts of *R. aquaticus*, and the *n*-hexane (A), chloroform (B) and aqueous (C) fractions of the roots of *R. aquaticus* possessed remarkable antimicrobial effects (Table 1). With the combination of different chromatographic methods (RP-VLC, prepTLC and HPLC), 19 compounds were isolated from *R. aquaticus*. The structure determination of the compounds was performed by 1D and 2D NMR, and HRMS investigations and with comparison of their spectral data with those reported in the literature. The identified compounds were the naphtalenes musizin (**1**) [27], musizin-8-*O*-glucoside (**2**) [28], and torachrysone-glucoside (**3**) [29]; the naphtoquinone 2-methoxystypandrone (**4**) [30]; the anthraquinones emodin (**5**), chrysophanol (**6**), physcion (**7**) [31], citreorosein (**8**) [32], emodin-8-*O*-glucoside (**9**) [33], and chrysophanol-8-*O*-glucoside (**10**) [34]; the stilbenes resveratrol (**11**) [35] and piceid (**12**) [36]; and the flavonoids quercetin (**13**), quercetin-3,3'-dimethylether (**14**) [37], isokaempferide (**15**) [38], quercetin 3-*O*-arabinoside (**16**), quercetin 3-*O*-galactoside (**17**), and catechin (**18**) [39]; and the monoacylglycerol 1-stearoylglycerol (**19**) [40]. Apart from musizin-8-*O*-glucoside, all compounds were isolated for the first time from *R. aquaticus*.

Among the isolated compounds musizin (MIC = 57.8 μM, in case of *M. catarrhalis*; MIC = 231.5 μM, in cases of *S. epidermidis*, *S. aureus* and *B. subtilis*; and MIC = 463 μM, in case of MRSA), and its glycoside (musizin-8-*O*-glucoside, MIC = 529.1 μM, in case of *B. subtilis*), and 2-methoxystypandrone (MIC = 48 μM, in case of *M. catarrhalis*; MIC = 96 μM, in cases of *S. aureus* and *B. subtilis*; and MIC = 192.3 μM, in cases of *S. epidermidis* and

MRSA) showed remarkable antibacterial activity, while other compounds proved to be inactive. The aglycon musizin was more active, than its glucoside.

Previously naphthalenes were also isolated from other *Rumex* species (*R. alpinus, R. crispus, R. dentatus, R. hastatus, R. japonicus, R. nepalensis* and *R. patientia*) [10]. The antimicrobial effect of 2-methoxystypandrone, musizin and torachrysone was tested and 2-methoxystypandrone proved to be active against *S. aureus*, *S. lutea* and *S. cerevisiae* [41].

In conclusion, the results of our screening study serve as a good starting point for selection of *Rumex* species and their different extracts with antibacterial properties for future work. These species, especially *R. acetosa, R. alpinus, R. aquaticus, R. conglomeratus* and *R. patientia,* are promising candidates for further activity-guided fractionation to find new antibacterial natural compounds. Phytochemical and pharmacological investigation of *R. aquaticus* resulted in the isolation of 19 compounds, among them naphthalenes (musizin, musizin-8-*O*-glucoside and 2-methoxystipandron) can be at least partly responsible for the antibacterial activity of the plant.

Conflict of interest

The authors have no conflict of interest to declare.

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Graphical abstract