

Evaluation of Antibacterial Activity of Extract of *Rumex alveollatus* Leaf against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Article information	Abstract
<p>Article history: Received: 3 June 2012 Accepted: 21 June 2012 Available online: 29 Oct 2012 ZJRMS 2013; 15(6): 58-61</p> <p>Keywords: Antibacterial <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Rumex</i> Plant extract Microbial sensitivity test</p> <p>*Corresponding author at: Department of Microbiology, Falavarjan Branch-Islamic Azad University, Isfahan, Iran. E-mail: mohamadi_m@iaufala.ac.ir</p>	<p>Background: There is considerable interest in alternative approaches for eradication of infections causes by pathogenic bacteria especially resistant bacteria. Methanol and ethanol extracts of <i>Rumex alveollatus</i> leaves were evaluated for in vitro antibacterial activity against <i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i> strains.</p> <p>Materials and Methods: In this empirical-experimental study, the agar plate well diffusion method was used for antibacterial assay of different sample. Broth microdilution method was used for evaluate Minimum inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) of extracts.</p> <p>Results: All extracts demonstrated significant inhibitory effect against selected bacterial strains. There were statistically significant correlations between antibacterial activity and concentration of the extracts ($p < 0.05$). The MIC values of the extracts ranged from 31.3 mg/ml to 62.5 mg/ml whereas the MBC values ranged from 62.5 mg/ml to 125.0 mg/ml.</p> <p>Conclusion: Result from these finding suggest that methanol and ethanol extracts of <i>Rumex alveollatus</i> leaves may be used as natural antibacterial for treatment of some diseases, especially local skin diseases.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

The use of natural products with therapeutic properties has a long history. In general, synthetic drugs are not expensive but are also often with side effects. On the other hand treatment of resistant infections is one of the most important problems in medicine. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria [1].

A renewed interest has occurred in the last decade to search for phytochemical of native plants for pharmaceutical and antimicrobial purposes. Many efforts have been made to discover new antimicrobial compounds from various kinds of plants can possess antimicrobial natural products that can be used against infections [2].

Medicinal plants represent a rich source of antimicrobial agents. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. *Rumex* species are widely distributed in worldwide and comprises of about 200 species of herbs. *Rumex* species belonging to family Polygonaceae contains large number of chemically complex and biologically active compounds. The root and aerial parts of members of the Polygonaceae family, including those of *Rumex*, have been used as traditional herbal medicines throughout the world for a variety of therapeutic uses, such as psychopharmacological, antioxidant, cytotoxic, anti fertility, anti-inflammatory, antimicrobial, purgative, anti diarrheal, antitumor,

astriagent and anti dermatitis, diuretic and antiviral activity [3-10]. Furthermore, Zhu and co-workers isolated ten compounds from *R. dentatus* while many important phytochemicals including emodin, aloe emodin, chrysophanol, physocin, chrysophanol, parietin and nepodine have previously been isolated from different species of *Rumex* genus [11]. Harshaw showed methanol extracts of the leaves of *R. obtusifolius* had the highest levels of free radical scavenging property and antibacterial activity against *Bacillus cereus*, *B. subtilis*, *E. coli*, *S. aureus* and *Salmonella typhi* [12]. Yadav and co-workers were evaluated antimicrobial activity of six crude extracts from the roots of *R. nepalensis* against two Grams positive (*S. aureus*, *Streptococcus mutans*) and two Grams negative bacteria (*E. coli*, *Pseudomonas aeruginosa*) and one fungi, *Candida albicans*. Their results indicated benzene and ethylacetate extracts demonstrated significant inhibitory effect against majority tested microorganisms [13]. Mostafa was found that ether extracts of *R. vesicarius* was most effective against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus pyogenes* [14].

Rumex alveollatus (Polygonaceae) is commonly known as "Torshak" in Iran. It grows in many parts of Iran especially west mountain domains at altitudes between 1200-1400 m. The use of *Rumex alveollatus* for various therapeutic purposes is well known in Iran traditional medicine. Ethnobotanical uses of *Rumex alveollatus* include its use as an antidote to nettle, depurative,

astringent, and in the treatment of sore, blisters and burns [15]. In the present study was aimed to evaluate the antibacterial activity of methanol and ethanol extract of *Rumex alveollatus* leaves.

Materials and Methods

This empirical-experimental study was conducted at Microbiology Research Laboratory, Department of Microbiology; Islamic Azad University of Falavarjan in 2011. Plants material and preparation of extracts: The fresh leaves of *Rumex alveollatus* were collected from Kohdasht, Lorestan, Iran in spring 2011. These plants were identified and deposited at the herbarium of Falavarjan Branch of Islamic Azad University, Isfahan, Iran.

The Fresh leaves were shade dried at room temperature. Then dried leave were chopped in to powder using an electric grinder. The 30 grams powdered leave was extracted with 100 ml methanol and ethanol in a Soxhlet apparatus separately. The extracts were filtered and excessive solvent was dried using Rotary flash evaporator for experimentation. These extracts were stored at 4°C. Dried extracts were dissolved in dimethylsulphoxide (DMSO, Merck) and serial dilutions were prepared to final concentration of 500, 50, 125, 62.5 and 31.3 mg/ml.

Microbial strains: The extracts of the leaves were assayed against *Staphylococcus aureus* (ATCC: 25923), *Pseudomonas aeruginosa* (ATCC: 9027), *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from infections.

Antibacterial assay: The well diffusion method was used to determine the antibacterial activity of all leaves extracts of *Rumex alveollatus*. Solution of different extracts of varying concentrations from 1000 to 31.3 mg/ml was prepared. The Muller Hinton agar plates were inoculated with different selected strains of bacteria separately. Wells were made on the agar prepared surface with 6mm cork borer. 100 µl of different concentration of extracts were poured into well aseptically. All plates were incubated at 37°C for 24 hours. The plates were inspected for the zone of inhibition around the wells. Zone of inhibition were compared with standard antibiotics like ceftizoxime.

Determination of MIC and MBC: The broth microdilution method was used to determine Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of different leaves concentration extracts of *Rumex alveollatus*. The test well of a 96-well microtiter plate were filled with 100 µl of exponentially growing culture (about 10⁸ CFU/ml) and added with 100 µl of different concentration of leaves extract (250, 125, 62/5, 31/3 mg/ml) separately.

The absorbance of each well was determined using an automatic ELISA tray reader adjusted at 630 nm. The plate was incubated at 37°C for 24 hr, agitated and the absorbance was read again in the reader at the same wavelength. These absorbance values were subtracted from those obtained before incubation [16, 17].

The experimental results were expressed as mean ± standard deviation (SD) of triplicates. The data were subjected to one-way analysis of variance (ANOVA), using the SPSS-17.0 software. P-valueless than 0.05 was regarded as significant.

Results

The results of antibacterial activity of *Rumex alveollatus* leaves extracts were shown in table 1. All extracts showed dose dependent activity which increases with increase in concentration (Table 1).

Maximum growth inhibition against *Staphylococcus aureus* (ATCC: 23923) was shown by ethanol extract (30.00±2.00 mm) while methanol extract showed maximum growth inhibition against *Pseudomonas aeruginosa* (23.00±2.65 mm) at 250 mg/ml. The methanol extract of *R. alveollatus* leaves possessed significant antibacterial activity against all test bacteria at 250 mg/ml. Results showed that methanol extract had inhibitory activity against *P. aeruginosa*, as a Gram negative bacterium, compared to *S. aureus*, as a Gram positive bacterium, at all concentration. Therefore *P. aeruginosa* and *S. aureus*, which are also resistant to different antibiotics, had their growth inhibited by methanol and ethanol extracts of *R. alveollatus*. Such results are very interesting, because these bacteria were isolated from hospital environment and their controls are very difficult by therapeutic means.

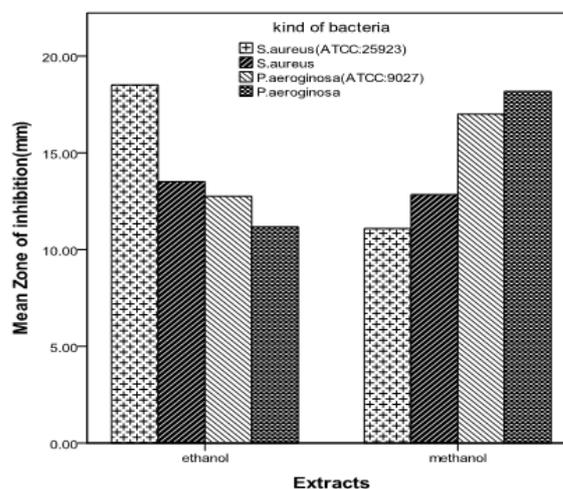


Figure 1. Antibacterial activity of *Rumex alveollatus* leaves extracts against selected bacteria

It is evident that ethanol extract showed significant activity against Gram positive bacteria, *S. aureus* which similar to inhibition zone observed for control ceftizoxime. This indicates that the Gram positive bacteria are more susceptible to ethanol extract compared to Gram negative bacteria. It is probably due to the presence of outer membrane which acts as effective barrier in Gram negative bacteria. Table 2 indicated the MIC and MBC of extracts that required to completely inhibiting the growth of all bacterial strains (Table 2).

Table 1. Antibacterial activity of *Rumex alveollatus* leaves extracts against tested bacteria (Inhibition zone \pm SD in mm)

Plant extracts and Positive control	Concentration of extracts (mg/ml)	Standard Bacteria		Isolated Bacteria	
		<i>S. aureus</i> (ATCC: 25923)	<i>P. aeruginosa</i> (ATCC: 9027)	<i>S. aureus</i>	<i>P. aeruginosa</i>
Methanol	250	12.67 \pm 0.58	20.67 \pm 0.58	16.33 \pm 2.31	23.00 \pm 2.65
	125	11.33 \pm 1.15	17.33 \pm 1.15	14.00 \pm 1.00	19.33 \pm 1.15
	62.5	10.33 \pm 0.58	16.33 \pm 1.15	11.00 \pm 0.00	16.33 \pm 1.15
	31.3	10.00 \pm 0.00	13.67 \pm 1.52	10.00 \pm 0.00	14.00 \pm 1.73
Ethanol	250	30.00 \pm 2.00	16.67 \pm 1.53	19.67 \pm 0.58	12.33 \pm 0.58
	125	21.00 \pm 3.61	14.33 \pm 1.53	13.00 \pm 1.00	11.00 \pm 1.00
	62.5	12.67 \pm 2.08	10.00 \pm 0.00	11.00 \pm 1.00	10.67 \pm 0.58
Ceftizoxime disc	31.3	10.33 \pm 0.58	10.00 \pm 0.00	10.33 \pm 0.58	10.67 \pm 0.58
	30 μ g	32.00 \pm 0.08	27.60 \pm 0.05	30.00 \pm 0.15	24.00 \pm 0.05

Table 2. MIC and MBC of *Rumex alveollatus* leaves extracts against tested bacteria (mg/ml)

	Ethanol extract		Methanol extract	
	MIC	MBC	MIC	MBC
Staph. aureus (ATCC: 25923)	62.5	125.0	31.3	62.5
Staph. aureus (isolated strain)	62.5	125.0	62.5	125.0
P. aeruginosa (ATCC: 9027)	62.5	125.0	62.5	125.0
P. aeruginosa (isolated strain)	31.3	62.5	62.5	125.0

Discussion

Moreover all extracts of *Rumex alveollatus* leaves extracts demonstrated significant inhibitory effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. There were statistically significant correlations between antibacterial activity and extracts concentration. The MIC values of the extracts ranged from 31.3 mg/ml to 62.5 mg/ml whereas the MBC values ranged from 62.5 mg/ml to 125.0 mg/ml.

The potential for developing antimicrobial from plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with fewer side effects that are often associated with synthetic antimicrobials. This study showed that all extracts of *Rumex alveollatus* leaves were effective against bacterial growth. Methanol extracts inhibited *S.aureus* better than *P. aeruginosa*. On the other hand, ethanol extracts inhibited *P. aeruginosa* better than *S. aureus*. Ahmad et al. reported that methanol extracts of different species of *Rumex* genus such as *R. persicaria*, *R. hastatus* and *R. dentatus* had antibacterial activities but their inhibitory effects varied against Gram negative and Gram positive bacteria [18]. Yildirim et al. research was comparable to our study [5]. They showed ethanol extracts of *Rumex crispus* leaves had antibacterial activity on *S. aureus* and *B. subtilis*. However, these extracts did not show detectible antibacterial activity on *E. coli* and *P. aeruginosa*, which are Gram negative bacteria. Fatima et al. also concluded that methanol extracts of roots and leaves of *Rumex dentatus* had antibacterial potential

References

- Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. *Lancet* 2005; 365(9459): 579-87.
- Cowan MM. Plant Products as Antimicrobial Agents. *Clin Microbiol Rev* 1999; 12(4): 564-82.
- Litvinenko YA, MuzychKina RA. Phytochemical investigation of biologically active substances in certain

against Gram positive and Gram negative bacteria [19]. These results suggested that there are bioactive compounds in *Rumex* genus. These compounds probably have high biological activity. Our results about antibacterial activity of *R. alveollatus* were parallel to findings of other researchers, who found that, ethanol, methanol and petroleum ether extracts of different species of *Rumex alveollatus* leaves have variable effects against both Gram positive and Gram negative bacteria [5, 18, 19]. In some study reported, aqueous extract of *Rumex* such as *R. vesicarius* had antibacterial activity [20], while in our study, aqueous leaves extract of *R. alveollatus* had not antibacterial activity against bacterial tested. This may be related to kind of bacterial species.

Several previous experiments on different plant parts of different species of *Rumex* confirm that, *Rumex* genus were potent antibacterial agents. Results of this study revealed that the ethanol and methanol extracts from leaves of *Rumex alveollatus* were exhibit antibacterial activity, which might be helpful in preventing the resistant bacterial infections and can be used in alternative agent of medicine. However, further studies are necessary to find the extract mechanism of antibacterial efficacy and to analyze the active compounds responsible for this biological activity.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

No conflict.

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- Kazakhstan Rumex species. Chem Nat Comp 2003; 39(5): 368-70.
4. Suleyman H, Demirezer L, Kuruuzum Z, et al. Antiinflammatory effect of the aqueous extract from Rumex patientia L. roots. J Ethnopharmacol 1999; 65(2): 141-8.
 5. Yildirim A, Mavi A, Kara AA. Determination of Antioxidant and Antimicrobial Activities of Rumex crispus L. Extracts. J Agri Food Chem 2001; 49(8): 4083-9.
 6. Demirezer LO. Comparison of two Rumex Species with a spectrophotometric method and chromatographic identification with regard to anthraquinone derivatives. Planta Med 1993; 59: 630.
 7. Midiwo JO, Yenesew A, Juma BF, et al. Bioactive compounds from some Kenyan Ethanomedicinal plants: Myrsinaceae, Polygonaceae and Psiadia. Phytochem Rev 2002; 1(3): 311-23.
 8. Ghosh L, Gayen JR, Sinha S, Pal S, Pal M, Saha BP. Antibacterial efficacy of Rumex nepalensis Spreng roots. Phytother Res 2003; 17(5): 558-9.
 9. Liu SY, Sporer F, Wink M, et al. Anthraquinones in Rheum palmatum and Rumex dentatus(Polygonaceae), and phorbol esters in Jatropha curcas(Euphorbiaceae) with molluscicidal activity against the schistosome vector snails ncomelania, Biomphalaria, and Bulinus. Trop Med Inter Health 1997; 2(2): 179-88.
 10. el-Mekkawy S, Meselhy MR, Kusumoto IT, Kadota S, Hattori M, Namba T. Inhibitory effects of Egyptian folk medicines on human immunodeficiency virus. Chem Pharm Bull 1995; 43(4): 641-8.
 11. Choi GJ, Lee S-W, Jang KS, Kim J-S, Cho KY, Kim J-C. Effects of chrysophanol, parietin, and nepodin of Rumex crispus on barley and cucumber powdery mildews. Crop Protection 2004; 23(12): 1215-21.
 12. Harshaw D, Nahar L, Vadla B, Saif-E-Naser GM, Sarker SD. Bioactivity of Rumex obtusifolius (Polygonaceae). Arch Biol Sci 2010; 62(2): 387-92.
 13. Yadav S, Kumar S, Jain P, et al. Antimicrobial activity of different extracts of roots of Rumex nepalensis Spreng. Indian J Nat Prod Resour 2011; 2(1): 65-9.
 14. Mostafa H, Elbakry A, Eman A. Evaluate antibacterial and antioxidant activities of different plant parts of Rumex vesicarius L. Int J Pharm Pharm Sci 2011; 3(2): 109-18.
 15. Rao KNV, Sunitha C, David B, Sandhya S, Mahesh V. A study on the nutraceuticals from the genus Rumex. Hygeia J D Med 2011; 3(1): 76-88.
 16. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protocols 2008; 3(2): 163-75.
 17. Devienne KF, Raddi MSG. Screening for antimicrobial activity of natural products using a microplate photometer. Braz J Microbiol 2002; 33: 166-8.
 18. Ahmad B, Hussain F, Hameed I, Dastagir G, Sanaullah P, Azam S. Antibacterial, antifungal and insecticidal activities of some selected medicinal plants of polygonaceae. Afr J biotechnol 2010; 9(31): 5032-6.
 19. Fatima N, Zia M, Rehman R, et al. Biological activities of Rumex dentatus L: evaluation of methanol and hexane extracts. Afr J Biotechnol 2009; 8(24): 6945-51.
 20. Panduraju T, Rao P, Kumar V. A study on antimicrobial activity of Rumex vesicarius Linn. Int J Pharm Technol 2009; 1(1): 21-5.

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