Zahedan J Res Med Sci. 2017 November; 19(11):e56009. doi: 10.5812/zjrms.56009.

Published online 2017 November 30. **Research Article**

Anti-Quorum Sensing and Antibacterial Activity of *Rumex alveolatus*

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Received 2017 July 15; **Revised** 2017 September 20; **Accepted** 2017 November 01.

Abstract

Background: Infections caused by resistant bacteria are spreading so that efficacy of antibiotics in curing diseases has decreased. Therefore, many attempts are made to find new active compounds of plant origin as suitable substitutes. This research intended to identify active compounds with antibacterial and anti-quorum sensing activities of aqueous and methanol extracts of *Rumex alveolatus* against a number of bacteria.

Methods: This empirical study was conducted against some of pathogenic bacteria. Using the Soxhlet method, extracts of *R. alveolatus* leaves and roots were prepared and antimicrobial effects of the extracts were evaluated by well diffusion method. Anti-quorum sensing activity of the methanol extract of *R. alveolatus* against pyocyanin production, proteases production and biofilm formation were also investigated. The active compounds in *R. alveolatus* were identified using the gas chromatography-mass spectrometry method.

Results: The inhibition zone produced by the methanol leaves extract at 500 mg/mL against *P. aeruginosa*, *S. aureus*, and *S. typhi* were 22.8 ± 0.8, 10.7 ± 1.3, and 12.1 ± 1.0 mm, respectively. The MICs and MBCs of the methanol leaves and roots extracts of this plant for *P. aeruginosa*, *S. aureus*, and *S. typhi* were similar, 125 and 250 mg/mL, respectively. The main phenolic compound obtained from *R. alveolatus* was 1, 2-benzenedicarboxylic acid (89.68%). The methanol leaves extract of *R. alveolatus* at 62.5 mg/mL prevented biofilm formation by *P. aeruginosa* and *S. aureus* and, when applied at 62.5 mg/mL, reduced pyocyanin production in *P. aeruginosa* by up to 66 percent. The aqueous extracts of *R. alveolatus* had not antibacterial and anti-quorum sensing activity.

Conclusions: The methanol extracts of *Rumex alveolatus* had antibacterial and anti-quorum sensing activity.

Keywords: Methanol Extract, Active Compounds, Antibacterial, Biofilm Formation, Anti-Quorum Sensing, *Rumex alveolatus*

1. Background

Resistance of pathogenic bacteria to antibiotics is a problem that has attracted interest all over the world. This resistance to various antimicrobial agents occurs through inherent and acquired resistance mechanisms. Acquired resistance results from contact with antimicrobial agents [1]. Utilization of the antibacterial property of plants has offered a new approach for coping with resistance to antibiotics and for their replacement. Nowadays, four billion people the world over use plants as a source of pharmaceutical materials, and 25 percent of the standard drugs prescribed by physicians are of plant origin. Plants have unlimited capability in synthesizing various aromatic compounds, phenolic compounds and their derivatives [2, 3]. Therefore, it is necessary to study the active compounds in plants of all geographical regions. *Rumex alveolatus* grows in the mountainous regions of western Iran at altitudes of 1200 - 1400 meters. It has long, straight roots, fleshy leaves, and green flowers that are carried above the leaves in clusters. In traditional medicine, this genus is used to treat tumors, hepatic diseases, constipation, heart problems, spleen diseases, hiccup, flatulence, asthma, bronchitis, indigestion, toothache, gall, and nausea [4, 5]. *Rumex* contains many bioactive materials such as flavonoids and anthrakinones and, especially in its roots, carotenoids, vitamins (particularly vitamin C), proteins, lipids, and organic acids have been identified [6, 7]. Antimicrobial properties of aqueous and methanol extracts of various plant species against a broad spectrum of bacteria have been reported from various geographical regions. We studied antibacterial activity of *R. alveolatus* before. We reported methanol extract of *R. alveolatus* leaves were effective against *Pseudomonas aeruginosa* at 31.3 mg/mL [8]. In another study reported that the minimum inhibitory concentrations (MIC) of ethanol extract of *R. alveolatus* against *Escherichia coli* and *Pseudomonas aeruginosa* were 50 and 25 mg/mL [9]. Results of research by Nisa demonstrated the butanol extract of *R. dentatus* had antibacterial effect against *E. coli*, *P. aeruginosa*, and *S. aureus*. Research on extract of *Rumex* attributed its antibacterial properties to the presence of compounds including flavonoids, glycosides, reducing sugars, anthrakinones, tannin, and alkaloids [10]. Therefore, this research was conducted to identify active compounds and antibacterial activity and anti-

quorum sensing effects of aqueous and methanol extracts of *R. alveolatus* leaves and roots against a number of bacteria.

2. Methods

This empirical study was carried out from April 2015 to October. The fresh plant, *R. alveolatus* was collected Lorestan Province (47°36', 33°32). This plant was identified by plant taxonomy experts (registration number 025/006/001). The dried plants were powdered and kept in dark-colored containers in a refrigerator at 4°C.

The 60 grams of powder were extracted with 300 mL of methanol using Soxhlet apparatus for eight hours. Then a rotary evaporator was used to dry the powder at 40°C. The concentrated extracts were diluted to 500, 250, 125, 62.5, 31.3 and 15.6 mg/mL by 5% dimethyl sulfoxide (DMSO, Merck) and were used in antibacterial experiments.

The strains of *S. aureus* (ATCC:25923), *E. coli* (ATTC:25922), *K. pneumoniae* (ATCC:10031), *S. typhi* (ATCC:1690), *S. sonnei* (ATCC:1188), *P. aeruginosa* (ATCC:1310), and *A. baumannii* (ATCC:10654) obtained from the Collection of Microorganisms at the Iranian research organization for Science and technology. The bacterial suspension turbidity was adjusted to be identical to that of 0.5 McFarland turbidity standards (equivalent to 1.5×10^8 CFU/mL). To make sure of the bacterial concentration, absorption was adjusted in a spectrophotometer (UNIC-UV-2100, USA) at 630 nm in the 0.08 - 0.1 range. Following that, 0.2 mL of the suspension was added to 19.8 mL of MHB so that the bacterial cell concentration reached 1.5×10^6 CFU/mL [11].

2.1. Antibacterial Activity

The well diffusion method was used to determine the antibacterial activity of the extract. One hundred μl of the bacterial suspension (1.5×10^6 cfu/mL) were spread on MHA medium, and then wells were made. Each well was inoculated with 100 μL of each prepared concentration of the extract. The negative control was the 5% solution of DMSO, and the positive control was imipenem solution (10 μg/mL). All of the plates were incubated at 37°C for 24 hours. The MIC and MBC of the extracts were determined using the microtiter plate method [12, 13].

2.2. Anti-Quorum Sensing Activity of Methanol Extract of R. alveolatus

The anti-quorum sensing assay was performed in sub-MIC concentrations of the methanol extract of *R. alveolatus* with no interference on bacterial growth.

2.3. Anti-Biofilm Activity

The methanol extract were first prepared at 62.5, 31.3 and 15.6 mg/mL in MHB that contained 1% glucose. One hundred μL of each extract concentration were poured into wells in columns 1 to 8 of the sterile flat bottom of 96 well. Then 150 μL of the bacterial suspension (1.5×10^8 CFU/mL) were added to each well, and the 100 μL of the culture medium containing 1% glucose were also transferred to each one. The 250 μL of sterile MHB were poured in column 11 as the negative control, and 150 μL of the bacterial suspension and 100 μL of the MHB containing 1% glucose to column number 12 as the positive control. After inoculation, the absorbance of the samples was read using ELISA tray reader at 492 nm. The microplates were placed in an incubator at 37°C for 72 hours, and then the contents in the wells were discarded. Following that, 250 μL of 95% ethanol were poured into each well to fix the cells. After 15 minutes, the ethanol in the wells was removed, and the wells were dried in the ambient air. The 200 μL of 2% crystal violet dye were added. After five minutes, the extra dye was then washed off under a gentle stream. The microplates were dried at room temperature, and then 100 μL of 33% glacial acetic acid were poured into each well. The microplates were put in an incubator at 37°C for 20 minutes. The absorbance by the wells was read at 492 nm. Reduction of biofilm formation was calculated [14, 15].

2.4. Pyocyanin Production Test

The effects of the methanol extract of *R. alveolatus* on pyocyanin production of *P. aeruginosa* were determined as Krishnan et al. [16]. The 250 μL of methanol extract of *R. alveolatus* at concentrations of 62.5, 31.3 and 15.6 mg/mL were prepared and 100 μL of the bacterial suspension (1.5×10^8 CFU/mL) was added. The test tube containing the bacterial suspension without the extract was considered as control. The tubes were incubated at 37°C for 24 hours, were then centrifuged at 8000 rpm at 4°C, and the supernatant was transferred to another sterile microtube. One mL of chloroform was added to 5 mL of the supernatant, and the supernatant was discarded. The absorbance of lower part of the microtubes was read at 690 nm. The reduction of pyocyanin production was calculated.

2.5. Protease Activity Test

The effect of the methanol extract of *R. alveolatus* on protease activity of *P. aeruginosa* was evaluated by Laurie method [17]. The 250 μL of the methanol extract of *R. alveolatus* at concentrations of 62.5, 31.3 and 15.6 mg/mL were prepared and 100 μL of the bacterial suspension (1.5×10^8 CFU/mL) were added. The tube containing the bacterial suspension without the extract was the control. The test

tubes were incubated at 37°C for 24 hours and centrifuged at 8000 rpm at 4°C for 10 minutes. Then 100 μL of the supernatant were added to 900 μL of 0.5% azocasein. After 30 minutes at 37°C, 250 μL of 15% trichloroacetic acid were added to the tubes. The tubes were centrifuged at 8000 rpm for 10 minutes at 4°C. The absorbance of supernatant was read at 440 nm. The reduction in protease activity was calculated.

2.6. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

An Agilent model 7890 GC interfaced to a 5975C mass selective detector was used for mass spectral identification of the elements of the extract.

2.7. Statistical Analysis

Data were analyzed using the statistical package for social sciences (SPSS-Ver.17). Normality of the data was evaluated by the Shapiro-Wilk test. Non-parametric kruskal wallis test was used to compare the values between the groups due to the lack of normal assumption. A P value less than 0.05 were considered significant.

3. Results

Sensitivity of the strains to methanol extracts of *R. alveolatus* leaves and roots was studied using the well diffusion method. Results were presented in Table 1. The root methanol extract of *R. alveolatus* did not exhibit any antibacterial effects, except against *S. aureus*.

The species of bacteria and extract concentration ($P < 0.05$) influenced zone of inhibition. Furthermore, increases in the concentration of the methanol extracts of leaves and roots improved their antibacterial activities. Among the studied concentrations of these extracts, zone of inhibition at 500 mg/mL were significantly larger compared to the other concentrations ($P < 0.05$). Based on Duncan post hoc test, all concentrations were compared in pairs and with negative and positive controls. In *P. aeruginosa*, *S. aureus*, and *S. typhi*, the various concentrations were significantly different from the negative control ($P < 0.05$), in all the studied bacterial strains significant differences were observed between zone of inhibition at 500 and 125 mg/mL concentrations of the methanol extracts of leaves and imipenem ($P < 0.05$). The methanol extracts of *R. alveolatus* leaves did not affect *E. coli*, *K. pneumoniae*, *S. sonnei* and *A. baumannii*.

In *S. aureus*, inhibition zone diameters created by methanol extract of *R. alveolatus* roots at 125, 250, and 500 mg/mL were significantly different, and all the paired comparisons between the concentrations were significant. Inhibition zone diameters of *S. aureus* produced by root extract were significantly larger compared to leaf extract ($P < 0.05$).

The MIC of the methanol leaves extract was 125 mg/mL for *P. aeruginosa*, *S. aureus*, and *S. typhi*, and the MBC was 250 mg/mL. The MIC of the root extract for *S. aureus* was 125 mg/mL and the MBC was 250 mg/mL. the aqueous extracts of *R. alveolatus* leaves and roots did not exhibit any antibacterial effects.

3.1. Anti-Quorum Sensing Activity of Methanol Extract of R. alveolatus

Results confirmed that leaves extract of *R. alveolatus* were capable of reducing biofilm formation by *P. aeruginosa* and *S. aureus* at sub-MIC of methanol extract. The methanol extract of *R. alveolatus* leaves and roots, at 62.5 mg/mL reduced biofilm production in *P. aeruginosa* by 60 and 80 percent, respectively. However, at 62.5 mg/mL, methanol root extract of *R. alveolatus* lowered biofilm production in *S. aureus* by 80 percent; and, at 32.3 mg/mL, the methanol extract of *R. alveolatus* leaves decreased biofilm production in *S. aureus* by 40 percent.

As shown in Figure 2, methanol extract of *R. alveolatus* leaves at 62.5 mg/mL reduced pyocyanin production by 66 %, while methanol extract of the roots of this plant at 62.5 mg/mL decreased pyocyanin production by up to 16 percent. Methanol extracts of *R. alveolatus* leaves and roots at 62.5 mg/mL reduced protease activity by up to 42% and 27%, respectively. The lower concentration of methanol extract did not show any effect on pyocyanin production and protease activity.

The methanol extract of *R. alveolatus* taken from GC / MS spectrum were analyzed. The components of methanol extract were identified in Table 2. The percentage of each product in the extract was shown in this table indicating Biocyclo (3.1.1) heptan-3- one, 2, 6, 6- trimethyl (1.alpha, 2alpha, 5a) with 97% had the most presence in our extract. Table 2 also reveals high probability of Alpha-Pinene, Sabinene and Eucalyptol (1, 8-cineole) that proved antimicrobial activity in the extract.

4. Discussion

In this research, a qualitative method (well diffusion) and a quantitative method (microdilution) showed antibacterial and anti-querumsensing of *R. alveolatus*. Results indicated that zone of inhibition had a direct relationship with extract concentration in the well diffusion method ($P < 0.05$). Furthermore, means of inhibition zone

Table 1. Antibacterial Activity of the Methanol Extracts of *R. alveolatus* Leaves and Roots

Sample	Concentrations of the Methanol Extract, mg/mL						Negative Control	Positive Control (Imipenem)
	Leaves			Roots				
	500	250	125	500	250	125		
P.aeruginosa	22.8 ± 0.6	21.4 ± 0.6	19.3 ± 1.5	-	-	-	-	26.0 ± 1.4
S.typhi	12.1 ± 1.0	9.3 ± 0.6	6.66 ± 0.6	-	-	-	-	30.0 ± 2.6
A.baumannii	-	-	-	-	-	-	-	27.0 ± 2.1
S.sonnei	-	-	-	-	-	-	-	28.0 ± 1.8
K.pneumoniae	-	-	-	-	-	-	-	26.0 ± 2.4
E. coli	-	-	-		-	-	-	26.0 ± 1.9
S. aureus	10.7 ± 1.3	9.7 ± 1.5	8.7 ± 1.5	22.0 ± 1.0	17.6 ± 0.6	11.7 ± 1.5	-	25.6 ± 2.1

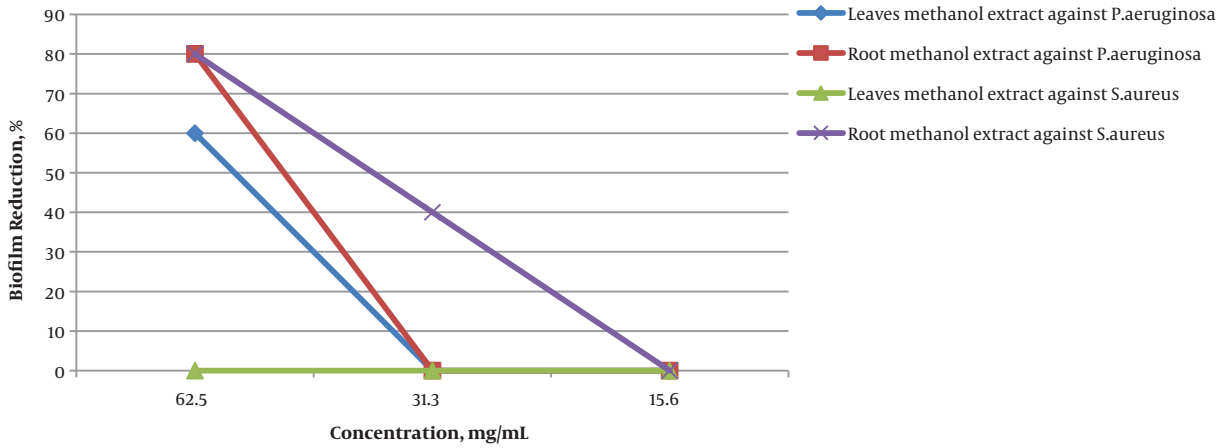

Figure 1. Reduction of Biofilm Formation at sub-MIC of *R. alveolatus*

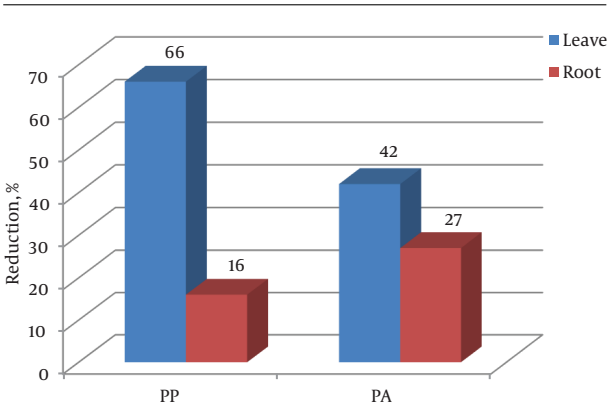

Figure 2. Effect of Methanol Extract of Leaves and Root of *R. alveolatus* (62.5 mg/mL) on Pyocyanin Production (PP) and Protease Activity (PA) in *P. aeruginosa*

diameters for the various bacterial species were significantly different in this method ($P < 0.05$). In other words, bacterial species influenced zone of inhibition.

P. aeruginosa was the most sensitive species to methanol extracts of *R. alveolatus* leaves, while *S. aureus* exhibited the maximum sensitivity to the methanol extract of *R. alveolatus* roots. This difference in sensitivity of the various bacteria to the antimicrobial compounds was probably due to differences in the cell wall of the microorganisms. McKeegan et al. showed that Gram-negative bacteria were more resistant to extracts compared to Gram-positive bacteria.

Our results conform to those previous research, who reported that methanol extracts of *R. alveolatus* leaves at 250 mg/mL created inhibition zone diameters of 12.5 and 20.67 mm for *S. aureus* and *P. aeruginosa*, respectively [18]. At 250 mg/mL, the methanol extract of this plant in the present research created inhibition zone diameters of 9.68 and 21.4 mm against *S. aureus* and *P. aeruginosa*, re-

Table 2. The Methanol Extracts Composition of *R. Alveolatus* by Using a GC-MS.

Identified Compounds	Percentage of Total	Kovats Retention Index	Release Time
5-Methyl-2-cyclohexanone	0.42	1156.03	16.765
4-Methyl-1-cyclohexane	0.28	1393.53	20.539
Alpha-pinene	0.21	929.94	7.988
5-Methyl-2-cyclohexanol	0.75	1275.08	17.409
Cyclononasiloxane-ctadecamethylcy	0.22	1826.72	30.009
1,2-benzenedicarboxylic acid	89.68	2519.18	34.264
Toluene	3.27	780. 25	4.147
1-Methyl-3 benzene	0.1	1024.52	11.425
Eucalyptol	0.09	1030.20	11.693
Caryophyllene	0.11	1420.33	23.198
ethylhexyl phthalate	0.27	2516.27	34.176
2,6-dimethyl cyclohexanol	0.20	1108.47	15.262
2-methyl-5H-dibenz	0.70	2747.41	35.116

spectively. These results indicate that the effective compounds in methanol extracts of *R. alveolatus* leaves have very good antimicrobial effects against *P. aeruginosa*, while they do not exhibit suitable antibacterial activity against *S. aureus*. It seems that the active compounds present in the methanol extract of *R. alveolatus* leaves have different mechanisms of action against Gram-negative and Gram-positive bacteria.

In research conducted by Moradi, ethanol extract of *R. alveolatus* at 300 mg/mL created inhibition zone diameters of 25.33 and 16.37 mm against *P. aeruginosa* (ATCC: 85327) and *S. aureus* (ACTT: 25923), respectively. However, results of the present study do not confirm this for other Gram-negative bacteria. The methanol extract of *R. alveolatus* leaves had no antimicrobial effects against Gram-negative bacilli of *E. coli*, *K. pneum*oniae, *S. sonnei*, and *A. baumannii*, and only created an inhibition zone diameter of 12.1 mm against *S. typhi*. Almost all known antimicrobial compounds of plant origin are either aromatic or organic substances, and most of them are extracted using methanol and ethanol solvents. The researchers have shown that extracts prepared by using organic solvents have greater and more stable antimicrobial effects because most known active antimicrobial compounds are insoluble in water and, therefore, extracts prepared by using organic solvents are more capable in extracting antimicrobial substances [18]. Researchers have attributed antibacterial properties of *R. alveolatus* to its phenolic compounds. This is probably why aqueous extracts lack antibacterial effects because they have concentrations of phenolic compounds dissolved in water. This is in agreement with studies conducted by Nisa and Yildirim [5, 19]. The differences in the mentioned results are due to the different plant species used in the first place and to differences in the tested parts of the plants, in the tested bacterial strains and/or in the habitats of the plants in the second place. Presence of phenolic compounds in plant extracts is one of the main reasons for their antimicrobial effects [20].

In the present research, 1, 2-benzenedicarboxylic acid constituted 89.68 percent of the main compounds isolated from *R. alveolatus* using the GC-MS method (Table 2). The large content of phenolic compounds in our research is related to the antibacterial properties of this extract. Studies have indicated that polyphenols including tannins, anthrakinones, and flavonoids have effective antimicrobial effects against various microorganisms in addition to their antioxidant properties [21, 22]. Therefore, considering the presence of anthraquinones and flavonoids in plants of the *Polygonaceae* family, antimicrobial effects of methanol extracts of *R. alveolatus* leaves and roots can be attributed to these compounds [23-27].

The efficacy of the methanol extract in reducing biofilm formation by the mentioned bacteria was confirmed. It is observed that presence of endol together with terpenes in extracts enhanced their bactericidal properties [28]. Considering the large content of phenolic compounds in *R. alveolatus*, its inhibition of biofilm formation seems to be logical.

In our research, extracts of *R. alveolatus* reduced activities of protease and decreased pyocyanin production in *P. aeruginosa*. Pathogenicity of bacteria is strongly associated with their ability in secreting various toxic and decompos-

ing enzymes into the related environment. Among these enzymes, lipase and protease become involved in bacterial pathogenicity through breaking down host defenses and non-specific physical barriers. As extracellular enzymes in *P. aeruginosa*, proteases provide the necessary nutrients for bacterial growth.

Acknowledgments

This work was a part of MSc thesis of N. Korkorian at Islamic Azad University, Isfahan, Iran (No: 17230507931032). The Authors would like to thank Research laboratory personnel for their cooperation in this investigation.

Footnotes

Authors' Contribution: Two authors had equal role in design, work, statistical analysis, and manuscript writing.

Conflict of Interests: The authors declare no conflict of interest.

Funding/Support: Islamic Azad University.

References

1. Furuya EY, Lowy FD. Antimicrobial-resistant bacteria in the community setting. *Nat Rev Microbiol.* 2006;**4**(1):36–45. doi: 10.1038/nrmicro1325. [PubMed: 16357859].
2. Dholwani KK, Saluja AK, Gupta AR, Shah DR. A review on plant-derived natural products and their analogs with anti-tumor activity. *Indian J Pharmacol.* 2008;**40**(2):49–58. doi: 10.4103/0253-7613.41038. [PubMed: 21279166].
3. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts. *J Nat Med.* 2008;**62**(2):259–62. doi: 10.1007/s11418-007-0216-x. [PubMed: 18404337].
4. Khan TH, Ganaie MA, Siddiqui NA, Alam A, Ansari MN. Antioxidant potential of Rumex vesicarius L.: in vitro approach. *Asian Pac J Trop Biomed.* 2014;**4**(7):538–44. doi: 10.12980/APJTB.4.2014C1168. [PubMed: 25183273].
5. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. *J Agric Food Chem.* 2001;**49**(8):4083–9. doi: 10.1021/jf0103572. [PubMed: 11513714].
6. Liang HX, Dai HQ, Fu HA, Dong XP, Adebayo AH, Zhang LX, et al. Bioactive compounds from Rumex plants. *Phytochem Lett.* 2010;**3**(4):181–4. doi: 10.1016/j.phytol.2010.05.005.
7. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour H. Phytochemical screening of some species of Iranian plants. *Iranian J Pharm Res.* 2010;**2**(2):77–82.
8. Mohammadi-Sichani M, Sadeghzadeh P, Madani M. Evaluation of antibacterial activity of extract of Rumex Alveollatus leaf against Staphylococcus aureus and Pseudomonas aeruginosa. *Zahedan J Res Med Sci.* 2013;**15**(6):58–61.
9. Saeidi S, Amini Boroujeni N, Ahmadi H, Hassanshahian M. Antibacterial Activity of Some Plant Extracts Against Extended- Spectrum Beta-Lactamase Producing Escherichia coli Isolates. *Jundishapur J Microbiol.* 2015;**8**(2):e15434. doi: 10.5812/jjm.15434. [PubMed: 25793093].
10. Moradi A, Ebrahimipour G, Karkhane M, Marzban A. Surveying the antioxidant and the antimicrobial effects of aqueous and ethanolic extract of Rumex alveollatus L. on In-vitro indicator microorganisms. *J Fasa Uni Med Sci.* 2015;**4**(4):418–26.
11. Valgas C, Souza S, Smânia EFA, Smânia Jr A. Screening methods to determine antibacterial activity of natural products. *Braz J Microbiol.* 2007;**38**(2):369–80. doi: 10.1590/s1517-83822007000200034.
12. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016;**6**(2):71–9. doi: 10.1016/j.jpha.2015.11.005.
13. Klancnik A, Piskernik S, Jersek B, Mozina SS. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *J Microbiol Methods.* 2010;**81**(2):121–6. doi: 10.1016/j.mimet.2010.02.004. [PubMed: 20171250].
14. Budzynska A, Wieckowska-Szakiel M, Sadowska B, Kalemba D, Rozalska B. Antibiofilm activity of selected plant essential oils and their major components. *Pol J Microbiol.* 2011;**60**(1):35–41. [PubMed: 21630572].
15. Chusri S, Phatthalung PN, Voravuthikunchai SP. Anti-biofilm activity of Quercus infectoria G. Olivier against methicillin-resistant Staphylococcus aureus. *Lett Appl Microbiol.* 2012;**54**(6):511–7. doi: 10.1111/j.1472-765X.2012.03236.x. [PubMed: 22486208].
16. Krishnan T, Yin WF, Chan KG. Inhibition of quorum sensing-controlled virulence factor production in Pseudomonas aeruginosa PAO1 by Ayurveda spice clove (Syzygium aromaticum) bud extract. *Sensors (Basel).* 2012;**12**(4):4016–30. doi: 10.3390/s120404016. [PubMed: 22666015].
17. Huerta V, Mihalik K, Crixell SH, Vattem DA. Herbs, spices and medicinal plants used in hispanic traditional medicine can decrease quorum sensing dependent virulence in Pseudomonas aeruginosa. *Int J Appl Res Nat Pro.* 2008;**1**(2):9–15.
18. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, et al. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethnopharmacol.* 2002;**79**(2):213–20. doi: 10.1016/S0378-8741(01)00384-1. [PubMed: 11801384].
19. Nisa H, Kamili AN, Bandh SA, Shajr ul A, Lone BA, Parray JA. Phytochemical screening, antimicrobial and antioxidant efficacy of different extracts of Rumex dentatus L. – A locally used medicinal herb of Kashmir Himalaya. *Asian Pac J Trop Dis.* 2013;**3**(6):434–40. doi: 10.1016/s2222-1808(13)60097-3.
20. Sahreen S, Khan MR, Khan RA, Hadda TB. Evaluation of phytochemical content, antimicrobial, cytotoxic and antitumor activities of extract from Rumex hastatus D. Don roots. *BMC Complement Altern Med.* 2015;**15**:211. doi: 10.1186/s12906-015-0736-y. [PubMed: 26138389].
21. Al-Asmari AR, Siddiqui YM, Athar MT, Al-Buraidi A, Al-Eid AS, Horaib GB. Antimicrobial activity of aqueous and organic extracts of a Saudi medicinal plant: Rumex nervosus. *J Pharm Bioallied Sci.* 2015;**7**(4):300–3. doi: 10.4103/0975-7406.168031. [PubMed: 26681888].
22. 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.
23. Semwal DK, Rawat U. Antimicrobial hasubanalactam alkaloid from Stephania glabra. *Planta Med.* 2009;**75**(4):378–80. doi: 10.1055/s-0028-1112223. [PubMed: 19148860].
24. Elzaawely AA, Xuan TD, Tawata S. Antioxidant and antibacterial activities of Rumex japonicus HOUTT. Aerial parts. *Biol Pharm Bull.* 2005;**28**(12):2225–30. [PubMed: 16327154].
25. Humeera N, Kamili AN, Bandh SA, Amin SU, Lone BA, Gousia N. Antimicrobial and antioxidant activities of alcoholic extracts of Rumex dentatus L. *Microb Pathog.* 2013;**57**:17–20. doi: 10.1016/j.micpath.2013.02.001. [PubMed: 23415966].
26. Pezeshki Najafabadi M, Mohammadi-Sichani M, Kazemi MJ, Shirsalimian M, Tavakoli M. Investigation of the chemical composition and different effects of a Rumex dentatus methanol extract against drug resistant pseudomonas aeruginosa Isolates. *Iran Red Crescent Med J.* 2016;**18**(12) doi: 10.5812/ircmj.27064.
27. Elzaawely AA, Tawata S. Antioxidant capacity and phenolic content of Rumex dentatus L. Grown in Egypt. *J Crop Sci Biotechnol.* 2012;**15**(1):59–64. doi: 10.1007/s12892-011-0063-x.

28. 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. doi: 10.1002/ptr.1162. [PubMed: 12748999].