

Antibacterial effects of methanolic extracts of *Reum ribes L.* and *Hyssopus officinalis* on some standard pathogenic bacteria

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Abstract

Background and objectives: As much as people become aware of the dangerous side effects of synthetic antibiotics, the demand for natural alternatives to these drugs increases. Natural ingredients, lower risk of complications and even have beneficial side effects. The aim of this study was to determine the antibacterial effect of herbs *Reum ribes L* and hyssop *Hyssopus officinalis* is on some pathogenic bacteria.

Methods: After collecting and confirming the scientific name, the methanolic extract of *R. ribes L.* and *H. officinalis* plants was prepared and the antimicrobial effects of the extracts by agar well diffusion and disk diffusion, as well as the determination of The minimum bactericidal concentration and the minimum inhibitory concentration (MIC / MBC) were dilution test on *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Results: The highest growth inhibitory zone in *S. aureus*, *B. cereus*, *P. aeruginosa*, and *E. coli* at concentrations of 400 mg / ml, respectively, in the disc method of 13.21, 13.41, 11.2 and 10.74 mm and the well method, respectively 13.64, 13.11, 10.67 mm, and 9.38 mm for the *R. ribes L* extract, and the disc method of 11.74, 10.2, 10.71, and 9.1 mm, and the well method of 12.41, 11.6, 10.2, and 9.9 respectively. 4.3 mm was observed for *H. officinalis* extract. The results of MBC / MIC showed that the extract of medicinal plants had the highest susceptibility to *B. cereus* bacteria and the least susceptibility to *E. coli*.

Conclusion: *R. ribes L.* and *H. officinalis* plants have significant inhibitory effects on the growth of pathogenic bacteria in vitro. Therefore, it can be expected that these extracts can be used for the treatment of bacterial infections and are a good alternative to the usual chemical treatments for the treatment of infections.

Keywords: Antibacterial effects, Pathogenic bacteria, Medicinal plants, Extract

Introduction

Medicinal plants during the development of all human civilizations, there has always been a close relationship between man and plant. Although most plant species are known to date, there is still much time left to discover new and valuable plant resources (1-4). As such, plants can be a useful source of chemicals that are only partially identified. These chemicals can be used as a drug but also as a unique starting point for the manufacture of pharmaceutical analogues, as well as an interesting tool to better understand a biological phenomena (5-7). *Rheum ribes L.* is a plant from the indigenous Asian Polygonaceae (probably Siberian or Himalayan) cultivated in Europe since the sixteenth century (8). *R. ribes L.* is abundant in the Binalud Neyshabur slopes, in the mountains of Azerbaijan and Kurdistan, and in the Alborz highlands in northern Tehran, as well as in the Khorasan Razavi province of Gonabad province and in the mountains of Rabat Turk village (9). *R. ribes L.* has both medicinal and oral uses. *R. ribes L.* is a carrier of bile salts, a stimulant of bile salt secretion from the liver, laxative, gastric tonic, blood purifier and anti-parasite [10-13]. *R. ribes L.* contains some elements such as potassium - calcium and is also rich in vitamins. The *R. ribes L.* petiole, which is a usable part, contains some organic acids, such as malic acid, which are useful for relieving thirst and facilitating digestion. It is also used in the traditional medicine of *R. ribes L.* to disperse some harmful bacteria [14-15]. However, antibacterial effects of this plant have been reported on some Gram-negative bacteria [16]. *Hyssopus officinalis* from the Lamiaceae family is a perennial, very aromatic, with numerous stems of 20 to 60 cm height that grow in southern Europe, Asia Minor, Iran and Russia. [17-18]. this plant has anti-inflammatory, antibacterial, anti-

inflammatory, anti-spasmodic, anti-hypertensive and anti-cancer properties [19]. The aim of this study was to investigate the antibacterial properties of *R. ribes L.* and *H. officinalis* in vitro on standard bacteria.

Materials and Methods

In this in vitro study, plants were collected from the natural areas of Marand city during two stages of one month interval from May to June 2019. Samples were collected carefully from a geographical area and hand-picked. After collection, nylon bags were used to transport the samples. The specimens are cleaned after collection and transport, and their waste materials such as pebbles, soil, other weeds, yellow leaves and stems and roots are separated from the specimens and in a large, convenient space and dried in the sun. After the specimens were completely dried and the shoots and leaves of the shoots were removed from the roots, they were prepared for grinding. Thus, to increase the level of contact of the plant parts with distilled water, they were powdered with electric milling. It is advisable to do this a few minutes before the extraction operation and to prevent the elevation of the mill temperature because high mill temperature affects the quality of the extract. Soxhlet extractor is used for extraction so that 300 g of the plant powder is poured into filter paper and soaked in a little methanol and put into the Soxhlet extractor. Laboratory flask containing 500 ml of methanol are attached to the Soxhlet and heated then rotary to obtain pure extract. Then, using 5% DMSO (CinnaGen Co, Iran) solvent, concentrations of 50 mg / ml, 100 mg / ml, 200 mg / ml, 400 mg / ml were prepared for use in disk diffusion test and MIC / MBC determination (according to CLSI protocol) (20). *Staphylococcus aureus* (ATCC: 25923), *Bacillus cereus* (PTCC:

1052), *Escherichia coli* (ATCC: 25922), *Pseudomonas aeruginosa* (ATCC: 27853) strains are lyophilized from Pasteur Institute of Iran. In this method, the bacteria first were tested with a standard suspension of McFarland's equivalent of standard 0.5 McFarland and dispersed by sterile swabs of each bacterial sample to the surface of the Mueller-Hinton agar medium (MERCK CO, Germany). Immediately above the culture medium, wells of 5 mm in diameter and 2 cm apart were introduced and a certain amount of the dilution of the extract first referred to was inoculated into the well. Streptomycin antibiotic was used as positive control and DMSO as negative control. After completion of the work, all media were incubated for 24 hours at 37 ° C and finally the diameter of the bacterial growth holes was measured by a caliper (21-22). The diameter of the auras in the culture medium is a reaction to the concentration of the extract tested. This phenomenon is a linear relationship between the halo and the logarithm of the concentration of the extract that is determined by measuring the diameter of the growth halo and comparing it to a specific standard, the antimicrobial potency of the extract being tested [23]. The disc diffusion method is similar to the diffusion method, with disks impregnated with different concentrations of methanolic extract rather than agar surface. The method is to first extract the concentrations of 50mg / ml, 100mg / ml, 200mg / ml, 400mg / ml, and sterilize the sterile blank disks and allow them to dry. After dipping the sterile swab in the microbial suspension, the excess solution was removed by pressing the swab to the side of the tube and then stretched across the plate. Streptomycin (PADTANTEB Co, Iran) antibiotic was used as positive control and DMSO as negative control. Plates were incubated at 37 ° C for 16–18 h, then

measured using a caliper for the growth zone diameter in millimeters [24]. Tests for determination of Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) were performed by Tube dilution. For determination of MIC from methanolic extract, dilution series of 0.78 and 1.56, 3.25, 6.25, 12.5, 25, 50, 100, 200, mg / ml in Muller Hinton broth culture medium (MERCK CO, Germany). Then, 1 ml of the prepared microbial suspension was added to each dilution. A positive control tube with microbes (culture medium containing bacterial, no extract) and a negative control tube with contents (bacterial culture medium) were also prepared. After completion, all tubes were transferred to an incubator at 37 ° C for 24–48 h. After incubation, the tubes were examined for opiate-induced inoculation of bacterial growth. The least dilution of the extract with no opacity (non-growth) was considered as MIC. To determine the minimum bactericidal concentration of extracts (MBC) from all tubes in which no growth was observed. The culture medium was cultured on Muller-Hinton Agar. The inoculated media were incubated for 24 hours at 37 ° C, a plate containing the lowest concentration of extract and no bacterial growth observed as MBC of the extract concentration [25]. SPSS software version 18 was used to analyze the data. In order to study the significant difference was found between the results of ANOVA and Chi-square and the difference between the groups was significant at the significance level of $p < 0.05$.

Results

Using the well diffusion and disk diffusion method and the effect of methanolic extracts of *Reum ribes L.* and *Hyssopus officinalis* on the tested bacteria, it was found that these extracts had a significant inhibitory effect on

the tested bacteria and the higher the concentration of the methanolic extract, the greater the inhibitory effect. This study showed that the inhibitory effect of *Reum ribes L.* and *Hyssopus officinalis* methanolic extracts on Gram-positive bacteria is more than gram-negative bacteria. The results of the effect of different concentrations of methanolic extracts by well diffusion and disk diffusion methods are presented in Tables 1

and 2. These two methods differed in the size of the inhibition zone of these bacteria. MBC / MIC analysis showed that the extract of medicinal plants had the highest susceptibility to *B. cereus* and the least susceptibility to *E. coli*. The results of MBC / MIC test of methanolic extracts against selected bacteria by tubular method are presented in Table 3.

Table 1. The diameter of inhibition zone at different concentrations of methanolic extracts by well diffusion method (mm)

Methanolic extracts of <i>Reum ribes L</i>						
Bacteria / Concentrate Extract	50 mg/ml	100mg/ml	200mg/ml	400mg/ml	Positive control	Negative control
<i>Staphylococcus aureus</i>	8	9.24	11.69	13.64	-	18.09
<i>Bacillus cereus</i>	7.68	9	11.38	13.11	-	17.3
<i>Pseudomonas aeruginosa</i>	--	7.4	8.55	10.67	-	13.6
<i>Escherichia coli</i>	--	--	7.69	9.38	-	14.57
Methanolic extracts of <i>Hyssopus officinalis</i>						
<i>Staphylococcus aureus</i>	--	8.29	10.46	12.41	-	17.41
<i>Bacillus cereus</i>	--	--	9.82	11.6	-	16.9
<i>Pseudomonas aeruginosa</i>	--	--	8.7	10.2	-	14.5
<i>Escherichia coli</i>	--	--	7.7	9.9	-	15

Table 2. The diameter of inhibition zone at different concentrations of methanolic extracts by disk diffusion method (mm)

Methanolic extracts of <i>Reum ribes L</i>						
Bacteria / Concentrate Extract	50 mg/ml	100mg/ml	200mg/ml	400mg/ml	Positive control	Negative control
<i>Staphylococcus aureus</i>	7.32	9.12	11	13.21	-	17.66
<i>Bacillus cereus</i>	7.2	9.24	11.85	13.41	-	17.17
<i>Pseudomonas aeruginosa</i>	--	8	9.74	11.2	-	14.55
<i>Escherichia coli</i>	--	--	8	10.74	-	14.73
Methanolic extracts of <i>Hyssopus officinalis</i>						
<i>Staphylococcus aureus</i>	--	7.65	9.87	11.74	-	18.21
<i>Bacillus cereus</i>	--	--	8.4	10.2	-	17.24
<i>Pseudomonas aeruginosa</i>	--	--	8.1	10.71	-	15.26
<i>Escherichia coli</i>	--	--	7.27	9.1	-	15.76

Table 3. MBC / MIC test of bacteria at different concentrations (mm)

Methanolic extracts of <i>Reum ribes L</i>		
Bacteria / Concentrate Extract	MIC mg/ml	MBC mg/ml
<i>Staphylococcus aureus</i>	12.5	50
<i>Bacillus cereus</i>	12.5	25
<i>Pseudomonas aeruginosa</i>	25	50
<i>Escherichia coli</i>	50	100
Methanolic extracts of <i>Hyssopus officinalis</i>		
<i>Staphylococcus aureus</i>	25	50
<i>Bacillus cereus</i>	25	50
<i>Pseudomonas aeruginosa</i>	50	100
<i>Escherichia coli</i>	50	100

Discussion:

Due to the increasing resistance of bacteria to a variety of antibiotics, efforts have been made to obtain and use the compounds present in plants and their application in the treatment of various diseases. Plants have played an important role in maintaining health and improving the quality of life of humans for thousands of years. Medicinal herbs have useful properties such as antimicrobial, antiparasitic, antifungal and antioxidant properties. Medicinal herbs are those groups of herbs that are used for medical, therapeutic, clinical, and pharmacological purposes [10]. According to research by Dehghanzadeh et al., Essential oils of *Reum ribes L* have carvacrol (7.73%) and thymol (18.95%) [26 - 29]. Carvacrol results in the depletion of proton flux and depletion of ATP, so that measurement of ATP levels inside and outside the cell indicates that after the presence of carvacrole in the medium the intracellular ATP level decreases and increases steadily outside the cell. According to the results of this study, gram-positive bacteria are more susceptible to plant extracts, which is similar to the results of previous studies on gram-positive and gram-negative bacteria [31-31]. The reason for the sensitivity of gram-positive bacteria to chemicals and essential oils and plant extracts is the difference in wall structure. Gram-positive bacteria have a mucopeptide in their cell wall, whereas gram-negative bacteria only have a thin layer of mucopeptide and most of the wall structure is lipoprotein and lipopolysaccharide. In fact, Gram-negative bacteria have an outer membrane around their cell wall, which makes them more resistant to antibacterial substances [32]. Degradation of the cell wall results in the leakage of cellular contents outward resulting in cell death. The effect of these compounds depends on the

dose and duration of their action. Higher concentrations will increase the rate of destruction of the microorganisms, As a result, more time should be used to produce similar antibacterial effects at low doses [30]. Hovadik and Chladek reported that *Hyssopus officinalis* had the highest antibacterial effect [33]. Hassanshahiyan et al., by analyzing the antimicrobial effects of *Hyssopus officinalis* extract, showed that the maximum inhibitory effect of these extracts was against *S. aureus* and *P. aeruginosa*. The inhibitoriest effects of ethanolic extract of *Hyssopus officinalis* on biofilm formation against *E. coli* (95%) were observed [34]. Burfer reported in a study in 2014 that the ethanolic extract of *Reum ribes L* has antibacterial properties on *S. aureus*, *K. pneumoniae* and *E. coli* [35]. One study showed that the extract obtained from *Reum ribes L* leaves had significant antibacterial activity [16]. Salehi et al. (2016), by studying the aqueous and ethanolic extracts of *Reum ribes L*, showed that the inhibitory effect of aqueous and ethanolic extracts of *Reum ribes L* stems and leaves on *S. aureus* was more than that of *E. coli*. The highest diameter of non-growth zone at concentration of 150 mg / ml was related to ethanolic and aqueous extracts of *Reum ribes L*. stalk on *S. aureus* which was 23 ± 1.3 and 16 ± 1.1 mm respectively [36].

Conclusion:

Since *Reum ribes L*. and *Hyssopus officinalis* extracts have higher antibacterial activity, identifying the effective material of these plants as an effective antimicrobial compound is the next step in this direction and it is necessary to identify the antimicrobial effects of other plants of the same family with these plants.

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Declarations***Conflict of interest***

We declare that we have no financial or nonfinancial conflicts of interest related to the subject matter or materials discussed in the article.

Authors' contributions

All authors contributed equally to this work

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