

Comparing the Effect of Ethanol Extracts of *Descurainia sophia* (L.) Seed and *Althaea officinalis* Root on *Streptococcus pyogenes*

Kian Aghaabbasi,*¹ Elham Dehghan,¹ Amin Baghizadeh,² Hossein Dashti³

1. Department of Biotechnology, Faculty of Sciences and Modern Technology, Kerman Graduate University of Technology, Kerman, Iran
2. Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran
3. Department of Plant Breeding, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran

Article information	Abstract
<p>Article history: Received: 13 Apr 2013 Accepted: 29 May 2013 Available online: 12 Oct 2013 ZJRMS 2014 Oct; 16(10): 27-32</p> <p>Keywords: <i>Descurainia sophia</i> (L.) Ethanol extract <i>Streptococcus pyogenes</i> Antibacterial activity</p> <p>*Corresponding author at: Department of Biotechnology, Faculty of Sciences and Modern Technology, Kerman Graduate University of Technology, Kerman, Iran. E-mail: kianaghaabasi@yahoo.com</p>	<p>Background: Owing to therapeutic properties, flaxweed and marsh mallow have traditionally been very important in Iran. In this research study the effect of various concentrations of ethanol flaxweed seed and marsh mallow root extracts, collected from different areas of Iran, was studied on <i>Streptococcus pyogenes</i>; the effect of the extract with antibiotics of penicillin, erythromycin and amoxicillin was compared in a completely randomized design with four replications.</p> <p>Materials and Methods: In this experimental study, discs impregnated with concentrations of 25, 50, 100, 250 mg/mL were prepared from ethanol extract of flaxweed concentration in Jiroft, Baghin, Rafsanjan and Kohbanan as well as marsh mallow root extract in Tehran, Isfahan, Yazd and Kerman. Then, they were placed on culture medium of blood agar that <i>S. pyogenes</i> has grown on. Finally, inhibitory effect was evaluated.</p> <p>Results: The results showed that, among different areas and available antibiotics, the highest inhibition zone was related to marsh mallow root extract of Yazd in concentration of 250 mg/mL with 14.5 mm. Beta hemolysis was observed on concentrations of 50, 100 and 250 mg/mL of flaxweed seed extracts in all areas; thus, these concentrations are not suitable for producing herbal medicine. Concentration of 25 mg/mL, however, showed no hemolysis in all areas. The best extract to produce herbal medicines with flaxweed seed was related to concentrations of 25 mg/mL. Penicillin had the highest inhibition zone with 8.31 mm.</p> <p>Conclusion: Considering the significant difference in the level of 0.01%, marsh mallow root extracts have more anti-bacterial effect than flaxweed seed extracts.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Numerous studies have been conducted on chemical medicine, emphasizing their undesirable effects. No chemical substances can be found without having adverse effects [1]. Antibiotic capsules which now use frequently in Iran increases bacterial resistance to these chemical medicine. In comparison with these chemical substances, the effective substances available in herbal always have a biological equilibrium because of having other substances [2]. Flaxweed with scientific name "*Descurainia sophia* (L.) web" belongs to cruciferae family. Some of its properties include stomach strengthening, voice clearing, skin clearing, appetizing, measles and scarlet fever treatment [3]. *Althaea officinalis* is one the most important mucilaginous herbal and belongs to malvaceae family [4]. It is amazing medicinal properties of this plant have been used for years in traditional medicine [5]. Marsh mallow root extract is used to treat irritation mouth and throat associated with dry cough [6], inflammations of the digestive and respiratory system [7], stimulatory coughs and bronchitis [8]. Gargle to relieve inflammation of the boiled roots and leaves of members such as inflammation of oral mucosa, gingival abscess, preventing from dry throat during fever

[9]. Wash impetigo with marsh mallow infusion is so useful that none of the common expensive medicine can replaced it [10]. *Streptococcus pyogenes* are considered one of the important Gram-positive bacteria in streptococci group A. The diseases caused by these bacteria are scarlet and impetigo [11, 12]. Impetigo is local infection of skin superficial layers, especially in children [13]. The most serious consequence of autoimmune are streptococcus infections, group A that have affected so many children around the world and have caused patients' disability and death [14, 15]. This research is conducted to study the inhibition effect of ethanol extract of flaxweed seed and marsh mallow roots on this bacterium caused scarlet fever and impetigo with regards to medicinal properties of *A. officinalis* and *D. sophia*, to increase use of herbal medicine due to their low side effects and their compatibility with human body, to the effects of abuse of chemical medicines and to resistance of some bacteria to antibiotics.

Materials and Methods

In this experimental study, samples of fresh flaxweed seed (*D. sophia*) were collected from 4 different areas in

Kerman province; Kohbanan, Jiroft, Rafsanjan and Baghin in spring. Marsh mallow (*A. officinalis*) roots were collected from Tehran, Isfahan, Yazd and Kerman in spring. The samples were dried completely and naturally at ambient temperature. In this in vitro and laboratory study, method of soaking seed and its ethanol solvents was used to extract of flixweed seed. One hundred grams of collected samples of flixweed seed was crushed in an electric grinder. Then, resultant powder was completely mixed in a shaker with average speed at a temperature of 24°C for a period of 28 h in 600 mL of 70% ethanol. Next, samples were filtered using Whatman filter paper number one; the remaining residual was discarded and yellow solution containing ethanol and flixweed ingredients was concentrated in the Rotary evaporator at 50°C for a period of 90 min. Concentrated extract was completely dried in an incubator at a temperature of 55°C for 24 h to complete separate from solvent. To extract from marsh mallow root, method of soaking and solvent related to ethanol was used. To do that, 20 g of plant root powder of each area was carefully weighed and each one was poured into a large flask separately, and 400 mL of ethanol, 70%, was added to each of the 4 flasks. After incubating for 48 h at 50°C, the extract was filtered through paper and residual was pressed to fully discharge. Ethanol was again added to the residual and previous step was repeated. Then, the extracts were concentrated using rotary evaporator, at 50°C [14]. Concentrated extracts of all four samples were completely dried in an incubator at temperature of 50°C for 24 h. Dried extracts of both plants were removed with a spatula and were powdered in a sterile mortar. Powder of flixweed and marsh mallow were to concentration of 25, 50, 100 and 250 mg/mL using solvent of dimethyl sulfoxide (DMSO); they were finally filtered to remove possible contamination using syringe filters with diameter of 0.22 µm. In this study, the standard strains of *S. pyogenes* (PTCC: 1447), as lyophilized ampoule, were prepared from Iranian research organization for science and technology (IROST). Moreover, 40 g of blood agar culture medium (made in German, by Merck Co.) was mixed in one liter of deionized water to prepare blood agar culture medium. Sheep defibrinated blood (5-8%) was added to blood agar culture medium in complete sterile conditions under the laminar hood; it was divided after mixing in 9 cm Petri dishes [14]. A jar with candle was used to supply CO₂ to grow *S. pyogenes* on blood agar culture medium. First, one gram of dried extract was dissolved with 4 mL of DMSO to prepare sterile discs including concentrations of 25, 50, 100 and 250 mg/mL from flixweed seed extract and marsh mallow root. Then, the concentration of 250 mg/mL was provided; concentration of 250 mg/mL was used to prepare lower concentration and their dilution was done. Thirty microliters of concentrations prepared from extract of each area were inoculated on sterile discs with pipette. Discs containing antibiotics purchased from Iran Padtan Tab Co. were included amoxicillin (25 µg), penicillin (10 µg) and erythromycin (15 µg). Standard method of dilution on agar culture medium was used to determine the minimum inhibition concentrations of

ethanol extract. Concentrations of 25, 50, 100 and 250 mg/mL of ethanol extract of flixweed seed were transferred to culture mediums and *S. pyogenes* was inoculated immediately. Process of bacterial growth in culture medium containing various concentrations of extract was studied to determine Minimum Inhibitory Concentration (MIC) level for extracts after being incubated for 24 h.

Colony of bacteria grown on blood agar culture medium was taken using a sterile loop after 24 h, and was thoroughly mixed in a sterile test tube containing 5 mL of sterile physiological serum. The suspension of bacteria inside tube was taken using a sterile swab and was cultured on Petri dishes as parallel lines in three dimensions on a uniform basis [16]. Then, discs containing various concentrations collected from different areas were cultured on Petri dishes containing bacteria and control disk with disks containing antibiotics of penicillin, amoxicillin and erythromycin were placed on culture medium in appropriate intervals. Samples were placed in an incubator with a temperature of 37°C for a period of 24 h to study antibacterial properties of each extract. It's worth mentioning that each of flixweed seed and marsh mallow root extract were placed in a Petri dish separately. This test was conducted through antibiogram method in a completely randomized design with 4 replications.

The inhibition zone of bacterial growth was measured with a ruler; SAS software (Statistical Analysis System software-version 9.2) was used to analyze the variance and Duncan's test to compare treatment means in level 0.01% and 0.05%.

Results

The results of this research showed that all concentrations prepared from ethanol extract of flixweed (*D. sophia*) and marsh mallow (*A. officinalis*) had inhibitory effect on *S. pyogenes*; it showed the antibacterial effect of flixweed seed and marsh mallow root. There was a significant difference in level 0.01% of Duncan's test on inhibition zone; concentration of 250 mg/mL of ethanol extract in Jiroft had the highest inhibition zone of bacteria growth, 13.5 mm, while the least inhibitory effect was related to extract of 25 mg/mL in Kerman with diameter of 4 mm (Table 1). Considering the results obtained from SAS software (Table 2), ethanol extract of flixweed seed found in Jiroft had greatest effect in similar extracts. Flixweed extract in all four areas with concentrations of 250 mg/mL had greater effect than three other used antibiotics. This extract has growth inhibitory effect on bacteria in high concentrations, but it caused complete hemolysis of blood in culture medium which showed that this extract couldn't be used in high concentrations. As a result, it affects bacteria and it also destroys and analyzes the blood inside culture medium. Therefore, flixweed seed, in high concentrations, is not good to prepare herbal medicine (Fig. 1 A).

Although concentration of 25 mg/mL flixweed seed has the least bacterial growth inhibitory effect, it is

recommended as the best concentration used in this research to control bacteria and to produce herbal medicine because of not causing blood hemolysis. Concentration 25 mg/mL in Jiroft with diameter of 8.75 mL has greater inhibitory effect on bacterial concentrations among concentration of 25 mg/mL of flaxweed seed.

Because of not causing blood hemolysis and having greater inhibitory effect than three used antibiotics, it can be suggested as the best concentration sample of flaxweed seed extract used in this research to control bacteria and to produce herbal medicine. Penicillin had the most growth inhibitory diameter effect on 8.31 mm bacteria among three used antibiotics. Results of variance analysis of areas and various concentrations of marsh mallow root extract showed a significant difference in level 0.01% in terms of inhibitory on bacterial growth.

Comparing means of concentration and areas, it was shown that concentration of 250 mg/mL of marsh mallow root extract had the greatest mean (Fig. 1 B) and its concentration of 25 mg/mL had the least mean in terms of growth inhibitory effect on *S. pyogenes* (Table 1).

Comparing areas, it was shown that marsh mallow extract in Yazd had the greatest inhibitory effect and that the least one was related to 25 mg/mL concentration of Isfahan and Tehran with diameter of 6.5 mm. In comparison with mean of growth inhibitory effect on *S. pyogenes*, marsh mallow root extract and flaxweed seed and various concentrations with antibiotics of erythromycin, amoxicillin and penicillin in level of 0.05% and 0.01% conducted using Dunstan's method, concentration of 250 mg/mL in marsh mallow extract in Yazd had the greatest effect on growth of *S. pyogenes* with inhibitory effect of 14.5 mm bacteria growth rather than other extracts as well as all studied antibiotics; the

least inhibition effect among used extracts was related to ethanol extract of flaxweed seed, concentration of 25 mg/mL in Baghin (Table 1). Bacterial growth trend in culture mediums included different concentrations was examined after incubating for 24 h in 37°C to determine the MIC level for extracts. The results showed that there was no growth in culture medium containing extract of flaxweed seed and marsh mallow root at concentrations of 25 mg/mL. General result of analysis of two extracts and comparison done using SAS software showed that marsh mallow root extract had greater inhibition effect than flaxweed seed extract in level of 0.01%. In many concentrations, marsh mallow root extract had greater mean of growth inhibition diameter than flaxweed seed extract in such a manner that concentration of 25 mg/mL of marsh mallow extract in Yazd had greater inhibition effect on *S. pyogenes* than concentration of 250 mg/mL of flaxweed extract in Rafsanjan (Fig. 2)

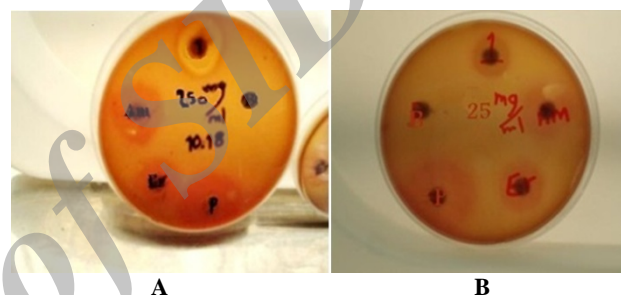


Figure 1. Effect of flaxweed seed extract in concentration of 250 mg/mL and Marsh mallow root extract in concentration of 25 mg/mL on *Streptococcus pyogenes* compared with common antibiotic. A: Marsh mallow root extract and B: Flixweed seed of extract, Er: erythromycin, Am: amoxicillin, P: penicillin, B: Blank disc or water disc.

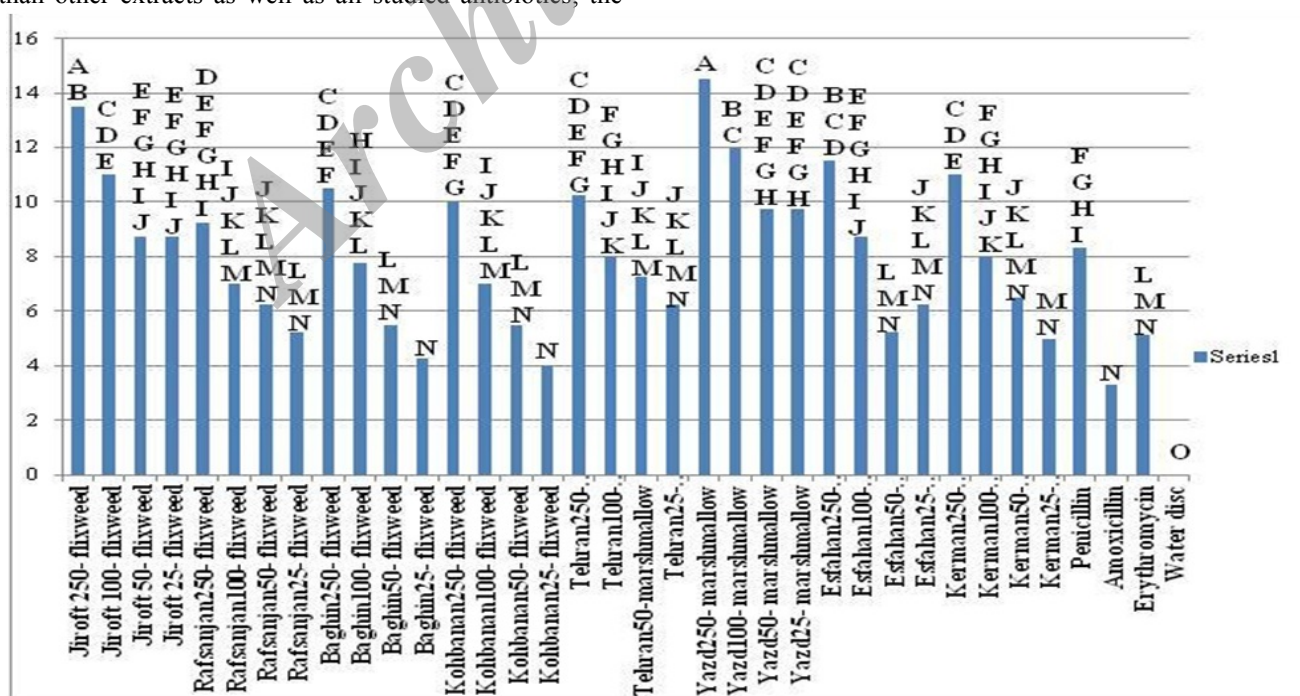


Figure 2. Grouping based on average bacterial growth inhibitory diameter in millimeters in different treatments and concentrations according to Duncan test, 0.01%

Table 1. Average bacterial growth inhibitory diameter in millimeters in different treatments and concentration according to Duncan's test, 0.01%

Source	DF	Sum of squares	Mean square	F Value	Pr>F
Model	35	1250.611627	35.731761	24.35	<0.0001
Error	108	158.475586	1.467367		
Corrected total	143	1409.087212			

Table 2. Results of variance analysis of comparison of inhibitory effect of flaxweed seed extract and marsh mallow roots of different regions and antibiotics erythromycin, amoxicillin and penicillin

Concentration mg/m	Average bacterial growth inhibitory diameter (mm)	Concentration mg/mL	Average bacterial growth inhibitory diameter (mm)
Tehran 50-marsh mallow	7.25	Jiroft 250- flaxweed	13.5
Tehran 25- marsh mallow	6.25	Jiroft 100- flaxweed	11
Yazd 250- marsh mallow	14.5	Jiroft 50- flaxweed	8.75
Yazd 100- marsh mallow	12	Jiroft 25- flaxweed	8.75
Yazd 50- marsh mallow	9.75	Rafsanjan 250- flaxweed	9.25
Yazd 25- marsh mallow	9.75	Rafsanjan 100- flaxweed	7
Isfahan 250- marsh mallow	11.5	Rafsanjan 50- flaxweed	6.25
Isfahan 100- marsh mallow	8.75	Rafsanjan 25- flaxweed	5.25
Isfahan 50- marsh mallow	5.25	Baghin 250- flaxweed	10.5
Isfahan 25- marsh mallow	6.25	Baghin 100- flaxweed	7.75
Kerman 250- marsh mallow	11	Baghin 50- flaxweed	5.5
Kerman 100- marsh mallow	8	Baghin 25- flaxweed	4.25
Kerman 50- marsh mallow	6.5	Kohbanan 250- flaxweed	10
Kerman 25- marsh mallow	5	Kohbanan 100- flaxweed	7
Penicillin	8.31	Kohbanan 50- flaxweed	5.5
Amoxicillin	3.31	Kohbanan 25- flaxweed	4
Erythromycin	5.125	Tehran 250- marsh mallow	10.25
Water disc	0	Tehran 100- marsh mallow	8

None of the concentrations had effects on blood of culture medium and hemolysis did not happen in it. Therefore, concentrations of 250 mg/mL of marsh mallow root extract in Yazd that had the highest inhibition zone of bacterial growth was considered the best concentration to prevent the bacteria growth among available concentrations and three antibiotics (erythromycin, penicillin and amoxicillin). Results have shown that by increasing concentration of extract, the antibacterial effect level of the 2 plants increases in a way that concentrations of 250 mg/mL will have the highest inhibitory effect on bacterial growth and concentration of 25 mg/mL will have the lowest inhibitory effect on bacterial growth.

Discussion

In this research, as mentioned above, both ethanol extract of marsh mallow root and flaxweed seed have antibacterial properties; however, since marsh mallow root extract had higher inhibitory effect on *S. pyogenes* and beta hemolysis of blood that was not seen in any of its extract concentrations, concentration of 250 mg/mL of marsh mallow root extract in Yazd was known as the best concentration among extracts and antibiotics of erythromycin, penicillin and amoxicillin.

Since many years ago when there were no chemicals to treat, herbal medicines were used to cure patients in various countries such as China, Ancient Rome, Greece, India and Iran. By developing science and producing medicine manufactured using chemicals, traditional medicine is gradually being forgotten [17]. However, nowadays a new approach is formed in using herbal medicines to treat infectious and bacterial diseases because herbal medicines have more antibacterial effects and fewer adverse effects; also, less money should be paid

for them than chemical medicines [18]. Among the compounds available in marsh mallow root extract, it can be pointed out to flavonoids quercetin, kaempferol, tannins, lecithin, pectin and astragal; these materials have different properties, for example, flavonoid quercetin effects on vasodilating contracted ring of thoracic aortic [19, 20].

In a study carried out by Abachi et al., the effect of extract of 10 Iranian herbal such as *D. sophia* (L.) was examined on *S. epidermidis*. All ten herbal extract with different ratios had inhibitory effect on bacteria; this bacteria is resistant to antibiotic of vancomycin [21]. Some studies were carried out by Lin et al. in Florida university, America, about antibacterial effect of allyl isothiocyanate extracted from cruciferae on *Escherichia coli* (K-12), *Salmonella montevideo* and *Listeria monocytogenes*; the bactericide effect of liquid extract of this composition and its vapor form similar to polymyxin B were introduced [22]. Mohammadinia et al. studied the effect of flaxweed on spontaneous labor in nullipars in late pregnancy. Results showed a positive effect of flaxweed on easier delivery in nulliparous women [23].

A research was conducted by Niestani et al. on the inhibitory effect of *Camellia sinensis* on *S. pyogenes* growth and its comparison with green tea in laboratory environment. The results showed that both *Camellia sinensis* and green tea had inhibition effect on growth of bacteria, while antioxidant capacity of green tea was significantly more [14]. Komeilizadeh et al. studied the effect of hydro-alcoholic extract of *Triticum sativum* Lam. on some Gram-positive and Gram-negative bacteria, including *S. pyogenes*. Aqueous extract of *T. sativum* Lam. did not have any effects on the above-mentioned bacteria. Ethanol extract and petroleum ether extract had a positive effect on bacteria. Chloroform extract had little

effect on bacteria [16]. The effect of flaxseed seed extract on inhabitation of standard strains growth PTCC 1222 of *E. coli* and *Staphylococcus aureus* was studied by Shahandeh et al. they concluded that lack of flaxseed extract effect may be because of having anti-bacterial combination with other compounds in extracts or it may be antagonism effect [24]. In a study carried out by Ono et al., 6-methyl sulfinyl-hexyl isothiocyanate available in extract of some of the members of cruciferae has antibacterial effect on *E. coli* and *S. aureus* [25].

Considering the antibacterial compounds available in flaxseed such as isothiocyanate, disulfide allyl, glycoside sulfide with phenol compounds known, and metabolites, effective materials available in marsh mallow, the antibacterial effect of these plants on the *S. pyogenes*, these two plants can be good replacements for chemical medicines. With regard to the significant difference available in level of 0.01%, marsh mallow root extract has greater antibacterial property than flaxseed seed extract. Carrying out more studies and research on these two plants and recognizing the available effective materials in lower concentrations than the studied research, we can prescribe the specific concentrations of these plants for

those who are in incubation of being affected by this bacterium instead of antibiotics that bacterium daily increase their strength against; In addition to have fewer side effects than chemical medicines, they save the time and expense.

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Authors' Contributions

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

Conflict of Interest

The authors declare no conflict of interest.

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