



A comparative study on the effect of ethanol extract of wild *Scrophularia deserti* and streptomycin on *Brucella melitensis*

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ABSTRACT

Introduction: Brucellosis or Malta fever is one of the most prevalent bacterial zoonosis which is transmitted to human being from animals. High fever, myalgia, and arthralgia of the large joints are the important symptoms. This study was aimed to evaluate the anti-brucellosis effect of ethanol extract of wild snapdragon on *Brucella melitensis* bacteria.

Methods: This is an experimental in which after preparing the ethanol extract of wild Snapdragon (*Scrophularia deserti*), the anti-Brucellosis impressions of the extract on *Brucella melitensis* which is separated from goat's abortive fetus were studied by serial dilution and disc diffusion method.

Results: In disc diffusion test, the disc containing 60 µg/ml extract the inhibition zone was 9.7mm after 72, for streptomycin (20 µg/disc) 13.3 mm and for streptomycin plus 60 µg/disc extract discs it was 21.5 mm, after 72 hours incubation at 37 centigrade.

After analyzing the data; the MIC for serial dilution test was 52% (576 µg/ml) for the first repetition and 50% (576 µg/ml) for the third repetition of the extract with $OD_{600}=1.56$. For standard medicine "streptomycin" with $OD_{600}=1.67$ it was 52% (360 µg/ml).

Conclusion: Ethanol extract of wild *Scrophularia deserti* is effective on *Brucella melitensis* and its consumption might be useful in these patients.

Implication for health policy/practice/research/medical education:

Ethanol extract of wild *Scrophularia deserti* is effective on *Brucella melitensis* and its consumption might be useful in Brucellosis.

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Introduction

Scrophularia deserti is in *Scrophulariaceae* family. It is found in areas such as Iran, Egypt, Palestine, Jordan, Saudi Arabia, Syria, Iraq, Bahrain and Kuwait. *Scrophularia deserti* is used in traditional medicine as an anti-pyretic, a remedy for kidney diseases, fever, tumors and lung cancer (1). In traditional veterinary medicine *Scrophularia deserti* is used for treatment of bovine mastitis (2). Traditional herders in south of Ilam province used *Scrophularia deserti* for the treatment of mastitis caused by *Staphylococcus aureus*, eye infections and

poisons (3). In phytochemical analysis of *Scrophularia deserti* eight compounds were identified (4). Compositions of the plant include 3(f)-hydroxy-octadeca-4(E), 6(Z)-dienoic acid, ajugoside, scropolioside B, 6-O-a-L-rhamno-pyranosylcatalpol, buddlejoside A8, scrospioside A, laterioside and 3R-1-octan-3-yl3Ob-D-glucopyranoside (4).

Brucella strains are optional intracellular bacteria that cause brucellosis. It can be transmitted from infected animals to humans and cause infection. Brucellosis or Malta fever is a disease with a variety of clinical symptoms such as high fever,

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myalgia, and arthralgia of the large joints. Three types of Malta fever have been found: Acute syndrome or ACS (under 8 weeks), chronic (over 8 weeks) and the billowing (recurrent attacks and healing). In severe form, flu-like symptoms are predominant. Symptoms of chronic form include chronic fatigue, depression, arthritis attacks undulant fever (which dates from 2 to 14 days). The risk of livestock brucellosis increases 4 to 6 times by having contact with livestock (5-7).

Herbal treatments are effective and cheap in controlling the disease. The antimicrobial effect of this plant in southern of Ilam was indicated. This study was aimed to evaluate the effect of methanol extract of *Scrophularia deserti* on *Brucella melitensis* in comparison with streptomycin.

Materials and Methods

Preparation of the plant

Scrophularia deserti was prepared from the southern margin of the Zagros Mountains in Ilam. Herbal sample of the plant was sent to the Natural Resources Research of Ilam and were determined by diagnostic keys of flora. *Scrophulariaceae* species had the length of 10 to 50 cm, short stem, in perfectly crack the pedicle, non-shoot crisp corners, thicker leaves and more or less thick, dense inflorescences flowery bracteole pistil-bearing, spherical capsules, hard, straw yellow and black beads and oval desert (8).

Preparation of the extract

100 gram of aerial parts of *Scrophularia deserti* was dried in 60°C (Oven: U632, Iran) and powdered. Then it was well mixed in 250 ml of ethanol (96 degrees) and was concentrated in vacuum distillation system via Rotary (Zirbus302®, Italy). Ultimately the extract was sterilized by filtration.

Preparation of antibiotics

360 mg of the streptomycin (Hayyan Jaber Pharmaceuticals, Iran) was dissolved in 1ml of sterile distilled water and 1% to 10% of this solution (21.6, 43.2, 64.8, 84.4, 108, 129.6, 151.2, 172.8, 194.4, 216 mg/μl) was added in each tube. Concentrations of 1% to 5% (12, 24, 36, 48, 60 μg/disc) of streptomycin were added to disks in disk diffusion method.

Preparation of bacterial strains

In this experimental study, the native strain of *Brucella melitensis* was isolated from an aborted fetal goat and was confirmed by PCR in Biotechnology Research Center of Islamic Azad University, Shahrekord. It was grown in brucella culture. Suspensions were prepared from bacterial suspensions and its turbidity was measured at a wavelength of 600 nm by spectrophotometer.

According to the company recommendations, the distilled water was added to the sterile brucella broth agar medium (Merck, Germany). For Serial Dilution Method, 5 ml of broth was added to each tube and the tubes containing 5 ml of Brucella broth and Brucella agar medium were sterilized by autoclaving for 15 minutes at 121°C. In disc diffusion method, 8 ml of Brucella agar medium was added to each plate. All liquid and solid media were kept in the refrigerator.

Serial dilution method

11 tubes were prepared in each stage. Each tube contained 5 ml of sterilized water and 1 ml of suspension of liquid bacteria. They were prepared on a fixed OD₆₀₀ and were added to each

series of tubes. The concentrations of 1% to 10% of the filtered extract (in μg/ml) of *Scrophularia deserti* were added to the tubes 1 to 10, respectively and one of the tubes considered as negative control tube. 48 hours after addition of the extract, opacities were surveyed at OD₆₀₀ nm by a spectrophotometer. Percentage of growth inhibition was calculated for each iteration in the following way (18):

$$\text{MIC\%} = \frac{\text{OD}_{600 \text{ Ct}} - \text{OD}_{600 \text{ st}}}{\text{OD}_{600 \text{ Ct}}}$$

Ct: control tube

St: sample tube

Results

First extract had OD₆₀₀ = 1.79, the second iteration OD₆₀₀ = 1.56, the third iteration of the extract and standard drug streptomycin had OD₆₀₀ = 1.67. The P value of 0.023 was found between the three replicate extracts of *Scrophularia deserti*. Results are shown in Table 1. MIC for the first iteration of the extraction on the tube for OD₆₀₀ = 1.79 was 51% (576 μg/ml), for the second iteration of the extract of tube with OD₆₀₀ = 1.56 was 52% (576 μg/ml), for the third iteration of the extract for OD₆₀₀ = 1.67 was 50% (576 μg/ml) and standard drug streptomycin control for OD₆₀₀ = 1.67 (108 μg/ml) was 52%. Disc diffusion method was 60 μg/ml for disk and 9.7 mm zone was achieved after using oven for 72 hours at 37°C. Pricing for the streptomycin disc (20 μg/disc) was 13.3 mm and for streptomycin (60 μg/disc) extract containing 60 μg/ml was 21.5 mm, respectively.

Disc diffusion method

100 ml of the bacterial suspension from negative control tube was added to brucella agar plates with OD₆₀₀ = 1.67 and incubated. Sterile discs (discs 6.4 mm) with 10 microliters of dimethyl sulfoxide (DMSO) were added on each plate. Then 1% to 5% (12, 24, 36, 48 and 60 μg/ml) of the extract containing 10 ml sterile dimethyl sulfoxide was added to the disc and incubated at 37°C. In order to determine the synergistic effects of the extract, streptomycin discs with concentrations of 1% to 5% were added to the extract. Zone diameter was measured at 24, 48 and 72 hours after the test by caliper (Table 2).

The percent of growth inhibition calculating in the first iteration of *S. deserti* for the first iteration to OD₆₀₀ = 1.79 in the tube 8 was 51% (576 μg/ml), for the second iteration of the tube 8 with OD₆₀₀ = 1.56 was 52% (576 μg/ml), for the third iteration of the tube 8 OD₆₀₀ = 1.67 was 50% (576 μg/ml) and streptomycin antibiotic (360 μg/ml) with OD₆₀₀ = 1.67 in tube No. 5 was 52%. Between the three replicates of *Scrophularia deserti* there were significant differences (p=0.023) in which analyses were performed two by two between iterations specified by the difference between repeats of one and three from *Scrophularia deserti* (p= 0.013), and to occurrence between two and three (p= 0.001), respectively.

Relationships between the concentration of ethanol extract of *Scrophularia deserti* to streptomycin for the first iteration (p= 0.022), for the second iteration (p= 0.029) and for third iteration (p= 0.01), indicated that there were significant differences between the three repeated of *S. deserti* (p=0.023).

Discussion

Brucellosis or Malta fever is of common bacterial diseases of humans and animals with symptoms such as fever raging conflict with the musculoskeletal system and brucella arthritis

Table 1. The MIC for different treatments and concentrations of the extract

Tubes	The first repeat <i>Scrophularia deserti</i> OD ₆₀₀ =1.79		Second iteration <i>Scrophularia deserti</i> OD ₆₀₀ =1.56		The third iteration <i>Scrophularia deserti</i> OD ₆₀₀ =1.67		Streptomycin OD ₆₀₀ =1.67		Concentration of extract (µg/ml)
	OD ₆₀₀	Percent inhibition of growth	OD ₆₀₀	Percent inhibition of growth	OD ₆₀₀	Percent inhibition of growth	OD ₆₀₀	Percent inhibition of growth	
1	1.26	29%	1.17	25%	1.24	25%	1.01	39%	72
2	1.19	33%	1.09	30%	1.16	30%	0.998	41%	144
3	1.11	37%	0.990	36%	1.08	35%	0.967	43%	216
4	1.05	41%	0.910	41%	1.02	38%	0.845	49%	288
5	0.980	45%	0.860	44%	0.950	42%	0.793	52%*	360
6	0.920	48%	0.810	48%	0.900	46%	0.617	63%	432
7	0.890	48%	0.780	50%*	0.870	47%	0.580	65%	504
8	0.860	51%*	0.740	52%	0.830	50%	0.433	74%	576
9	0.840	53%	0.710	54%	0.810	51%	0.397	76%	648
10	0.810	54%	0.630	60%	0.730	56%	0.321	80%	720

*: MIC

Table 2. Concentration of extract based on microgram/ ml and the zone of inhibition of growth at 24, 48 and 72 h after incubation in millimeters

Extract concentration	Extract Concentration of extract						Antibiotic Zone streptomycin
	After 24 hours	After 48 hours	After 72 hours	After 24 hours	After 24 hours	After 24 hours	
12	6	6.5	6.6	0	0	8.3	6.8
24	7	7.5	7.7	8.1	8	8.5	7.3
36	7	7.5	8.9	8.1	12	12	9.6
48	8	8.5	9	10	12.5	12.5	10.6
60	9	9.5	9.7	11	20	21.5	13.3

associated (9,10,11). In this study the antimicrobial effects of different concentrations of the extract on *Brucella melitensis* strain isolated from aborted goat fetuses with assay of Serial Dilution and Disc Diffusion methods carried out in which the results for the discs containing 60th µg/ml extract the inhibition zone was 9.7 mm after 72 and for streptomycin was 13.3 mm and for streptomycin plus 60 µg/disc extract was 21.5 mm. Ghasemi-pirbalouti *et al.* indicated the Camel thorn plant extract at a concentration of 500 micrograms/ml had greatest inhibition of growth and ethanol extract of *Scrophularia deserti* to concentration of 125 micrograms/milliliter had lowest inhibition of bacterial growth (12).

After 72 hours of incubation *S. deserti* formed inhibition zone equal to 5.21 mm, which represents appropriate antimicrobial effect of ethanolic extracts of *S. deserti*.

Ghasemi-pirbalouti in a study conducted in 2009 on the effects of some native medicinal plants on *Candida albicans* isolated from recurrent vaginitis by disk diffusion method, the extract and essential oil of *Scrophularia deserti* caused the most zone of

inhibition of growth (11).

Bahmani *et al.* has proven that *S. deserti* ethanol extract had good efficacy against *Saprolegnia Parasitologica* (12). In the present study for the first iteration was 51%, the second iteration 52% and for the third iteration was 50% which may indicate that *Saprolegnia parasitica* has antibacterial effect. Ethno-veterinary in of southern Ilam province is referred *Scrophularia deserti* for gangrainous mastitis and disinfects the body of toxins and is used for eye infections (13,14), which in part these effects are confirmed.

In other study the effect of *Teucrium polium L.* on *B. abortus* was evaluated. It had no effect on the inhibition of bacterial growth (15). In another study the effect of eight combinations of the plant on *staphylococcus* was evaluated and was found that three combinations of 8 had the antimicrobial effects (4).

Results of this study indicate that inhibition of the growth amount increases with increasing concentration. According to the studies on the antimicrobial effects of the extract on microorganisms such as *Candida albicans*, *Staphylococcus aureus*, *Saprolegnia*

parasitica, *Brucella abortus* and *Brucella melitensis* the effects are remarkable. More studies are recommended to identify the main compound of the extract with antibacterial activity.

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Authors' contributions

NVS, RM, HM, KS, BK, and FE prepared the main draft. MB, AS and JS edited the paper.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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