Original Paper

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The antibacterial activities of six organic solvent extracts of *Salicornia iranica* against *Salmonella typhimurium*

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Keywords

Salicornia iranica, antibacterial, Salmonella

Abstract

The overuse of antibiotics in poultry industries may lead to a rise in antibiotic-resistance, in humans and animals. Natural antibiotics degrade in living tissues, and therefore, they do not induce antibiotic-resistance. In this study, the antibacterial activity of six organic solvent extracts of *S. iranica* were investigated against *S. typhimurium*. The results indicate a significant antibacterial activity of *S. iranica* extracts against S.typhimurium ($p < 0.01$). The maximum and minimum inhibitory activity of organic solvent extracts compared with 0.5 mg/ml of tetracycline were detected for butanol and acetyl acetate (50 mg/ml) treatments, respectively.

Abbreviations

TSB: Tryptic Soy Broth
GLM: Generalized Linear Model

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Introduction

Salicornioideae family comprises approximately 15 genera and 80 species (Shepherd et al. 2005). *Salicornia* genus is known as a halophytic, annual glasswort plant, grows in salt marshes area worldwide (kim and song, 1983). Different species of *Salicornia* growing in northern, northwestern, southern, and central part of Iran. Two species including, *S. persica* and *S. iranica*, were reported as native species in Iran (Akhani. 2008; Mohammadi et al. 2013).

Plant natural products offer potential as new therapeutic agents against human diseases. Several studies have demonstrated that the antibacterial effects of plants relate to secondary metabolites such as: alkaloids, flavonoids, tannins, and polyphenolic compounds (Brantner et al. 1996; Manikandan et al. 2009). These are a diverse group of molecules which are involved in the adaptation of plants to their environment. These secondary metabolites are not part of the primary biochemical pathways of cell growth and reproduction. (Makkar et al. 2007).

Antibiotics are widely used in farm animals, especially poultry productions. For increasing the growth rate of chickens, the birds are fed antibiotics for a short time (45-60 days). This time frame leads to antibiotics which remains in tissues, with the potential to be transferred to humans through meat and eggs. Natural antibacterial compounds of plants are preferred to synthetic antibiotics, because natural compounds do not remain in tissues and break down rapidly. Previous studies demonstrated that the methanolic extract of S.brachiata had significant antibacterial activity against gram-negative bacteria, such as *E. coli* (Feroz khan et al. 2013). Additionally, the antibacterial activity of *S. brachiata* was related to the presence of phenols in the plant extract (kumar et al. 2009). *S. herbacea* extracts have also been shown to have an inhibitory effect on Streptomyces (Yu et al. 2011). *Salmonella typhimurium* is gram-negative bacteria, and it can be transferred from eggshells to humans. It is the causal agent of Salmonellosis in humans.

In this study the antibacterial activities of six organic solvent extracts of *S. iranica* have been investigated against *S. typhimurium*.

Material and Methods

Plant material and extractions

The shoots of *S. iranica* were obtained from the Salt Lake (Maharloo Lake 52°46'18.00"E- 29°32'17.00"N) in Fars province (Iran). Temperature and relative humidity at the time of sampling were, 13.6 °C and 56%. The fresh shoots were dried at room temperature for two weeks, then 25 g of the dried material was finely ground, and extracted with 200 ml of 96% ethanol for 8 hours using the Soxhlet apparatus followed by filtration through filter paper. The plant extracts were lyophilized. Twenty gram of the lyophilized material was dissolved in 200 ml of the following solvent solution: Acetyl acetate, N-hexan, Butanol, Ethanol, Methanol and Dichloromethane for 24 hours, and filtered through number four paper filter. Organic solvents were removed by evaporation at 50°C.

Antibacterial activity assay

The extracts after removal of the organic solvents, were dissolved to a final concentration of 50 mg/ml, in 200 ml sterile Tryptic Soy Broth (TSB, Merck, Germany). The suspension was slowly filtered through a size: 0.1 micrometers filter (Biofil Company). Bacterial suspension (*Salmonella typhymurium* ATCC 14028) was prepared by the School of Veterinary Medicine, Shiraz University. The treatments, TSB, bacteria, and extracts, were incubated (incubation size: 105) overnight at 37 °C in a microplate reader system (BioTek's PowerWave XS2 with GEN5 data analysis software, USA). The absorbance was measured at 600 nm at 11 time points during the 24 h incubation time. TSB medium containing bacteria without antibiotic or plant extracts was considered as a negative control. TSB medium with bacteria and 0.5 mg/ml tetracycline was prepared as positive control. The antibacterial activity of the plant extracts were compared with the positive control.

Statistical analysis

Statistical analysis was performed using the repeated measurement model for the antibacterial activity of plant extracts data, from eight treatments with three replications at 11 time points. Differences between treatments were tested using the GLM procedure of the SPSS software (SPSS, Version 16). Tukey's test was used for pairwise mean comparisons. The antibacterial activity of antibiotic treatment was compared to other treatment by the (A-B/B) ×100 formula, where A is average of each treatment and B is the average of antibiotic treatment (Casey et al. 2004).

Results

The results of the statistical analysis, showed that there is significant effect of the plant extracts against *Salmonella* bacteria ($p < 0.01$). The summary of statistical analysis and Tukey's test are shown in Table 1.

No significant difference was observed between ethanol, methanol and dichloromethane plant extracts in antibacterial activity (Table 1). The antibacterial activity of different organic fractions in different incubation times were shown in Figure 1. The effect of Butanole fractions were compare to the positive control. The positive control had the strongest inhibitory effect against all treatments.

The growth inhibition effects against *S. iranica*, reported as percent inhibiton compared with tetracycline (Fig-

Table 1
The mean absorbance (at 600 nm) compared among different treatments.

Treatment	Acetyl acetate	N-hexan	Butanol	Ethanol	Methanol	Dichloromethane
Means	0.4825 ± 0.06 ^{A}	0.3544 ± 0.04 ^{B}	0.1439 ± 0.03 ^{C}	0.2281 ± 0.02 ^{D}	0.2615 ± 0.04 ^{D}	0.2156 ± 0.02 ^{D}

Means have been statistically analyzed using Tukey's test. There is no significant differences between groups with common letters.

ure 2). The results showed that all treatments were very effective against *S. typhymurium*. The highest and lowest inhibitory effects were for butanol and acetyl acetate respectively. The treatments ordered starting from the most effective are as follows: butanol, dichloromethane, ethanol, methanol, n-hexan and acetyl acetate.

Discussion

According to the results, the antibacterial effect of *S. iranica* against *Salmonella* growth was significant. This result is consistent with other studies. Coban et al. (2009) that showed that the extracts of *S. europaea* are very effective against *S. typhymurium* (ATCC 14028). Sarker et al. (2010) reported *S. herbacea* could be replaceable as an antibiotic for broiler chickens. In addition they indicated the efficiency of *S. herbacea* extract against bacteria growth is comparable with antibiotic. In addition, Manikandan et al. (2009) reported the comparable results about the antibacterial activity of *S. brachiata*. They reported the methanolic extract of this species was more active than the water extract against *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*. In addition, they reported the shoot extracts of *S. brachiata* were very effective against gram-negative bacteria. However, there are different reports on the antibacterial properties of *Salicornia* species. Lellau and Liebezeit (2003) demonstrated no significant association between the antibacterial activity of *S. europaea* and gram-negative bacteria. The different results of studies can be attributed to the instability of secondary plant metabolites. In fact, there is association between the amounts of secondary metabolites and sampling conditions such as

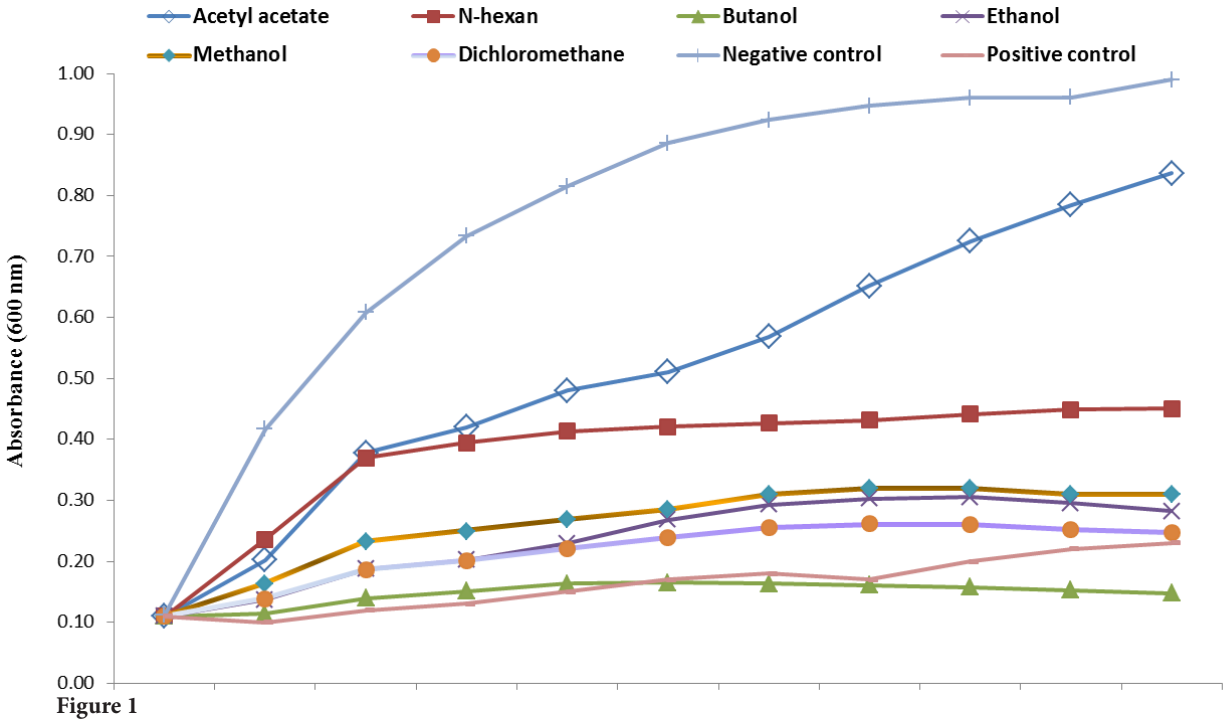

Figure 1
Antibacterial activity of six organic solvent extracts of S. iranica (50 mg/ml) against *S. typhymurium* at 37 °C. Negative control (TSB medium, bacteria suspension without antibiotic and extracts) and positive control (TSB medium, bacteria suspension and tetracycline (0.5 mg/ml).

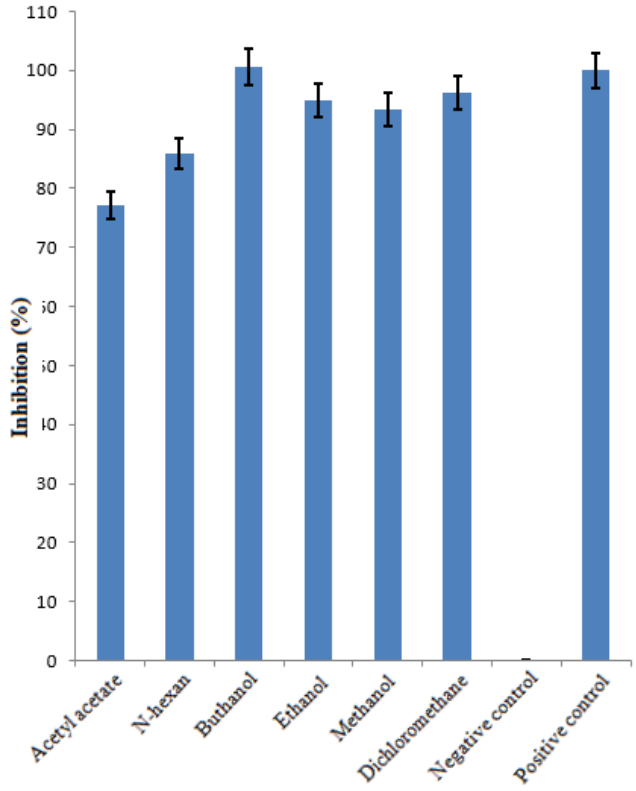

Figure 2
The percent inhibition of six organic solvent extracts of *S. iranica* against *S.typhymurium* in comparison with tetracycline. Negative control (TSB medium and bacteria suspension, without antibiotic or a extracts) and positive control contain TSB medium with bacteria suspension and 0.5 mg/ml of tetracycline.

temperature, relative humidity and age of plant.

Our results show that the organic solvent extract of *S. iranica* is very effective against *S. thyphymurium*. Butanol treatment had the same inhibitory effect in comparison with thetracycline antibiotic. Therefore the use of this extract can be recommended as an alternative to antibiotic usage in poultry productions. *In vivo* studies will be needed to further explore the suggested application.

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