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Evaluation of the Antibacterial Potential of *Scrophularia Striata* against Plant Pathogenic Bacteria

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Abstract: *The present study was conducted with a view to evaluate the antibacterial potential of Pseudomonas syringae used in Iran against plant pathogenic bacteria. For evaluation of antibacterial effects of the methanol, disc diffusion method through the measurement of the inhibitory zone diameter was used. The antimicrobial methanol extract in three concentrations (20, 40, 50 mg/ml) were tested on Escherichia coli, Rathayi bacter tritici, Xanthomonas campestris and Pseudomonas syringae. Results of research showed that both concentrations had a positive effect on aforementioned bacteria. It also revealed higher concentration had direct influence on the size of inhibitor zone. In 50 mg/ml concentration, the maximum zone of inhibition (ZOI) was 23.98 mm in Pseudomonas syringae. The acceptable minimal inhibitory concentration (MIC) and minimum lethal concentration (MLC) were 10 mg/ml and 22mg/ml respectively. The results demonstrate the antibacterial potential of this plant and hence lend support for the use of them to control plant pathogenic bacteria.*

Keywords: *Scrophularia striata, medicinal plant, pathogenic bacteria, antibacterial effect, disk diffusion test.*

1. INTRODUCTION

Medicinal plants are considered as rich genetic resources and one of the most valuable national assets. Currently, some species of medicinal plants are in danger (known as red species) because of the pressure on natural resources and too many valuable species have been extinct so far (Shoohani 2010). The conducted studies have shown that secondary metabolites as natural materials have important ecological roles in plant defense reactions. Many metabolites are effective in plant defense against pests and diseases (Cowan 1999). Identifying and studying these metabolites can effectively help to control pests and diseases (Azlan 2003).

Scrophulariastriata (figwort) is a species of *Scrophulariaceae*. The straight trees height is 30-90 cm without trichome, which grow in Ilam and some parts of Khuzestan, Iran. The plant flowers from May to June. The chemical compositions of this species have not been identified yet, but people living in Ilam province are empirically using it in different forms such as edible sodden, fumigation and ointment to treat several diseases including inflammation and infection of eyes and ears, skin burns, infected wounds, episiotomy, gastrointestinal pain and disorders, cold, hemorrhoids, blotch, etc (Azadbakht, 2000). The branches are used as a stomach tonic (Jallali et al., 2007). Today, with advances in organic chemistry and significant developments in methods of extraction, purification and identification of natural compounds of plants, the value of drugs derived from plant sources is becoming more obvious (Nariman et al., 2004)

Several decades of experience have shown that chemical drugs with remarkable efficiency have many adverse and side effects. Given the increased antibiotic resistance due to using antimicrobial drugs for prevention and treatment of infections as well as different side effects and adverse results, studying medicinal plants in order to discover new sources of drugs against bacterial infections with antimicrobial activity seems necessary (Baron, et al., 1990; Jawetz, et al., 1991). Some studies have been done about the antimicrobial property of *Scrophulariastriata.* Abbasi et al (2006) stated that the mean diameter of zone against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was more than antibiotics of chemical drugs and the mean diameter of zone increased by increasing in extract concentration from 50 to 90 mg.

Shoohaniet al. (2010) in a study about the effect of hydro alcoholic extract of *Scrophulariastriata* on wound healing in rabbit found that *Scrophularia striata* 10% extract and phenytoin 1% had the best results. Sharafti et al. (2010) in a study about antimicrobial effect of aqueous and ethanolic extracts of *Scrophularia striata* on E. coli reported that the ethanolic extract in both agar and macro dilution diffusion methods had inhibitory effects on mentioned bacteria and the MIC was 90 mg/ml while the MBC was 100 mg/ml. The aim of this study was to evaluate antimicrobial effects of methanolic extracts of this species on *Escherichia coli* due to the presence of various phyto chemical materials and the antibacterial potential of this plant based on plant pathogenic bacteria.

2. MATERIALS AND METHODS

2.1. Preparation of Plant Material

The plant parts of *Scrophulariastriata* such as stem and flower were collected from Ilam. This plant parts were separated, washed with distilled water, dried and powdered using a blender.

2.2. Preparation of Plant Extracts

Plant parts powder was soaked in 70% methanol for 48 h. After extraction each solution was filter using what man No1 filter paper. The filtrate was concentrated in incubation at 20ºC for 24 h and was store at until when required.

2.3. Test Organisms

Clinical isolates of the following: *Escherichia coli* ،*Rathayibacter tritici* ،*Xanthomonas campestris* و*Pseudomonas syringae* were collected from Microbiology Laboratory, Sari university of Iran.

2.4. Bioassay

The antibacterial test was carried out through the agar diffusion method of Nair and Chanta (2005). The test organisms were inoculated on nutrient agar plates and were spread evenly. Four pathogenic bacteria cultured on Nutrient Agar plates and were incubated for 24 h at 37 °C. Nutrient Agar was used to maintain the clinical isolates of the bacteria. Bacterial strains grown on nutrient agar at 37 °C for 24 h were suspended in 20, 40 and 50 mg/ml concentration of methanol extract of plant stem and flower. Wells of 6.5mm diameter was made on nutrient agar by the use of forceps sterilized by flaming. To each well Bacterial strain on 20, 40 and 50 mg/ml concentration of methanol extract of plant stem and flower were introduced. Control experiment by the use of discs wet in methanol and tetracycline (10µg) as negative and positive control were introduced into the well. The dishes were pre incubated at 4°C for 1 h to allow uniform diffusion into the agar. Then, the plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed. The experiments were performed in triplicate.

2.5. Determination of Minimal Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined for *Scrophulariastriata* plant which showed antibacterial acitivity against*Escherichia coli* ،*Rathayibacter tritici* ،*Xanthomonas campestris* و*Pseudomonas syringae*. Microorganism inoculation was prepared after 12 hours of cultivation in liquid environment and was diluted to achieve a density of 0.5Mac Farond Standard (zgooda and Porter, 2001).The highest concentration of plant extract (1000 mg/ml) was prepared for testing and then successive dilutions were prepared in different concentration ranges of (22, 20, 15, 13, 10 mg/ml) MIC and MBC were determined against bacterial strains based on micro-well dilution.

2.6. Statistical Treatment of the Results:

The data were subjected to a one-way analysis of variance (ANOVA) and the mean values were separated using Duncan Multiple range test by SAS software.

3. RESULTS AND DISCUSSION

All the treatment that is, solvent in 1 level, concentration at 3 levels and bacterial at 4 levels were arranged in factorial completely randomized design with three replicates. Saturated disk with extract was negative control and saturated disc with tetracycline was positive control of the test.

Disk diffusion technique was applied to measure the antibiotic role of *Scrophulariastriata* through its inhibition zone. The figures obtained for the inhibition zones at the concentrations of 50, 40, and 20

(mg/ml) were respectively 20.44-23.98,18.73- 20.40, and 10.34-15.70. Tetracycline inhibition zone was measured 17-19mm which is significantly different (P<0.05) from all the concetrations considered (table 1).

Table 1. antibacterial effect of *Scrophularia striata* methanol extract on bacterial growth

Concentration					Bacteria
50 mg/ml	40 mg/ml	20 mg/ml	Tetracycline	Methanol	
20/44	20/40	10/34	18	0	*Escherichia coli*
22/06	17/67	14/24	18	0	*Rathayibacter tritici*
21/43	17/49	15/90	19	0	*Xanthomonas campestris*
23/98	18/73	15/70	17	0	*Pseudomonas syringae*

Different letter show significant differences (p< 0.05).

The result suggested that the inhibitory zone of *Scrophulariastriata* methanol extracts at the concentrations of 50,40 and 20 (mg/ml) are significantly different (P<0.05) from the mean value. The highest values concerning inhibition zone were obtained for the concentration of 50 mg/ml, however all concentrations were significantly different (P<0.05) from the mean obtained for the tetracycline (Fig.1)

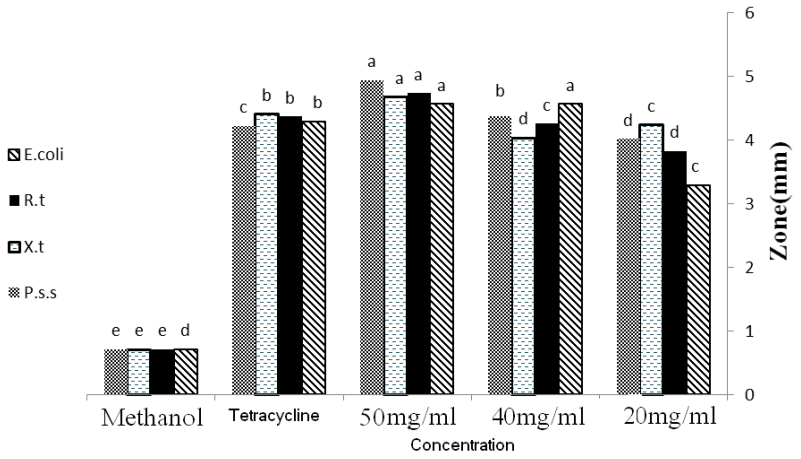

Figure1. *average inhibition zone of different concentration of methanol extract of Scrophulariastriatacompared withtetracylcline. Bars labled with different letters show significant differences (p<0.05).*

Methanol extract of *Scrophulariastriata* at concentration of 50 mg/ml with 23.98 mm diameter of growth inhibition had the greatest influence on the bacteria of Pseudomonas syringae. Interaction with treatment and bacteria related to the variance analysis ANOVA was examined in Table 2. According to Table 2, all elements of A, B and the interaction is significant.

Table 2. *variance analysis to evaluate the significance of F test of each factor and their interactions with each other*

F	SS	df	Sources of variation
***32.35	129.41	3	Concentration(A)
***0.07	0.22	3	Bacteria(B)
***0.16	1.98	12	A*B
0.001	0.06	40	Erorr

Significant at <.0001, ^{ns} indicates no signification ***

The antibacterial activity of the extracts was quantified according to the MIC and MBC. The lowest MIC and MBC values obtained for the *Scrophulariastriata* methanol extracts were respectively 20 and 22 mg/ml (Table 3).

Table3. *MIC, MBC values of active extract on test bacteria (mg/ml).*

20	13-		Escherichia coli
20	10	+	Rathayibacter tritici
22	15	-	Xanthomonas campestris
20	10	-	Pseudomonas syringae

Results of the study have clearly showed the significant antibiotic role of Tetracylcline, and meanwhile, the inhibition zone of *Scrophulariastriata* at concentration 50 (mg/ml)has been analogus to the obtained results for Tetracylcline. The minimum inhibitive concentration (MIC) of the *Scrophulariastriata* was obtained 20 mg/ml. the results of this study showed that positive warm bacteria have been comparatively more sensitive to the antibacterial activity at higher MBC of the extract. Findings of this paper is consistent with that of Mahboubi et al 2013 who reported the inhibitory effects of *S. striata* ondifferent gram negative bacteria, excluding *P. aeruginosa*, filamentous fungi and yeast.

In addition to peptides and glycans, negatie warm bacteria have also an outer membrane layer, which its hydrophilic surface is rich in lipopolysaccharide molecules and acts as a barrier to antibiotics. In addition, enzymes in the periplasmic space can take the incoming molecules from outside but in the case of positive warm bacteria, the cell wall and cytoplasmic membrane could be easily destroyed, which would result in leakage andcoagulation of cytoplasm (Kalemba and Kunicka, 2003). The higher sensitivity of positive warm bacteria than negative warm bacteria against plants extracts(Lee et al.,2008). This study indicates the ability of this plant in controlling plant pathogens.Extractingplants' extract at different growth stages may has different results. Also knowing that different solvents can extract different amounts and kinds of metabolites in plants, the repetition of experiments conducted in this study with other solvents would be recommended.The results of this study showed that the type of solvent is effective in extraction of active metabolites of plants and plant extract in different solvents can have different effects on plant disease control.

4. CONCLUSION

Results of current research suggest that *Scrophulariastriata* possess antebacterial properties against other pathogenic bacteria. Sampling and analysis have been carried out in Northwestern Iran, and the findings corroborate the effective role of plants agains pathogenic agents that could be the basis for further studies to assess the active compound of *Scrophulariastriata* against other microorganisms.

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