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Comparison of antibacterial activities of *Salvia* sp. and *Ballota platyloma* extracts on pathogenic bacteria

Ehteram Sadat Rahimi[1*], Saeid Soltani[1], Rahil Khoshrooei[1], Bahman Eslami[1], Zahra Rahmani[2]

1-Department of Biology, Qaemshahr branch, Islamic Azad University, Qaemshahr, Iran.
2- Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

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ABSTRACT

Salvia and *Ballota* genus of Lamiaceae have been used in traditional medicine. In this study, the antibacterial effects of extract of two *Salvia* and *Ballota* plants from Lamiaceae on the pathogenic gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) have studied. For evaluating these effects, two methods, Disc diffusion and microbroth dilution have been used. In the each used two methods, extracts have demonstrated the maximum effect on the gram-positive bacteria and the minimum effect on the gram-negative bacteria. In the disc diffusion method, the largest inhibition zone of with 185 mm diameter has distinguished from the effect of Salvia extract with 200 mg/ml concentration against *Staphylococcus ureus* bacteria. Salvia extract with owning values MIC=2 mg/ml and MBC=4 mg/ml against *Bacillus cereus*, has displayed very good result. Due to the strong antibacterial effect of Salvia extract on *Staphylococcus aureus* and *Bacillus cereus* in this research, it is recommended that additional studies be done on it in the treatment of bacterial infections and food poisoning.

1. Introduction

In the recent years, drug resistance to the human pathogenic bacteria has been extensively prevalent and reported (Daris, 1994; Robin et al., 1998). Because of side effects and resistance of pathogenic microorganisms against antibiotics, many of scientist in the recent years, have displayed the more attention to the herbal extracts and active biological compounds isolated from plant species that are used in the tradition medicine (Essuwi and Srour, 2000). Plants have compounds such as tannins, steroids and phenol compounds that can have important role in the anti-microbial activities and in this way, have application in the medical sciences and pharmacy (Hammer et al., 1999). Isolated anti-microbial compounds from plants may restrain bacteria growth through different mechanisms in comparison with used methods (Eloff, 1998). Among plants that have anti-microbial attributes, species of Lamiaceae are important, because of have better anti-microbial effect against pathogens and are easier available. The mint family have 236 genera (Raymond et al., 2004) and 6900 to 7200 species (Vernon et al., 2004) that from past time these plants naturally have used as tea, spice or in the medicine. Moreover, plants of Lamiaceae can be used as soothing, anti-inflammation, anti-spasm, anti-parasite and other and also have role in the treatment of gastric diseases, cardiac diseases,

*Corresponding author: Dr. Ehteram Sadat Rahimi
Tel: 00989113538675
E-mail address: etiraimi_24@yahoo.com

and headache (Sarac and Ugur, 2007). In this research, the anti-bacterial effects of plants extract from *Ballota* and *Salvia* genera from Lamiaceae against four human pathogens have surveyed.

2. Materials and Methods

2.1. Chemicals

Muller Hinton Agar, Nutrient broth and DMSO were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

2.2. Collection and preparation of sample

Plant Biomass was collected from the Gadouk area, north of Sari, Iran, in 2012. The samples were identified by Dr. Bahman Eslami (Assistant. Prof.of plant systems, Islamic Azad university of Qaemshahr, Iran). Voucher specimens are deposited in herbarium (Qaemshahr branch, Islamic azad university, Qaemshahr, Iran). The Samples were dried at room temperature and coarsely ground before extraction.

2.3. Preparation of plant extracts

Dried materials were coarsely ground before extraction. 5g of dried materials was extracted by maceration with 70% ethanol (1h sonication, filtered; repeated 2 times). The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper. The resultant extracts were concentrated in a rotary evaporator under reduced pressure until a crude solid extract was obtained which were then freeze-dried for complete solvent removal (0.9 g).

2.4. Microorganisms used and determination of antibacterial activity

Five bacterial strains (gram positive and negative) were selected for the study. Gram positive species were *Bacillus cereus* and *Staphylococcus aureus* while the gram negative species were *Pseudomonas aeruginosa* and *Escherichia coli*. Each bacterial strain was incubated in nutrient broth at 37 ˚C overnight (14 h), and test bacterial solutions were prepared with the same broth to give a concentration 1.5×10^{8} CFU ml^{-1}. Suspensions of microorganisms were transferred onto the surface of Muller Hinton Agar media and spread evenly over the entire surface of the plates. Blank discs (6.4 mm, Padtan Teb, Iran) impregnated with 20 µl of a serial 20-fold dilution of extract compounds (200, 150, 100, 50 mg ml^{-1}) were prepared using 50% DMSO. The plates spread with bacteria were incubated at 37 ºC for 24 h. After incubation, the inhibition zones formed around the disks were measured (Androw, 2001). Gentamicin disc (30 µg), and Chloramphenicol disc (30µg) were used as positive control.

2.5. The minimum bacterial lethal concentration (MIC)

For determination of this concentration, the method of dilution in 96-Well Standard Microplate has used. The used medium, MHB and the processes of experiment performing have described below: The dilution series from each extract in MHB was prepared (0.06-128 mg/ml and with 100 ml final volume). The suspension was prepared from 18-20 hours cultivation of normal saline bacteria. This suspension was diluted with MHB to 1:100 proportions and then 100 ml there from was added to each well. With adding bacterial suspension, the final concentration of surveyed material in each well decreased to half. After warming up, wells checked from viewpoint of having obscurity and MIC was determined and recorded based on mg/ml. wherein extracts that were caused obscurity after solving in medium culture, Resazurine reagent was used for differentiating sinks having growth from sinks without growth. The stock and sterile reagent solution with 4 mg/ml concentration, with 5 ml quantity added to each well and to the control well and pellet was located on the shaker. Upon changing color of positive control sink (violet to chromatic pink), MIC has been determined and recorded. All of MIC determination experiments conducted in three replications. For quality control of MIC determination experiment, positive control (the well that contained 100 ml medium culture and 100 ml diluted bacterial suspension), negative control (the well that contained only 200 ml liquid medium culture) and chloramphenicol as a standard antibiotic have been used.

2.6. The minimum bacterial lethal concentration (MBC)

For determination of this concentration, 100 ml from content of each sink with no growth, after disarranging, cultured on the one plate. After warming up for 24 hours in 37 ºC temperature, the minimum concentration that caused 99.9% destruction of bacteria primary count recorded as MBC. The bacteria count was determined with consideration of the results of quality control of MIC determination experiment.

2.7. Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by analysis of variance ($P<0.05$) and the means separated by Duncan's multiple range test.

3. Results

The obtained results from ethanol extract's effect of *Salvia* and *Ballota* plants on the studied bacteria have adduced in tables 1 to 3. As are observed in the table 1, in the all used concentrations of *Salvia* extract, the maximum inhibitory operation saw on the *Staphylococcus aureus*. As well, in 200 mg/ml concentration of this extract, any inhibitory effect on *Escherichia coli* was not seeing. In addition, this extract in 150 mg/ml and 50 mg/ml concentrations had no inhibitory effect on *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. As are observed in the table 2, in the all used concentrations of *Ballota platyloma*, the maximum inhibitory function saw on *Staphylococcus aureus*. In 150 mg/ml concentration of this extract, any inhibitory effect was not seeing on *Bacillus cereus*. In 100 mg/ml and 50 mg/ml concentrations of this extract, inhibitory effect was seeing only on the bacteria *Staphylococcus aureus*. With respect to table 3, the obtained results from MIC of *Saliva* plant's extract shows that the minimum concentration of growth inhibitory in gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) has the minimum value (2 mg/ml) and in *Pseudomonas aeruginosa* has the maximum value (32 mg/ml). Also, the obtained results from MBC of Saliva plant's extract shows that the lethal minimum concentration in Bacillus cereus has the minimum value (4 mg/ml) and in *Pseudomonas aeruginosa* has the maximum value (more than 64 mg/ml). With respect to the table 3, the obtained results from MIC of *Ballota* plant's extract shows that the minimum concentration of growth inhibitory in *Bacillus cereus* has the minimum value (4 mg/ml) and in *Pseudomonas aeruginosa* has the maximum value (32 mg/ml). As well, the obtained results from MBC of *Ballota* plant's extract shows that the lethal minimum concentration in *Bacillus cereus* has the minimum value (8 mg/ml) and in *Pseudomonas aeruginosa* has the maximum value (64 mg/ml). Thus, in the both used plant extracts, MIC and MBC had the minimum and maximum values for *Bacillus cereus* and *Pseudomonas areuginosa*, respectively.

4. Discussion

In the recent years, many researches on antimicrobial effects of different plants has been carried out and it was specified that some of plants have influences such as chemical drugs or more of their (Moshafi et al., 2004). The country of Iran enumerates among the richest regions of world because of ample dispersal of medicinal plants and as well as, from viewpoint of climatic conditions, geographical location and growth background of these plants (Zargari, 1999). Studying these plants from viewpoint of their antibacterial properties, obtains the appropriate context that from their results we can use for replacing drugs with natural origin for control and treatment of bacterial infection and this matter can cause reduction of chemical drugs consumption and the complication due to their usage. In addition, the antibacterial resistances are considering as a strong threat for human health especially people with immune deficiency. Therefore, finding the cheap and drastic antimicrobial matters is necessary. The medicinal plants possessing antimicrobial effects restrain growth of bacteria with different mechanism from antibiotics. This matter necessitates requisiteness of the more comprehensive researches about medicinal plants (Eloff, 1998). In this research, the antibacterial effects of *Salvia* and *Ballota* plant's extract from Lamiaceae were studied. Comparison the inhibitory effect of used plant's extract showed that had the more effect on the gram-positive bacteria than gram-negative bacteria.

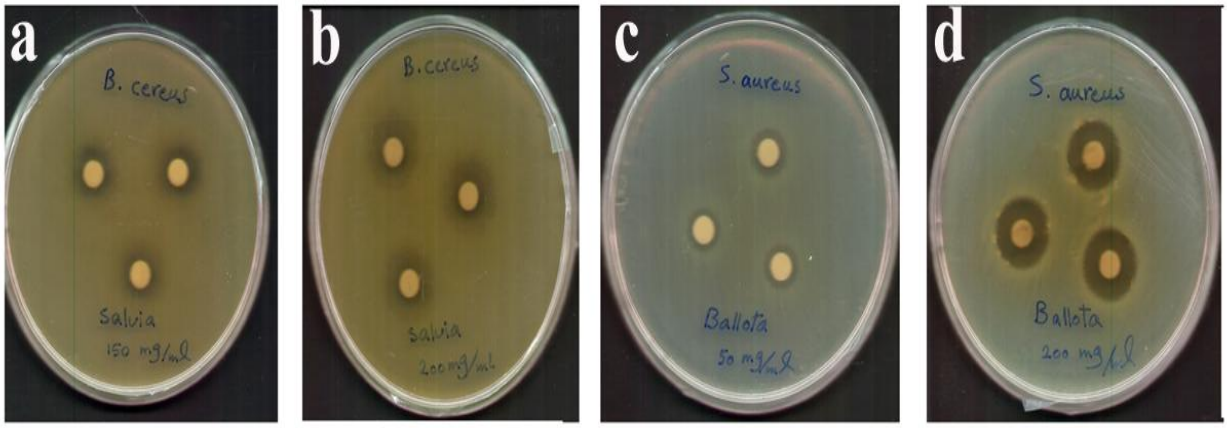

Fig 1. Disc diffusion assay (a and b =Disc impregnated with 150 and 200 mg/ml Salvia sp. extract on Bacillus cereus respectively; c and d =Disc impregnated with 50 and 200 mg/ml Ballota platyloma extract on Staphylococcus aureus respectively).

Table 1. Antibacterial activity of Salvia sp. extract.

Concentration of extract (mg/ml)	200	150	100	50	Positive control	
Bacteria name	Inhibition zone(mm)				Gentamicin	Chloramphenicol
Staphylococcus aureus	18.5	16.66	11.5	8	19.33	21.5
Pseudomonasa aeruginosa	7.33	8	8.66	-	19.83	-
Bacillus cereus	8.33	9	7	7	19.66	19.33
Escherichia coli	-	7	7.3	-	21.83	24

- no activity

Table 2. Antibacterial activity of Ballota platyloma extract.

Concentration of extract (mg/ml)	200	150	100	50	Positive control	
Bacteria name	Inhibition zone(mm)				Gentamicin	Chloramphenicol
Staphylococcus aureus	11.33	10.33	7	7	19.33	21.5
Pseudomonasa aeruginosa	7	7	-	-	19.83	-
Bacillus cereus	7	-	-	-	19.66	19.33
Escherichia coli	7	7	-	-	21.83	24

- no activity

Table3. Results of MIC and MBC.

Bacteria name	*Ballota platyloma* extract (mg/ml)		*Salvia* sp. extract (mg/ml)		Chloramphenicol (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	8	16	2	8	0.5	4
Pseudomonasa aeruginosa	32	>64	32	>64	16	64
Bacillus cereus	4	8	2	4	0.25	1
Escherichia coli	16	32	4	32	2	8

The *Ballota* genus from Lamiaceae consists of about 90 species in world. In Iran 3 genera from this genus are growing, those have medicinal properties and are used in traditional medicine. In some species of this genus, the antioxidant, antimicrobial, anti-inflammatory, anti-spasm, tranquilizer, disinfectant properties and the rheumatic pain therapy have been

proven by researches (Principa, 1988). In a study in Pakistan, the antibacterial activity of aqueous extract of *B. limbata* plant against *B. subtilis* checked out and showed that this extract can be used in eye infection treatment. In another study in Pakistan, the antibacterial effect of ethanol extract of *B. limbata* plant against *S. aureus, E. faecalis, S. epidermidis* and *S. saprophyticus* bacteria studied and was shown that this extract can be used in treatment of different diseases (Chaudhary et al., 2009). With respect to the table (2), the obtained results from antibacterial effect of *Ballota* plant on *Staphylococcus* in present research are consistent with the above study. In studying previous researches, any report from antibacterial effect of *Ballota platyloma* plant has not seen, and with respect to that this plant in one of the species with significant growth in Iran, it is hoped that more researches on antibacterial effect of this plant upon different microbial species was done, preparation of different medicinal forms from it become possible and worthwhile action with aimed to improvement of infectious diseases resulted from variant bacteria species was done.

Salvia genus from mint family because of existence different terpenoides compounds, essence, phenol compounds and flavonoids from viewpoint of antimicrobial effects is of great interest (Miski et al., 1983). Fifty-eight species of Salvia genus have identified in Iran (Hedge, 1986). According to different sources, these plants have good medicinal properties. According to a study in Turkey, methanol extract of *S. verticillata* howed the good antibacterial effect on *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* (Özkan et al., 2009). In addition, in a study in Iran showed that *S. leriifolia* plant's extract on the food pathogens such as *Staphylococcus aureus* had the appropriate antibacterial effect (Mahdavianmehr et al., 2010). In another study in Iran showed that S. officinalis plant's extract had the good antibacterial effect against Bacillus cereus, *Bacillus anthracis* and *Escherichia coli* (Jafari et al., 2011). With respect to tables 2 and 3, the results of antibacterial effect's extract of used plants in this research on *Staphylococcus aureus* and *Bacillus cereus* is consistent with results of previous studies. Of course, the effect on *Escherichia coli* and *Pseudomonas aeruginosa* bacteria that are gram-negative, were lower that can be due to differences in call wall structures of gram-negative and gram-positive bacteria. Gram-positive bacteria have thick layer of muco-peptid in their cell wall, but, gram-negative bacteria have only a thin layer of it and major section of their wall structure is lipoproteins and lipopolysaccharides. Of course, also existence of outer membrane in gram-negative bacteria is effective in resistance of these bacteria against antibacterial compounds. Also, the being sensitive gram-positive bacteria against antibacterial agents was confirmed in the result of MIC of *Salvia* and *Ballota* plant's extract, as, the extracts of both plants have showed good antibacterial effects against *Staphylococcus aureus* and *Bacillus cereus*. The minimum inhibitory concentration in *Salvia* extract assigned a lower number to itself than *Ballota* extract that it is a marker of stronger antimicrobial effect in *Salvia*.

Conclusion

Due to the strong antibacterial effect of Salvia extract on *Staphylococcus aureus* and *Bacillus cereus* in this research, it is recommended that additional studies be done on it in the treatment of bacterial infections and food poisoning.

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