International Scholars Journals

International Journal of Diseases and Disorders ISSN 2329-9835 Vol. 7 (1), pp. 001-004, January, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

The anti-bacterial effects of *Zataria multiflora* extract on common pathogenic Gram positive cocci, pathogenic Gram negative bacilli and non - pathogenic bacteria

Motevasel M.[1*], Zomorodian K.[2], Ashraf Mansouri M. A.[1], Farshad S. H.[3], Haghighhat A. R.[4], Hadaegh M. G.[5] and Takhshid M. A.[1]

[1]Diagnostic Laboratory Sciences and Technology Research Center, Faculty of Paramedical Siences, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Mycology, Medical Faculty, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[4]Department of Pharmacognosy, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shira, Iran.
[5]Faghihi hospital, Shiraz University of Medical Sciences, Shiraz, Iran.

Accepted 25 October, 2018

Due to increased bacterial resistance to common antibiotics, tendency towards using herbal extracts like *Zataria multiflora* from Lamiaceae family is increasing. In this study, antibacterial effects of *Z. multiflora* on several common pathogenic and non pathogenic bacteria were evaluated. Several clinical Gram positive cocci and Gram negative bacilli isolated from patients and healthy humans were identified with standard methods. The extract of *Z. multiflora* was prepared from dried leaves with maceration method. The antibacterial activity of *Zataria* extract with initial concentration of 200 µg/ml was determined by micro dilution method. Results obtained showed that the minimum inhibitory concentration varied from 2 to 32 µg/ml for all Gram positive cocci and Gram negative rods while it was 512 µg/ml for *Pseudomonas aeroginosa* and *Shewanella putrifaciance*. The minimum bactericidal concentration varied from 4 to 512 µg/ml for examined bacteria. In conclusion, it seems that *Zataria* extract could inhibit the growth of some clinical Gram positive and Gram negative bacteria. However, the inhibitory effects of *Zataria* extract for *Pseudomonas aeroginsa* and *Shewanella putrifacians* are lower than its inhibitory effects for other Gram negative bacilli in the examined extract concentrations. We noticed that the bactericidal effect of *Zataria* extract was less than its bacteriostatic effect.

Key words: Ethanolic, *Zataria multiflora* extract, antibacterial effect, pathogenic bacteria.

INTRODUCTION

Bacterial antibiotic resistance is one of the most serious hindrances for infectious disease treatment. The replacement of antibacterial agents with herbal medicines may overcome this problem (Dorman and Deans, 2000; Schuls and Hansel, 1998).

*Corresponding author. E-mail: maryam.motevasel@ymail.com. Fax: 00987112289113.

Zataria multiflora with common Persian name “Avishan – e – shirazi” is one of the most famous herbal remedies in Iranian folk medicine. Zataria is from *lamiaceae* family and the most effective compounds in this remedy are thymal and caracrol which have antibacterial effect (Shaffiee and Javidnia, 1997). Avishan – e – shirazi is native to Iran, Afghanistan and Pakistan (Shaffiee and Javidnia, 1997).

The dried leaves of plant have been used in food and hygiene industries (Gandomian et al., 2009). *Zataria*

multiflora extract is used as an antibacterial, antifungal or anti inflammatory agent. (Shaffiee and Javidnia, 1997). *Zataria multiflora* which is used as a preservative in food industry also stimulates inate immunity (Shokri et al., 2006).

Moreover reports the extract of *Zataria multiflora* inhibited the growth of bacteria associated with gastro-intestinal infections including *staphylococcus aureus* (Akhondzadeh et al., 2007), enterohemorrhagic *Escherichia coli* (Fazlara et al., 2008), *Salmonella thyphi, Salmonella paratyphi*, *Salmonella typhimurium* (Fazeli et al., 2007), and *Shigella* species (Mayrhofer et al., 2004, Abbasgholizadeh et al., 2008).

Some of these organisms have been resisted to anti-biotics such as methicillin- resistant *S. aureus* (MRSA) (Nuria et al., 1998), vancomycin resistant enterococcus (VRE) (Ravanshad et al., 2007), resistant *Pseudomonas aeroginsa* (Mujeeb et al., 2008) and antibacterial resistant salmonella and *Shigella* species (Mayrhofer et al., 2004). Therefore, it is important to assay the antimicrobial effects of some of the herbal extracts such as *Zataria multiflura* against these organisms.

The present study was performed to evaluate the inhibitory effects of the extract of Avishan – e- shirazi on several isolated clinical Gram positive cocci and Gram negative *Bacilli.*

MATHERIALS AND METHODS

Bacteria isolation

The bacteria were isolated from patients who attend in Faghihi Hospital (Shiraz University of Medical Sciences, Iran). The bacteria were then identified as the Gram positive or Gram negative by the use of standard methods (Washington and Stephen, 2006). The bacteria were isolated from wound, nose, stool, urine, and skin samples which were classified into three groups as follows:

a) Pathogen and non - pathogen Gram positive cocci.
b) Pathogen and non - pathogen Gram negative rods.
c) Non - fermentative Gram negative *Bacilli* (N.F.B).

Extract preparation

Preparing of *Zataria* extract was performed by maceration method (Nairn. 1990). First, some dried leaves of *Zataria multiflora* were ground. Next it was mixed with 80% ethanol and kept in a dark bottle.

It was filtered after 48 h incubation in a dark room and totally concentrated by rotary evaporator (Germany, HEIDOLPH company model VB 2000). The alcohol free extract was obtained and frozen in – 25°C. Finally the frozen extract was powdered by freeze dryer (Germany ZERBUS company, model VACO serial number D-37539).

Determination of antimicrobial activities of the extract

The antibacterial activities of the extract against the bacteria in this study were examined in two parts as follows:

Determination of minimum inhibitory concentration (MIC)

The MICs of the extract against bacteria were determined by using the broth micro dilution method recommended by the CLSI protocol with some modification (Clinical and Laboratory Standards Institute., 2006). To determine the antimicrobial activities of Zataria extract against the bacteria, the initial concentration of extract was prepared with 200 µg/ml in Dimethylsulfoxid (DMSO) as a solvent (MERK Schuchardt OHG 85662 Hohenbrunn, Germany). Then, the serial dilution of the extract from 2 to 512 µg/ml were prepared in 96 wells microtitere plates (Sigma, St.Louis, USA) using Muller Hinton broth (Merck, Darmstat. Germany).

100 µl of bacterial suspension in 1.5×10^5 cfu/ml (Baily et al., 1990) concentration was added to each well except negative control and the initial concentration of the extract. Then the micro plate was incubated in 35°C for 18 h. The tests were studied after 24 h incubation. The mixture of media, bacterial suspension and maximum concentration of solvent was used as a positive control.

The negative control well contained media and solvent without bacteria. The wells without sediment or turbidity indicated no growth of bacteria. The first well without any growth was considered as MIC.

Determination of minimum bactericidal concentration (MBC)

10 µl of the MIC of extract and the previous concentrations were sub cultured in spotted form on Muller Hinton Agar (MERK, Darmstat. Germany) for determining the minimum bactericidal concentration (MBC).

All of the inoculated plates were incubated at 35°C for 18 h. The MBC was the first concentration which could not grow on M.H.A. These procedures were performed for all of the isolated bacteria.

RESULTS

Table 1 shows that the MIC and MBC have been determined for some Gram positive cocci. The MIC for MRSA was 16 µg/ml and MBC was over 512 µg/ml.
The MIC for *Streptococcus pyogenes* was 2 µ / and MBC was 128µg/ml. For *Listeria monocytogenes* the MIC was 8 µg/ml and the MBC was over 512 µg/ml.

The MIC and MBC for Entrtococci were 4 and 128 µg/ml respectively.

The MIC and MBC of Gram negative pathogenic and non pathogenic bacteria in this study are shown in Table 2. The MIC for Salmonella thyphi, Salmonella parathphi B and *Shigella flexnerri* were 16, 64 and 8 µgml respectively.

The MBCs for above bacteria were over 512 µg/ml. *In vibrio* cholera MIC was 8 µg/ml and MBC was upto 64 µg/ml. In present study we found that the MIC and MBC for *Pseudomonas aeroginosa* was over 512 µg/ml. The similar result was found for *Showanella putrifaciants* as well. The range of the MICs and MBCs of *Acinetobacter baumannii, Alcaligenes* and *Chryseobacterium meningosepticum* were from 2 to 16 µg/ml.

DISCUSSION

Based on the previous studies, the avishen-e-shirazi

Table 1. The comparison of MIC and MBC (µg/ml) of alcohol extract of *Zataria multiflora* on Gram positive cocci.

Bacteria		MIC (µg/ml)	MBC (µg/ml)
Pathogens	MRSA	16	>512
	MSSA	8	>512
	Streptococcus pyogenes	2	128
	Listeria monocytogenes	8	>512
Non pathogens or opportunist	*Staphylococcus epidermidis*	8	256
	Staphylococcus saprophyticus	2	4
	Enterococci	4	128

MIC = Minimum Inhibitory Concentration. MBC = Minimum Bactericidal Concentration. MRSA = Methicillin Resistant *S. aureus* MSSA = Methicillin Sensitive *S. aureus*.

Table 2. The comparison of MIC and MBC (µg/ml) of alcohol extract of *Zataria multiflora* on Gram negative rods.

Bacteria		MIC (µg/ml)	MBC (µg/ml)
Entero pathogens	*Enterohemorrhagic E. coli*	32	>512
	Salmonella thyphi	16	>512
	Salmonella parayhyphi B	64	>512
	Shigella flexneri	8	>512
	Yersinia enterocolitica	16	>512
	Vibiro cholera (ogawa)	8	64
	Vibrio cholera (Inaba)	8	32
	Aeromonas hydrophilia	8	>512
Non Enteropathogens or Normal flora	*Enterobacter aerogenes*	32	>512
	Klebsiella pneumoniae	32	>512
	Citrobacter freundii	64	512
	Morganella morgani	64	128
	Proteus mirabilis	64	>512
Non fermentative Gram Negative *Bacilli* (NFB)	*P. aeruginosa*	512	>512
	Shewanella putrefaciens	512	>512
	Acinetobacter baumannii	8	16
	Alcaligenes	32	64
	Chryseobacterium meningosepticum	2	4
	Enterohemorrhagic E. coli		

NFB= Non - Fermentative Gram Negative *Bacilli*.

extract inhibits the growth of enterohemorrhagic *E. coli* (Fazlara et al., 2008; Fazeli et al., 2007; Mayerhofer et al., 2004), *Salmonella* sp and *Shigella* sp (Fazeli et al., 2007; Abbasgholizadeh et al., 2008, Dakhili et al., 2006), *Staphylococcus aurous* (Zahraei et al., 2005; Klebsiella (Abbasgholizadeh et al., 2008), *Enterococcus* (NURIA et al., 1998) and *Pseudomonas aeroginosa* (Owlia et al., 2009).

The results of this study show that the alcoholic *Zataria* extract in low concentrations can inhibit the growth of Gram positive cocci. In high concentrations, it can destroy all bacteria in this group. There is no difference between normal flora and pathogenic Gram positive cocci regarding their inhibitory and bactericidal effects. It is found that the bactericidal activity of the extract is higher than its bacteriostatic activity.

Similar results about extract effects were observed for pathogenic and non - pathogenic Gram negative bacteria except for *Pseudomonas aeroginosa* and *Shewanella putrifacians* which are resistant to the extract. Our findings are against owlia et al. (2009) research which showed that *Z. multiflora* extract inhibits the growth of *Pseudomonas* very well. We though this difference depends on their method and examined organism. That organism was standard but our organisms were clinical isolates. Also we used micro broth dilution method but

they used tube dilution method. As *Pseudomonas aeroginosa* becomes resistant to the most of the current antibiotics (MUJEEB et al*.,* 2008), its sensitivity to avishen-e-shirazi is interesting. Although the concentration used of the extract in this research could not inhibit the growth of *P. aeroginosa,* it is suggested that higher concentration of extract can be tested. We believe that the effect of the extract is not acceptable for prevention of growth of *Pseudomonas* and *Shewanella* in examined concentrations. *Shewanella purifacians* was named *Pseudomonas putrifations* previously. This may be a reason for its resistancy to the extract. Because of heavy oral usage of the avishen-e-shirazi in Iranian folk, we suggest that large amount of the extract can be used in future investigations.

We observed that *Acinetobacter baumannii*, *Alcaligenes and Chryseobacterium meningosepticum* in NFB group are very sensitive to the avishen-e-shirazi. These results may be noticeable for Acinetobacter which its resistance to some antibiotics have been published.

It is suggested that the same experiment can be performed *in vivo* by using the extract in the form of lotions or pastes on cutaneous, subcutaneous and skin lesions of lab animals. Although Avishen-e-shirazi is frequently eaten, more studies should be performed regarding its systemic usage.

Conclusion

In this study we found that the high concentration of *Zataria* extract shows the best antimicrobial activities and kill much type of bacteria with no difference between pathogens or non pathogens. All Gram negative bacteria were affected by *Zataria* extract more than Gram positive bacteria except *P. aeroginosa* and *S. putrifaciens*.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge Mr. Farzaneh Aboualizadeh and Mrs. Fatemeh Sedigh for their helpful comments in editoring the text.

REFERENCES

Abbasgholizadeh N, Ettehad GH, Arab A, Nemati A, Barak M, Pirzadeh A (2008). Antibacterial effects of *Zataria multiflura* Boiss (Shiraz organo essence) on Enterobacteriaceae species. Res. J. Biol. Sci., 3: 345-7.

Akhondzadeh A, Misaghi A, Khaschabi D (2007). Growth response and modeling of the effects of Zataria multiflora Bois. Essential oil, PH and Tempreture on *Salmonella typhimurium* and *Staphylococcus* aureus. Food Sci. Technol., 40: 973-81.

Baily S, Ellen Jo Baron, Sydney M, Finegold (1990). Diagnostic Microbiology, 8th Edition, pp. 171-193

Clinical and Laboratory Standards Institute (CLSI) (2006). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobicaly; Approved Standard. 7th Edition. Wayne, PA: Clinical and Laboratory Standards Institute, CLSI M7-A7.

Dakhili M, Zahraei Salehi T, Torabi Goodarzi M, Khavari A (2006). Evaluation of antimicrobial effects of medical plants against *Salmonella typhimurium* and composition them with common antibiotics in veterinary medicine. J. Med. Plants, 5: 21-6.

Dorman HID, Deans SG (2000). Antimicrobial agents from plants: antimicrobial activity of plant volatile oils. J. Appl. Microbial., 88: 308-316.

Fazeli MR, Gholamreza A, Ahmadian Attri MM (2007). Antimicrobial activity of sumac and Avishen-e-shirazi (*Zatiria multiflora*) against some food-born bacteria. Food Control., 8: 646-9.

Fazlara A, Najafzadeh H, Lak E (2008). The potential application of plant essential oil as natural preservatives *Escherichia coli* O157:H7. Pak. J. Biol. Sci., 11: 2054-61.

Gandomian H, Misaghi A, Akhondzadeh BA, Bokaeib S, Abbasifar A (2009). Efectof *Zataria multiflora* Boiss. Essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. Food Chem. Toxicol., 47: 2397-400.

Mayrhofer S, Paulsen P, Smulders FJ, Hilbert F (2004). Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. Int. J. Food Microbial., 97(1):23-9.

Nairn JG (1990). Remington's pharmaceutical sciences, 18th edition, 83: 1543.

Nuria Mir, Miguel S, Fernando B, Blanca L, Celia C, Raeael C (1998). Soft Salt-Manitol Agar-Cloxacillin Test: a Highly Spesific Bedside Screening Test for Detection of Clonisation with Methicillin-Resistant *Staphylococcus aureus*. J. Clin. Microbiol., pp. 986-989.

Mujeeb UR, Shereen G, Mohamad ZU, Irfan H, Mohammad A (2008). Inhibitory Effects of *Zataria multiflora* Boiss on Multiple Drug Resistant *Pseudomonas aeroginosa*. J. Chem. Soc., 30(4): 626-629.a

Owlia P, Saderi H, Rasooli I (2009). Antimicrobial characteristics of some herbal oils on *Pseudomonas eroginosa* with special refrence to their chemical compositions. Iranian. J. Pharmaceut Res., 107-14

Ravanshad Sh, Baseri E, Dastgheib B (2007). Antimicrobial activity of consentrations of essential oil of *Zataria multiflora* on *Enterococcus faecalis*. Shiraz Univ. Dent. J., 8: 28-36.

Schuls VR, Hansel VT (1998). A Physicia guide to herbal medicine, 3th ed. Berliu. Germany. p. 185.

Shaffiee A, Javidnia K (1997). Composition of essential oil of *Zataria multiflora*. Planta Medica, 63:371-2.

Shokri H, Asadi F, Bahonar AR, Khosrav AR (2006). The role of *Zataria multiflora* essence (Iranian herb) on innate immunity of animal model. Iran J. Immunol., 3164-168.

Washington Winn, Stephen Allen (2006). Koneman'scolor Atlas and Textbook of Diagnostic Microbiology. SIXTH EDITION. USA. chapters: 6, 7, 12, 14.

Zahraei MT, Vojgani M, Tarshizi H, Akhoundzadeh Bastia A (2005). Determination of minimum inhibitory cocenration (MIC) of extract of *Zataria multiflora* against the clinical isolates of *Streptococcus agalactiae*, *Staphylococcus aureus* and *E. coli*. J. Vet. Res., 60: 107-10.