



Antimicrobial Effects of *Zataria multiflora* Essential Oils on *Acinetobacter* Strains Isolated from Clinical Specimens

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ABSTRACT

Background & Aim: The aim of this study was to investigate the antimicrobial effects of *Zataria multiflora* against some *Acinetobacter baumannii* strains isolated from clinical samples.

Experimental: Twelve strains of *Acinetobacter baumannii* were isolated from referred patients in Zabol hospital. Essential oil of *Zataria multiflora* species was extracted using Clevenger device. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of essential oil on mentioned bacteria were determined using micro dilution broth method at six different concentrations.

Results: The results of this study showed that the lowest inhibitory concentration of essential oil against bacteria was 0.31 mg/ml, and only one strain of bacteria was inhibited. However, the highest inhibitory concentration was estimated 10 mg/ml.

Recommended applications/industries: The results showed that the antimicrobial effects increased with increasing in essential oil concentration and the essence showed good antimicrobial activity even at low concentrations. With the use of essential oil of *Zataria multiflora* against bacterial pathogens, a good antimicrobial agent can be obtained without any side effects.

1. Introduction

Traditional medicine has been used for thousands of years for therapeutic purposes. Among these, the use of essential oils and herbs has a remarkable place. Thyme is also a mint of dark herbs that is used by all air organs, especially flowered plants that have antiseptic

effects (Dean, 1992). Phytochemical studies on this species indicate the presence of flavonoids such as lutein (Ali et al., 2000) and quercetin (Murray et al., 1999), phenolic acids such as Rosmarinic Acid (Javidnia et al., 1999), benzoic acid derivatives (Shaiq et al., 1999), tocopherolquinone (Ali et al., 2000) and terpenes that is one of Parasmine derivatives.

Thymidine is a phenolic compound and its most important ingredient. Another effective combination is carvacrol, which is well soluble in alcohol and organic solvents. These materials are stored in young leaves during plant growth. The alcoholic extract has an antiseptic and spongy effect (Newall *et al.*, 1996; Zargari, 1989). Some properties of this plant as anti-fungi (Zarei *et al.*, 2007), pain and inflammation (Ramezani *et al.*, 2004), treatment and control of pests recurrent oral (Jafari *et al.*, 2003); the antioxidant effects (Babaie *et al.*, 2007) and effects on digestive and cardiovascular disorders (Mokhberi *et al.*, 2004) are mentioned in several researches.

Actinobacteria are a gram-negative fungus and polymorph, non-movable, aerobic and usually capsular, which grow easily on normal laboratory environments, the size of the colony is between 1 and 2 mm, without a smooth patch to mucoid. Actinobacteria are negative oxidase, negative nitrogen, positive catalase and they do not have the ability to regenerate nitrates.

Acinetobacter Baumanniis widely distributed in nature and can be separated from water, soil, human skin, food and sewage. The low level of this bacterium in food and its ability to use different sources of carbon has increased its frequency in different parts of the hospital and the surrounding area (Peleg *et al.*, 2008).

This bacterium is considered as one of the most problematic pathogens in the intensive care unit all over the world. Due to its remarkable clinical properties, especially in recent years, its ability to gain drug resistance is considered as one of the most threatening micro-organisms in the treatment of antimicrobial drugs (Fouriner and Richet, 2006). *Acinetobacter Baumanni* strains have shown resistance to most antibiotics that have been reported so far.

Acinetobacter bromine produces various diseases such as pneumonia, septicemia, skin and ulcers infections, meningitis, endocarditis, and urinary tract infections.

The aim of this study was to investigate the antimicrobial effects of *Zataria multiflora* essential oils on *Acinetobacter baumannii* bacteria isolated from clinical specimens.

2. Materials and Methods

2.1. Essential Oil

The leaves of *Zataria multiflora* were collected from Zabol (south-eastern, Iran) and were planted in Kerman Islamic Azad University herbarium received

approval and then dried at room temperature in dark place. Samples were crashed and transferred into glass container and preserved for extraction procedure in the laboratory. Extraction of herbal essential oils was performed using steam distillation and Clevenger apparatus. First, 200 g of dried plant powder was poured into a two-liter balloon and water was added about two thirds of the balloon. Then, balloon was attached to the Clevenger apparatus to allow distillation to be performed for 4 hours. After extraction of essential oil, dehydration was performed and the essential oil was stored in a dark container.

2.2. Determining the dry weight of the essential oil

After weighing the test tube, 1 ml of extracted oil was transferred into it. The essential oil was dried at room temperature. After drying, the essential oil of the test tube was re-weighed. The tube weight difference was 1 ml of the essential oil. The average of three times of recurrence was calculated as dry weight of essential oil.

2.3. Separation of *Acinetobacter baumannii*

The samples were cultured on agar blast agglomerates, BHI and agar nitrite. Then, they were incubated for 24 hours at 37 °C. After 24 hours, the presence of gram-negative bacteria coco-bacilli was confirmed by microscopy. To detect different species of *Acinetobacter*, the biochemical tests of urea-az-catalase and oxidase were performed.

Determination of susceptibility of bacterial isolates to plant essential oil was done by dilution method in wells. Six wells were created in a solid culture medium, and 100 µl of Nutrient Muller Hinton (MHB) was added to each well. Then, to the first well, 100 µl of dilute solution of the extracts of plants was added and after mixing, 100 µl of the first well was added to the second well, and this was done until the last well. From the final well, 100 µl of the medium was extracted, and the amount of 10 µl of the microbial suspension containing 107 µg/ml equivalent to 0.5 McFarland was added and incubated at 37 °C for 24 hours. The first well which prevented the growth of the bacteria after insertion in the incubator was considered as the minimum inhibitor concentration. In order to ensure this finding, 10 µl of wells were transferred to the Muller Hinton Agar medium, and after 24 hours the first crop that was able to kill 99.9% of the bacteria was shown as the least lethal concentration.

3. Results and discussion

The results of this study showed that the lowest inhibitor concentration of essential oil against bacteria was 0.31 mg/ml, which was only one strain of bacteria inhibited, while the highest inhibitor concentration was 10 mg/ml and a single strain of bacteria was inhibited in this inhibitory concentration (Table 1).

Table 1. MIC and MBC (mg/ml) of essential oil against *Acinetobacter baumannii*.

Bacterial strain	MIC	MBC
<i>A. baumannii</i> strain 1	5	10
<i>A. baumannii</i> strain 2	2.5	5
<i>A. baumannii</i> strain 3	0.62	1.25
<i>A. baumannii</i> strain 4	5	5
<i>A. baumannii</i> strain 5	0.62	1.25
<i>A. baumannii</i> strain 6	5	10
<i>A. baumannii</i> strain 7	10	20
<i>A. baumannii</i> strain 8	0.31	0.62
<i>A. baumannii</i> strain 9	2.5	5
<i>A. baumannii</i> strain 10	5	10
<i>A. baumannii</i> strain 11	1.25	2.5
<i>A. baumannii</i> strain 12	1.25	1.25

Medicinal herbs have long been used to treat bacterial infections. Recently, due to the resistance of the drug to plant sources, more attention has been paid to natural reservoirs. The number of metabolites expressed in different organs of the plant varies according to ecological conditions. Accordingly, it is necessary to evaluate the effective ingredients of medicinal plants in terms of geographical areas under their cultivation. *Zataria multiflora* a Labiate family has antimicrobial, antiviral, and antifungal properties. Caracole and Thymol have a large antimicrobial activity against bacteria such as *C. albicans* (Mahboubi *et al.*, 2008), *S. pneumonia*, *E. faecalis*, *S. agalactiae*, *S. pyogenes*, *S. sanguis*, *S. salivarius*, *S. mutans* (Mahboubi and Feizabadi, 2008), methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus* (MSSA) (Mahboubi and Ghazian Bidgoli, 2010), *A. niger* and *A. flavus* (Mahboubi and Kazempour, 2009), *K. pneumonia*, *P. aeruginosa*, *B. cereus*, *E. coli* and *S. typhimurium* (Mahboubi and Kazempour, 2009). *Listeria monocytogenes* has shown the highest resistance to thyme (MIC, MBC = 2, 4 µg/ml) (Mahboubi and Feizabadi, 2009).

Essential oil of *Zataria multiflora* has been extracted by distillation with water and water vapor. The essential oil yield was reported to be 3.3% (Babakhanlu *et al.*, 1998). The antimicrobial effect of Shirazi *multiflora* essential oil on *Staphylococcus aureus* has been studied and strong effect has been reported (Rasooli and Rezaei, 2002). The study of Ur Rahman *et al.* (2010) on the antimicrobial activity of the alcoholic extracts of *Zataria multiflora* against bacteria showed that the inhibition zone of the ethanolic extract against *S. typhimurium*, *E. coli* ATCC 8739, *E. coli* ATCC 25922, *P. aeruginosa* ATCC27853, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 25923, *S. aureus* ATCC 2923 and *B. subtilis* ATCC6633 were $0.37 \pm 14.49 \pm 12.4$, 0.40 ± 11.8 , 0.40 ± 15.2 , 0.65 ± 13.8 , 26.6 ± 0.64 , 24.8 ± 0.40 and 23.25 ± 0.52 , respectively, while the inhibition zone of methanol extract against these bacteria was 14.2 ± 0.54 , $\pm 11.8 \pm 0.40$, ± 12.2 , ± 0.63 , $\pm 0.71 \pm 11.8$, 29.6 ± 0.71 , 26 ± 0.73 , 30 ± 0.37 mm, respectively (Ur Rahman *et al.*, 2010).

In another study, antimicrobial activity of *Zataria multiflora* oil against food bacteria was explored and results showed that the minimum inhibitory concentration of essential oil against *E. coli*, *S. enteritidis*, *S. aureus* and *L. monocytogenes* were 6250, 6250, 3125 and 3125 ppm, respectively (Ghasemi *et al.*, 2012). In the study of Shamsalzadeh *et al.* (2011) antimicrobial activity against *Staphylococcus aureus* has been proven.

Zataria multiflora has an antimicrobial effect on *E. coli*, *S. enteritidis* and *S. dysenteriae* (Khalili and Vahidi, 2006). In other studies, the essential oil of *Zataria multiflora* has been proven to be a potent inhibitor against of *E. coli*, *S. aureus*, *S. typhimurium* (Rasooli and Rezaei, 2002; Basti *et al.*, 2004).

In the study of Shakeri *et al.* (2011) the antimicrobial activity of *Zataria multiflora* essential oil against pathogenic bacteria was investigated and the results showed that the inhibitory shield diameter at a concentration of 4% against *E. coli*, *S. enteritidis*, *S. aureus* and *B. cereus* was 19, 19.1, 14.33 and 21.79 mm; respectively (Shakeri *et al.*, 2011). Motevasel *et al.* (2013) studied the antimicrobial activity of the alfalfa extract of *Zataria multiflora* and the results showed that the minimum inhibitory concentration against *S. aureus* (ATCC25933, *S. aureus* (S.SAURA), *S. epidermidis* and *S. saprophyticus* was 16, 16 and 8 µg/ml, respectively, while the minimum bacterial

concentration was 512, 256 and 512 µg/ml (Motevasel *et al.*, 2013).

In previous studies, thyme extract inhibited the growth of bacteria such as *E.coli* (Fazlara *et al.*, 2008), *Salmonella* and *Shigella* (Shokri *et al.*, 2006), *S.aureus* (Motevasel *et al.*, 2011), *Klebsiella* (Abbasgholizadeh *et al.*, 2008), *Enterococcus* (Mir *et al.*, 1998), *Pseudomonas aeruginosa* (Parviz *et al.*, 2010) and *A.baumannii* (Motevasel *et al.*, 2011). The antimicrobial activity and phytochemical texture of *Z.multiflora* were explored and the results showed that the essential oil contains 25 compounds, the most important of which are caracole (50.57%), thymol (13.38%) and parasimon (8.27%)(Eftekhar *et al.*, 2011). The antimicrobial activity of *Z. multiflora* has been reported against a wide range of gram-negative bacteria (Saleem *et al.*, 2004; Abbasgholizadeh *et al.*, 2008; Ettehad *et al.*, 2007).

4. Conclusion

The results of this study showed that *Zataria multiflora* has good antimicrobial effects against *Acinetobacter baumannii*. Although the clinical application of herbal extracts and essential oils due to their lower side effects and their lower cost of production is beneficial and cost effective, but it seems that for clinical application of *Zataria multiflora* essential oil and extracts, more studies and researches should be undertaken on the mechanism of effective compounds action and also further studies should be conducted on microbial agents, pharmacological activity and pharmacokinetics of this plant.

5. References

- Ali, M.S., Saleem, M., Ali, Z. and Ahmad, V.U. 2000. Chemistry of *Zataria multiflora* (Lamiaceae). *Phytochemistry*, 55: 933 - 936.
- Abbasgholizadeh, N., Ettehad, G.H., Arab, R., Nemati, A., Barak, M. and Pirzadeh, A. 2008. Antibacterial effects of *Zataria multiflora* Boiss (Shiraz organo essence) on Enterobacteriaceae species. *Research Journal of Biological Sciences*, 3:345-347.
- Basti, A.A., Razavilar, V., Misaghi, A., Radmehr, B., Abbasifar, B. and Yazani, D. 2009. Effect of *Zataria multiflora* Boiss essential oil on probability of growth inhibition of *Staphylococcus aureus* in a brain heart infusion broth. *Journal of Medicinal plants*, 3: 53-60.
- Babaie, M., Yasa, N., Mohammadirad, A., Khorasani, R. and Abdollahi, M. 2007. On the anti oxidative stress potential of *Zataria multiflora* Boiss (Avishanshirazi) in Rats. *International Journal of Pharmacology*, 3: 510 - 4.
- Babakhanloo, P., Mirza, M., Sefidkon, F., Ahmadi, L., Barazande, M. and Asgari, F. 1998. Research on medicinal and aromatic herbs. *Research Institute of Forests and Rangelands*, 141-92.
- Deans, S.G. 1992. Natural antioxidant from thymus vulgaris (thyme) volatile oil. *Acta Horticulural*, 322: 171-182.
- Eftekhar, E., Zamani, S., Yusefzadi, M., Hadian, J. and Nejad Ebrahimi, S. 2011. Antibacterial activity of *Zataria multiflora* Boiss essential oil against extended spectrum β lactamase produced by urinary isolates of *Klebsiella pneumonia*. *Jundishapur Journal of Microbiology*, 4(1): S43-S49.
- Ettehad, G.H. and Arab, R. 2007. Evaluation of antibacterial effects of Shiraz oregano essence (*Zataria multiflora* Boiss) on *Salmonella typhi* and comparing to antibiotics. *Research Journal of Biological Sciences*, 2: 674-76.
- Fazlara, A., Najafzadeh, H. and Lak, E. 2008. The potential application of plant essential oils as natural preservatives against *Escherichia coli* O157:H7. *Pakistan Journal of Biological Sciences*, 11(17): 2054-61.
- Fouriner, P.E. and Richet, H. 2006. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clinical Infectious Diseases*, 42(5): 692-699.
- Ghasemi, S., SeyedJavadi, N.H., Esmaeili, S. and Khosravi- Darani, K.. 2012. Minimum inhibitory concentration of *Zataria multiflora* Boiss. essential oil on *Salmonella enteritidis*, *Listeria monocytogenes*, *Escherichia coli* 0157: H7 and *Staphylococcus aureus*. *Asian Journal of Chemistry*, 24(12): 5943-5944
- Javidnia, K., Tabatabai, M. and Shafiee, A. 1999. Volatile constituents and antimicrobial activity of *Zataria multiflora* population Iran. *Iranian Journal of Chemistry and Chemical Engineering*, 18: 1 - 5.
- Jafari, S., Amanlou, M., Borhan-Mohabi, K. and Farsam, H., 2003. Comparative study of *Zataria multiflora* and *Anthemismobelis* extracts with *Myrthuscommunis* preparation in the treatment of recurrent aphthous stomatitis. *Daru*, 11: 1 – 5.

- Khalili, M. and Vahidi, A. 2006. The anti-microbial effects of *Zataria multiflora* drops on three bacterial species cause gastrointestinal disease. *World Journal of Medicinal Science*, 2: 162-163.
- Motevasel, M., Zomorodian, K., Ashraf Mansouri, M.A., Farshad, S.H., Haghighat, A.R. and Hadaegh, M.G. 2011. The anti-bacterial effects of *Zataria multiflora* extract on common pathogenic Gram positive cocci, pathogenic Gram negative bacilli and non-pathogenic bacteria. *African Journal of Microbiology Research*, 5(28):4993-4996.
- Mir, N., Sanchez, M., Baquero, F., Lopez, B., Calderon, C., and Canton, R. 1998. Soft salt-mannitol agar-cloxacillin test: a highly specific bedside screening test for detection of colonization with methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 36(4): 986-989.
- Mahboubi, M., Feizabadi, M.M., Haghi, G. and Hosseini, H. 2008. Antimicrobial activity and chemical composition of essential oil from *Oliveria decumbens* Vent. *Iranian Journal of Medicinal and Aromatic Plants*, 24: 56-65
- Mahboubi, M. and Feizabadi, M.M. 2008. Antifungal activity of essential oil from *Oliveria decumbens* Vent and its synergy with amphotericin B. *International Journal of Essential Oil Therapeutics*, 2: 26-28.
- Mahboubi, M. and Ghazianbidgoli, F. 2010. Antistaphylococcal activity of *Zataria multiflora* essential oil and its synergy with vancomycin. *Phytomedicine*, 17:548- 550
- Mokhberi, M., Shams Lahijani, M., Monsefi, M., and Kamalinejad, M., 2004. The study of effects of aqueous extracts of *Zataria multiflora* (ZM) and *Elaeagnus angustifolia* (EA) on the volume of stomach of mouse fetus. *Iranian Journal. Pharmacology. Research*, 2: 58.
- Mahboubi, M. and Kazempour, N., 2009. The antimicrobial activity of essential oil from *Perovskia abrotanoides* Karel and its main components. *Indian Journal of Pharmaceutical Sciences*, 71:343-347.
- Mahboubi, M. and Farzin, N., 2009. Antimicrobial activity of *Artemisia sieberi* essential oil from central of Iran. *Iranian Journal of Microbiology*, 1: 43-48.
- Murray, M.T., Pizzorno, J.E., Pizzorno, J.E. and Murray, M.T. 1999. Aphthous stomatitis: text book of natural medicine. 2nd ed. Churchill Livingstone, U.K, 2: 1085 - 1087.
- Motevasel, M., Okhovat, M.A., Zomorodian, K. and Farshad, S. 2013. A Study of the effect of *Zataria multiflora* extract on methicillin resistant *Staphylococcus aureus*. *Jundishapur Journal of Microbiology*, 6(5): e5453
- Newall, C., Anderson, L.A. and Philipson, J.D. 1996. Herbal medicines a guide for health care professionals. The pharmaceutical press .London .
- Parviz, O., Horieh, S., Iraj, R. and Fatemeh, S., 2010. Antimicrobial characteristics of some herbal oils on *Pseudomonas aeruginosa* with special reference to their chemical compositions. *Iran Journal of Pharmaceutical Research*, 8(2):107-114.
- Peleg, A.P., Seifer, H. and Paterson, D.L. 2008. *Acinetobacter baumannii* : Emergence of a successful pathogen. *Clinical Microbiology Reviews*, 21(3): 538-582.
- Ramezani, M., Hosseinzadeh, H. and Samizadeh, S.H. 2004. Antinociceptive effects of *Zataria multiflora* Boiss fractions in mice. *Journal Ethnopharmacology*, 91: 167 - 70.
- Rasooli, I. and Rezaei, M.B. 2002. Bioactivity and chemical properties of essential oils from *Zataria multiflora* and *Mentha longifolia*. *Journal of Essential oil Research*, 14: 141-146.
- Saleem, M., Nazli, R., Afza, N., Sami, A. and Ali, M.S. 2004. Biological significance of essential oil of *Zataria multiflora* Boiss. *Natural Products Research*, 18: 493-97.
- Shokri, H., Asadi, F., Bahonar, A.R. and Khosravi, A.R. 2006. The role of *Zataria multiflora* essence (Iranian herb) on innate immunity of animal model. *Iranian Journal of Immunology*, 3(4):164-8.
- Shakeri, M.S., Shahidi, F., Beiraghi-Toosi, S. and Bahrami, A. 2011. Antimicrobial activity of *Zataria multiflora* Boiss. essential oil incorporated with whey protein based films on pathogenic and probiotic bacteria. *International Journal of Food Science and Technology*, 46: 549-554.
- Shaiq, A.M., Saleema, M., Akhtara, F., Jahangira, M., Parvez, M. and Uddin, A.V. 1999. Three p-cymene derivatives from *Zataria multiflora*. *Phytochemistry*, 52: 685 - 8.
- Shamsalizadeh, N., MerghatiKhoei, E., Sheikhan, F. and Fazelimanesh, M. 2011. Anti *Staphylococcus aureus* activity of *Zataria multiflora* Boiss on urinary tract *Staphylococcus aureus* infections. Heomparative study. *Research Journal of Biological Sciences*, 6(4): 187-191.

- Ur Rahman, M., Gul, S., Ali Odhano, E., Ali Soomro, U. and Hafeez, I. 2010. Affectivity of Zataria multiflora Boiss alcoholic extracts against bacteria. *Libyan agriculture research center journal international*, 1(3): 147-152.
- Zarei, M.A., Dabbagh, M.A. and Fouladi, Z. 2007. In Vitro Anti-Candida Activity of Zataria multiflora Boiss. *eCAM*, 4: 351 - 3.