Available online at http://www.ijabbr.com

International journal of Advanced Biological and Biomedical Research

Volume 2, Issue 2, 2014: 286-294

Antibacterial effect of medicinal plant essence (Thymus vulgaris) on major bacterial mastitis pathogen in vitro

Mahdi Khodaei Motlagh[1], Mahdi Kazemi[1], Hossein Ali Ghasemi[1], Amir Hossein Khaltabadi Farahani[1], Mohhamad Yahyaei[1], Mohhamad Rezaei[2], Fabio De Rensis[3] and Simone Taddei[3]

1- Department of Animal Science, Faculty of Agriculture and natural resource, Arak University, Iran.

2- Department of Food Safety and Hygiene, School of Public Health, Tehran University of Medical Sciences, Iran.

3- Department of Food Science U.O. Animal Production via Del Taglio 10, Parma, 43121 University of Parma.

ABSTRACT

Mastitis is one of the most costly diseases in the dairy industry. The use of antibiotics in the treatment of mastitis Leads to bacterial resistance and health problems for consumers. This study aimed to determine the antibacterial properties of essential oil of Thymus vulgaris on the bacteria Staphylococcus aureus, Escherichia coli and Staphylococcus were agalactia. Antibacterial properties of the essential oils were analyzed by disc diffusion method. The MIC and MBC were determined. By different concentration oils (10, 30 and 50%) significant difference in inhibition zone size was observed between 10%, 30% and 50%. There are no significant differences between concentrations of 30% and 50%.

Keywords: Bacteria, Mastaitis, Thyme plants essential oil.

INTRODUCTION

Mastitis as a health problem induces greatest economic losses and necessitates prescription of antibiotics in dairy farms (1). Antibiotic is the most commonly used treatment for the problem, though in long run may cause serious problems such as development of antibiotic-resistant microbes. Such evolution may be transferred to other dangerous types of microbes especially those common between man and animal. Add to that traces of the drugs in the final products (milk and meat) and negative consequences for the final users such as affecting population of microbes in the digestion system. Thanks to antibacterial and antioxidant characteristics of herbal medicines, they

Corresponding Author E-mail: M-motlagh@araku.ac.ir

are considered one of the good candidates as antibiotics for animal and poultry farms. Economic losses caused by mastitis are comprised of treatment and veterinary cost, reduction of milk production, and increase in mortality rate. Low milk production, low reproduction rate and mastitis are the main three causes of removing the cow from the herd. Different herds show different relation between quality of produced milk and removal of herd members. Sigers et al. (2003) reported that 5-17% of total removal of herd members is due to udder disorders and this figures reaches to 28.5% when high somatic cell count (SCC) and damages to the breast is taken into account (2). Milk of the cow that receives treatment due to mastitis must not be used for 8 days on average (3). Staphylococcus aureus is one of the main causes of mastitis, which usually induce chronic and clinical symptoms and poorly treated by common treatment. In addition, it excretes coagulase and collagenase enzymes (4).

Because of permanent interaction with other elements, the effective elements in herbal medicine enjoy a biologic balance so that no aggregation of such elements is formed in the body and no side effect is expected. This hints an important advantage over chemical-based drugs (5). Moreover, development of resistant microbial necessitates finding new antibiotics. Side effects and resistance to chemical treatment along with finding new materials such as vitamins, hormones, anti-microbial/viral, tumor elements in plants all power recent popularity of herbal products. Traditional medicine and medical herb hold great promises for finding treatment with fewer side effects (6). The main three groups of effective materials are terpenes, phenyl propanoids, and S and N kantians. Medial herbs are effective on a wide range of bacteria. However, resistance is observed to some extents among negative warm bacteria due to lipopolysaccharide on outer membrane (7). Thymus vulgaris is one of the main herbs with a history of medical usage (8). Thymol as the effective element of the plant is characterized with anti-bacteria, fungus and parasite effects. Amount of the essence in thymus vulgaris varies while the main chemical combinations are thymol ,carvacrol and camphor (>50%), which are found with different rate in leaf, flower, and root (8). Furthermore, growth environment is another factor of the effective combinations in the plant so that one study reported that carvacrol constitute greater portion than thymol in essential oil of thymus vulgaris, while another study reported that thymol is the main ingredient followed by carvacrol (9). According to Kasravi study (2009) average number of somatic cells in cow milk in Iran was about 450000 cell/ml and cell count for about 88% of the herds was more than 200000. Analysis on 670 samples taken for bulk tank milk from 150 herds in 17 provinces (2009) showed that count of somatic cells in the samples varies from 93000 to 4559000 (on average 368300 cell/ml). Furthermore, staphylococcus aureus and streptococcus agalactiae was observed in 83.7 and 27.2% of the samples, respectively. Reduction of milk production (due to subclinical mastitis) was about 150000MT in 2006 nationwide (about 420kg. per each milking period) (10). Knowing this and given ever increasing limitation on using antibiotics for the disease, and given necessity to use replacement combination and paucity of pertinent studies in the field, This study is aimed to survey effect of antibacterial effect and minimum inhibitory concentration (MIC) of thymus vulgaris essence on the main causes of mastitis (Escherichia coli, staphylococcus aureus, staphylococcus agalactiae) among the cow at the in-vitro situation.

MATERIAL AND METHODS

Bacterial Strains: standard and clinical treatments including staphylococcus aureus (PTCC 1113, staphylococcus epidermis (PTCC 1114), Escherichia coli (PTCC 1114), and staphylococcus agalactiae (PTCC 1399) supplied by Iran Technological and Scientific Research Center were used. Preparation of essences: The essences were obtained from Eksir Tabiat Kohsar Company.

Antibacterial activity

The antibacterial assay of essences was performed by Bauer et al. (11). The bacterial suspensions were adjusted with sterile normal saline to a concentration of 1×10^{8} CFU/ml. The inocula were prepared daily and stored at +4°C until use. The inoculum (1×10^{8} CFU/ml) was spread on sterile Mueller Hinton plate and sterile study discs were placed on the inoculated surface. The discs were saturated with 10%, 30% and 50% concentration essence were prepared by adding dimethyl sulfoxide solvent (10µlit) on the disks, kept at room temperature for absorption of extract in the medium and then incubated at 37^{0}c in the incubator for 24h. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth (12).

Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC)

Quantitative tests to obtain MIC and MBC were carried out using 1-9% concentrations of essences in Moler-Hinton Broth environment. Each concentration was added with 1×10^{6} CFU/ml of the bacteria. Positive control (culture in presence of the bacterium and absence of the essence) and negative control (culture in absence of the bacterium) were used along the tube. Afterward, the tubes were placed in incubator at 37ºC for 24h. Before reading the results the last concentration without opaqueness (no growth) was adopted as MIC for each essence; all the samples without opaqueness were cultured in Moler-Hinton Broth environment. After 24hrs incubation at 37ºC, the last concentration capable to be used as bactericide was adopted as MBC (12).

Data analysis

The model of this study was completely randomized design With 3 replications for each treatment.

$$\mathbf{Y_{ij}= \mu+ T_I+ e_{ij}}$$

Where, Y_{ij} is Diameter of the zone of inhibition for i^{th} treatment at j^{th} replication; µ is total average of diameter of the zone of inhibition; T_1 is the effect of i^{th} treatment (the essence), and e_{ij} is experimental error. Comparison of the means was done with Tukey test.

RESULTS

Comparison between average diameters of the zone of inhibition of staphylococcus aurous (mm) showed significant differences for different concentration of thymus vulgaris essence at three different levels (Table 1).

Table 1- Comparison of average diameter of the zone of inhibition (mm) of staphylococcus aureus for different concentration essence of thymus vulgaris

	staphylococcus aureus					
	Concentration	Samples	Min	Max	mean±SD.	Antibacterial effect
Thymus vulgaris	10	3	16	19.5	17.83±1.01a	Active
	30	3	26	35	29±3b	Very active
	50	3	33	45	37.5±3.72c	Very active

As presented in Table 2, the three concentrations of the essence have antibacterial effects on streptococcus agalactiae.

Table 2- Comparison of average diameter of the zone of inhibition (mm) of staphylococcus agalactiae for different concentration essence of thymus vulgaris

	staphylococcus agalactiae					
	Concentration	Samples	Min	Max	Mean±SD	Antibacterial effect
Thymus vulgaris	10	3	13	14	13.5±.28a	Active
	30	3	20	22.5	21.16±.72b	Very active
	50	3	30.5	31	30.83±.16c	Very active

Moreover, as listed in Table 3, no significant difference between 10 and 30% concentration of the essence on Escherichia bacterium was found however, significant difference was showed between 10% and 30% with 50% concentration.

Table 3- comparison of average diameter of the zone of inhibition (mm) of Escherichia coli for different concentration essence of thymus vulgaris

	Escherichia coli					
	Concentration	Samples	Min	Max	Mean±SD	Antibacterial effect

Thymus vulgaris	10	3	13	16	14.66±.88[a]	Active
	30	3	20	24	22.16±1.16[a]	Very active
	50	3	26	40	34.33±4.25[b]	Very active

The MIC and MBC values

The result of MIC and MBC are listed in Table 4. As the results show, MIC of thymus vulgaris essence for Escherichia bacterium was lower than that of the two others. This shows considerable antibacterial effect of the essence on Escherichia. No significant difference was found between MIC and MBC of the essence.

Table 4- The MIC and MBC of the essences on the bacteria under consideration

		Thymus vulgaris
staphylococcus aureus	MIC	5%
	MBC	5%
Escherichia coli	MIC	1%
	MBC	1%
staphylococcus agalactiae	MIC	4%
	MBC	5%

DISCOISSION

Our results showed that higher concentration of thymus vulgaris essence increased its antibacterial effect. The effective combinations of the essence were carvacrol and thymol which were known as strong antibacterial agents (8). Sokovis et al (2007) reported that carvacrol and thymol is much stronger antibacterial agent comparing with cineole and camphor to inhibit staphylococcus. They found that average diameters of the zone of inhibition for thymol and carvacrol were 22 and 32mm respectively and for both cineole and camphor this figure was 18mm (13). On the other hand, variance analysis on average diameters of the zone of inhibition for different concentrations of the essence showed significant difference between concentration of 10% and concentrations of 30 and 50%; while no significant difference was found between the two last concentrations concerning the characteristic. The results showed that the higher concentration of the essence has stronger antibacterial effect. This is due to increase in concentration of the effective elements. Karmen et al. (2001) studied antibacterial effects of thymus vulgaris essence on negative gram bacteria and showed that the strong antibacterial effect is due to presence of carvacrol in the essence (14). Sadeghzade et al. (2008) illustrated that in comparison with rosemary essence, Shirazi thymus vulgaris essence with average diameters of the zone of inhibition of 25mm was stronger antibacterial agent against Salmonella bacterium (15). Fazeli et al. (2007) surveyed antimicrobial effect of thymus vulgaris on staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella Typhi, and Proteus vulgaris through disk diffusion and Vol diffusion. They obtained MIC between 0.4-0.8percent (14). Another study obtained diameter of the zone of inhibition of 32mm for thymus vulgaris essence at concentration of 159µL/mg to inhibit Escherichia coli (15).

Zahraei Salehi et al. (2005) showed considerable effect of thymus vulgaris on standard treatment of Escherichia coli, staphylococcus aureus and Streptococcus agalactiae (16). Sokovice et al. (2007) worked on antimicrobial effect (human bacteria) of the essence and combination of 10 herbal plants in the Netherland and reported that thymus vulgaris (1µgr/milt) with average diameters of the zone of inhibition of 28mm was the second most effective among the 10 species. Among the effective combinations, carvacrol made the highest effect on staphylococcus aureus by diameters of the zone of inhibition of 32 mm. In this regard, thymol with average diameter of 22 mm has the second position. It is noticeable that thymus vulgaris essence with 48.92% and 3.45% thymol has the first and second places, respectively regarding effectiveness (13). The results imply that the main reason of antibacterial effect of thymus vulgaris lies with the two combinations; the results of the present study are consistent with. The differences between our results and that of other studies are probably due to genotype difference of thymus vulgaris used in the study along with growth ecosystem (habitat, temperature, height), and experiment condition (pH and temperature).Growth and performance of the plants in the ecosystems is under effect of many factors such as type, habitat, soil, height, geographical position. Each one of the factors may be considerably effective on quality and quantity of the result (17). Habibi et al. (2006) (17), showed that wild thymus vulgaris of Taleghan region, Iran bears different rates of carbonate (has to do positively with some of the effective materials) and that some oil rich combination are significantly a factor of environmental features. Moreover, significant relation was found between rate of essence and organic combination in the soil. Place of growth has been found significantly effective on effective combination found in thymus vulgaris. In this regard, it was showed that main element in evasive oil of thymus vulgaris is carvacrol followed by thymol; while in one study it was found that thymol is the main element and carvacrol is in the second place. Furthermore, inhibitory concentration, solvent, and procedure for extracting the essence, and culture medium used for preparing microbial samples were effective on the results (9 and 15). Along with these factors, genetic factors also have a role to play. There are results and rate of α-terpinene L combination in Iranian Menttha pulegium is higher for 10-20 times. Moreover, volume of pulegium in the essence of Iranian Mentha pulegium is 4 times of that of Turkish Mentha pulegium (18). In Compared antimicrobial effect of aqueous and methanol essence of sintenisii one study was carried on 12 treatments of bacteria and found no antimicrobial effect in the aqueous essence, while the alcoholic

essence of the type of Achillea millefolium showed considerable antimicrobial effect (19). We used the effective material in essence form, while it is common to use aqueous, alcoholic, or warm/cold essences. Mohammad Mobarak et al. (21 and 22) showed that difference between aqueous and methanol essence of some Thai herbal plants for mastitis causing bacterial including Escherichia coli, staphylococcus aureus and streptococcus agalactiae; so that antibacterial effect of methanol essence was higher than aqueous essence (20). In some cases, aqueous essence of some plants had no antibacterial effect (21).
Effectiveness of thymus vulgaris essence of Morocco city for negative gram Escherichai coli with MIC was 0.33mgr/lit higher than that for staphylococcus aureus (1.33mgr/milt), streptococcus (2.67mgr/milt), and Staphylococcus epidermidis (1.33mgr/milt) (22). In the present study, we found less effectiveness of thymus vulgaris essence MIC on Escherichai coli bacteria comparing with two positive gram bacteria (staphylococcus aureus and sterptoccucs agalacite. MIC and MBC of thymus vulgaris essence on Estaphiloccucus aureus bacteria was 0.5 and 1µg/lm respectively and this figures for Escherchia coli were 1 and 1.5µg/lm respectively (13). The study found MIC and MBC for staphylococcus aureus through microdilution equal with 0.25 and 1.5 µg/lm for thymol, 5 and 6 µg/lm for cineol, 0.5 and 0.5 µg/lm for carvacrol. Another study by AnandaBaskaran (23) on MIC and MBC of thymol and carvacrol on mastitits inducing bacteria found MIC and MBC for staphylococcus aureus equal with 0.6 and 12 µg/lm for thymol and 0.5 and 1.2% for carvacrol; these figures for Escherchia coli were 0.8 and 1.5 µg/lm and 0.8 and 1.2 µg/lm respectively and for Streptococcus agalactia 0.4 and 0.9 µg/lm and 0.4 and 0.8 µg/lm respectively. The MIC and MBC figure in our study are generally lower, which might be due use of pure material in AnandaBaskaran (24) study while we used essence of thymol and carvacrol.

CONCLOUSION

Given the results in this study, use of herbal essence may reduce chemical side effect of antibiotics and microorganisms resistance to a great extent. Moreover, use of herbal effective materials for treating diseases lessens the amount of chemical materials aggregation in milk and other product. Apparently, promoting use of herbal combination based on further studies paves the way for commercialization of such products by replacing chemical combinations.

REFERENCES

1. Firouzi, R., Rajaian, H., MansourianTabaee, I and Saeedzadeh, A. 2010. In vitro antibacterial effect of marbofloxacin on microorganisms causing mastitis in cows. J. Vet. Res., 65: 51-55.

2. Seegers, H., Fourichon, C and Beaudeau, F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res., 34: 475-491.

3. Østerås, O. 2000. The cost of mastitis- an opportunity to gain more money. Proceedings of British Mastitis Conference. 67-77.

4. Taghvaei Moghadam, E. Mastitis. Available From: URL: http://www.animal-nutrition.blogfa.com.

5. Soltaninejad, S.H., Satanimokhtari, T and Rahbarian, P. 2010. The study of antibacterial effect of the essential oil and methanol extracts of ziziphoracliniopodiodes on some pathogenic bacteria. J. of .Micro. Biotechnol., 2: 1-6.

6. AkhondzadehBasti, A. 2000. Iranian Medicinal Plants Encyclopedia.First Ed. Tehran. Arjmand Publication. 64: 561-566.

7. Daka, D. 2011. Antibacterial effect of garlic (Allium sativum) on Staphyloccusaureus: An in vitro study. Afr. J. Biotechnol., 10 (4): 666-669.

8. Mosavian, H and Basiri, M.T. 2009. Consideration of temperature and air velocity on narrow leaves traces thyme essential oil extraction during the industrial drying. 18th Food Science and Technology Congress (Mashhad). 225-230.

9. Khanavi, M., Norruzi, M., Tabatabaei, H., Ssalehinoudeh, A.R., Barzegarsafavi, S and Shafiei, A. 2009. Identification of volatile oil components and antiviral effects of thyme and oregano plants. J. of Medic. Plants., 33: 128-137.

10. Kasravi, R. 2011. Mastitis in dairy cattle. 16th Iranian Veterinary Congress.

11. Bauer, A.W., Kirby, W.M., Sherris, J.C and Turck, M. 1996. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493- 496.

12. Khosravi, A and Malekan, M.A. 2004. Determination of Alcoholic and aqueous extract of Lavender Astvkas on Staphylococcus aureus and other Gram-negative bacteria. The. J. Qazvin Univ. of Med. Sci., 29: 3-9.

13. Sokovic, m., Marin, P.d., Brkić, D and van Griensven, L.J.D. 2007. Chemical Composition and Antibacterial Activity of Essential Oils of Ten Aromatic Plants against Human Pathogenic Bacteria. Bacteria. Food., 1 (1): 220-226.

14. Hosseinzade, A., Mohajerfar, T., Akhondzade, A., Khanjari, E., Qandominasrabadi, H., Misaghi, A and Sadeghi, S. 2011. Detrmination of MIC and MBC Lysozymes and thymus vulgaris on E. coli O :$_{157}$H$_{7}$. J. of Medic. Plants., 8: 208-217.

15. Falahi, j., Ebadi, M.T., Rezvanimoghada, P., Hedayati, M and Tarighi, S. 2010. Comparison of the inhibitory effect of 6 medial plant essential oils with stereptomycin antibiotic on Salmonella. Iranian J. Vet. Res., 6: 25-33.

16. ZahraeiSalehi, T., Vojgani, M., Bayat, M., Torshizi, H and Akhondzadeh, A. 2005. Determination of Minimum Inhibitory Concentrarion (MIC) of Extract of Zataria,multiflora, against the clinical isolates of Streptococcus agalactiaeStaphylococcus,aureus and E.coli). J. Vet. Res., 60: 107-110.

17. Habibi, H., Mazaheri, D., MajnoonHosseini, N., Chaeechi, M.R., Fakhr-Tabatabaee, M and Bigdeli, M. 2006. Effect of altitude on essential oil and components in wild thyme (Thymus kotschyanusBoiss) Taleghan region. Pajouhesh & Sazandegi. 73: 2-10.

18. Kamkar, A. 2009. The study of antioxidant activity of essential oil and extract of Iranian Anethumgraveloens. Quarterly Journal of gonabad Univ. of Med. Sci., 15: 11-17.

19. Orojalian, F and Kasrakermanshahi, R. 2001. Study of Phytochemical and antibacterial properties of the Achilleaeriophora essential oil with Microdilution Method. J. of Horticultural Sci., 24: 109-115.

20. Muhamed Mubarack, H., Doss, A., Dhanabalan, R and Venkataswamy, R. 2011. Activity of some selected medicinal plant extracts against boving mastitis pathogens. J. Anim. Vet. Adv., 10: 738-741.

21. Muhamed Mubarack, H., Doss, A., Dhanabalan, R and Venkataswamy, R. 2011. In-Vitro Antimicrobial Effects of Some Selected Plants against Bovine Mastitis Pathogens. Hygeia. J. D. Med., 3: 71 – 75.

22. Imelouane, B., Amhamdi, A., Wathelet, J.P., Ankit, M., Khedid, K and Elbachiri, A. 2011. Chemical composition and antimicrobial activity of essential oil of thyme (thymus vulgaris) from eastern morocco. Int. J. Agric. Biol., 11: 205-208.

23. Velag, J and Studlla, G. 2005. The Medicinal Plants. Persian Translation by Zaman S. Sixth Ed. Tehran.Naghsh Iran publication. 9-10.

24. AnandaBaskaran, S., Kazmer, G.W., Hinckley, L., Andrew, S.M and Venkitanarayanan, K. 2009. Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens in vitro. J. of Dairy. Sci., 92: 1423–1429.