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HONEY AND ESSENTIAL OIL OF THYME (*Thymus fontanesii*) SYNERGETIC ANTIMICROBIAL EFFECTS AGAINST *Klebsiella sp.*, *S. xylosus*, *Pseudomonas aeruginosa* AND *Bacillus sp.* ISOLATED FROM GOAT'S MASTITIS MILK

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ABSTRACT

The resistance of some bacterial strains to commonly used antibiotics in goat's mastitis made it necessary to search some novel types of antimicrobial agents. The aim of this work was to investigate the antimicrobial activities of multi-floral honey and the essential oil of *Thymus fontanesii* when used jointly by determining the MIC (Minimum Inhibitory Concentration) against some bacterial agents isolated from goat's subclinical mastitis milk. The isolated bacteria were *Klebsiella sp, Staphylococcus xylosus, Pseudomonas aeruginosa and Bacillus sp.* The inoculum suspension of bacterial strains was taken from five colonies of 24 hours old cultures, grown on specific media. The results indicated the efficiency of multi-floral honey and essential oil of *Thymus fontanesii* against the isolated bacterial strains. -The MIC values for honey were 16%, 25%, 15% and 16% (vol/vol) for *Klebsiella sp, Staphylococcus xylosus, Pseudomonas aeruginosa and Bacillus sp.,* respectively, whereas for Thymus essential oil these values were 0.03%, 0.03%, 0.03% and 0,01 (vol/vol), respectively. In this study when honey and essential oil were used jointly, a decrease of the MIC values was noticed, which may be due to a synergetic effect. These results suggested that multi-floral honey and essential oil of *Thymus fontanesii* could be used together to manage mastitis in goats.

Keywords: Honey; *Thymus fontanesii;* synergetic; mastitis; Goat's milk.

INTRODUCTION

Indiscriminate use of antibiotics has led to the emergence of multidrug-resistant bacterial strains and it is a global public health problem [1]. To solve this problem, alternative antimicrobial strategies like plants and plant-based products such as honey have currently got more attention [2].

A variety of plants and their extracts have been used for the treatment requiring antimicrobial activity, and some of the popular natural antimicrobial substances described in the ancient medicine are honey

[3], and essential oils from aromatic plants [4,5].

Many bacterial strains implicated in goat's mastitis were resistant to commonly used antibiotics in Tiaret region of Algeria [6]. In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat the microbial resistance. The essential oil have been reported to possess significant antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal, and insecticidal activities [7,8,9]. Therefore, essential oils can serve as a powerful tool to reduce the bacterial resistance [10]. The composition, structure, as well as functional groups of the oils, play an important role in determining their antimicrobial activity [11]. The antimicrobial activity of Algerian *Thymus genus* essential oil as well as multi-floral honey was also demonstrated [12,13]. The aim of this study was to evaluate the antimicrobial capacity of *Thymus fontanesii* oil and Algerian multi floral honey, when used jointly, against goat's subclinical mastitis bacterial agents.

MATERIALS AND METHODS

Bacterial Strains and the Inoculum Standardisation

The bacterial strains were isolated from goat's subclinical mastitis milk. The isolated strains were *Klebsiella sp, Staphylococcus xylosus, Pseudomonas aeruginosa and Bacillus sp.* The inoculum suspensions of bacterial strains were taken from five colonies grown on specific media and 24 hours old cultures. It was suspended in 5 ml 0.85%. NaCl solution. The obtained inoculum suspensions were shaken for 15 seconds and the density was adjusted to a turbidity of 0.5 MacFarland Standard (equivalent to 1-5 x 106 CFU/ml).

Honey Samples

Honey sample was collected from the beekeepers in the region of Tiaret (Algeria). It was stored in dark at 4ºC temperature.

The Extraction of Essential Oil

Thyme was collected from the region of Tiaret (Algeria), washed with water, and leaves were removed and weighed. 100g of *Thymus fontanesii* were subjected to hydrodistillation process for 3 hours. After distillation, the obtained oil was isolated from water and dried over anhydrous sodium sulfate [14]. The essential oil was kept at 4°C until further tests.

The Minimum Inhibitory Concentration (MIC)

MIC of honey and thymus were determined separately by using an agar incorporation technique method. Honey and essential oil of *Thymus fontanesii* were added in increasing quantities (vol/vol) into media for a final volume of 5 ml, after that, the mixture was shaked with a vortex then poured into plates. Petri dishes were inoculated by the tested bacterial strains and then incubated at 37°C for 24 hours. The lowest concentration of honey and thymus that inhibited the growth of the tested organisms was recorded as the MIC.

Minimum Synergistic Inhibitory Concentration (MSIC)

In aseptic condition, the volumes lower than the MIC, determined in the first step of honey and the essential oil of *Thymus fontanesii*, were mixed and then incorporated with *Agar agar* media. The

mixture was shaked moderately and poured into plates then standard inoculums of 0.5 McFarland of bacterial strains were poured in the plates and incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

The results indicated that honey and essential oil of thyme had an antibacterial property. Antibacterial activities of honey against *Klebsiella sp., S. Xylosus, Pseudomonas aeruginosa and Bacillus sp* isolated from mastitis goat's milk were found at the concentrations of 16%, 25%, 15% and 16%, respectively. The concentration of thyme essential oil was 0.03% for all the strains except *Bacillus* sp. which was 0.01%.

The additive effect between honey and essential oil of thyme against all tested strains showed that the MIC was decreasing as shown in Table 2. The MIC of both honey and essential oil of *Thymus fontanesii* against the bacterial strains isolated from subclinical goat's mastitis are reported in Table 1 and Table 2. This indicated the synergistic effect of honey and thyme powder when used jointly.

Great attention has been paid to the screening of antimicrobial activity and its evaluation methods. Several bioassays such as well diffusion, disk-diffusion, and broth or agar dilution are well-known and commonly used methods [15].

The lowest concentration of antimicrobial agent that completely inhibits the growth of the organism in microdilution wells or tubes as detected by the unaided eye is called minimum inhibitory concentration (MIC) [16]. In the present study, the MIC value of honey against *Klebsiella sp., S. xylosus, Pseudomonas aeruginosa and Bacillus sp.,* isolated from mastitis goat's milk was 16%, 25%, 15% and 16%, respectively.

Table 1. MIC values of honey and essential oil of *Thymus fontanesii*

Bacterial strains	MIC of honey, %	MIC of essential oil, %
Klebsiella sp.	16%	0.03%
S. xylosus	25%	0.03%
Pseudomonas aeruginosa	15%	0.03%
Bacillus sp.	16%	0.01%

Table 2. Values of MSIC against bacterial strains

Klebsiella sp.		*S. xylosus*		*Pseudomonas aeruginosa*		*Bacillus sp.*	
Honey (%)	**Essential oil (%)**	**Honey (%)**	**Essential oil (%)**	**Honey (%)**	**Essential oil (%)**	**Honey (%)**	**Essential oil (%)**
11	0.001	15	0.001	14	0.001	15	0.001
11	0.08	14	0.001	14	0.08	14	0.001
11	0.06	12	0.08	13	0.08	14	0.06
11	0.04	10	0.08	12	0.08	13	0.06
10	0.04	9	0.06	12	0.06	12	0.04
9	0.04	9	0.04	12	0.04	10	0.04
9	0.04	8	0.04	11	0.04	7	0.02

Honey and essential oil are among the natural products, that are widely used in the treatments of infections that show resistance to conventional drugs [17,18]. The antibacterial activity of honey is attributed to its high osmolarity, low pH, hydrogen peroxide and the presence of other uncharacterised compounds [19]. The major antimicrobial properties are correlated to the hydrogen peroxide level determined by relative levels of glucose oxidase and catalase [20] whereas, other factors such as antioxidant, lysozyme, phenolic acids and flavonoids contribute to honey antibacterial activity [21]. In human medicine, bees' products have been researched for their antimicrobial, antifungal, antiviral, anticancer and antioxidant properties [22]. These reports are in concordance with our results.

In this study, the essential oil of *Thymus fontanesii* had shown a strong antibacterial effect and the MIC value was 0.03% for all the strains except *Bacillus* sp., for which the value was 0.01%. This activity could be due to the active components present in it, which may act synergistically against the bacteria. The factors determining the activity of essential oils are composition, functional groups present in active components, and their synergistic interactions [23]. The antimicrobial mechanism of action varies with the type of essential oil or the strain of the microorganism.

It is well known that in comparison to Gram-negative bacteria, Gram-positive bacteria are more susceptible to essential oil [24,25]. This can be attributed to the fact that Gram-negative bacteria have an outer membrane which is rigid, rich in lipopolysaccharide (LPS) and more complex, thereby limiting the diffusion of hydrophobic compounds through it. This extra complex membrane is absent in Gram-positive bacteria which instead are surrounded by a thick peptidoglycan wall not dense enough to resist small antimicrobial molecules, facilitating the access to the cell membrane [26]. It was reported that the carvacol and thymol, the main com-pounds of *Thymus,* have antibacterial properties [27].

It is expected that the antimicrobial action of phenolic compounds such as thymol and carvacrol is attributed to the structural and functional damages in the cytoplasmic membrane [28]. In another study, an enhancement in the antimicrobial activity of carvacrol loaded in polylactic glycolic acid nano-capsules was reported due to a significant transformation in the rheological characteristics of bacterial biofilm that potentially facilitated the activity of carvacrol [29].

The results of the present study are supported by many previous studies testing the synergistic effect of honey and other compounds [30]. An overview pertaining to synergism between plant metabolites and antibiotics has been provided and promising adjuvants of antibiotics may be represented by phytochemicals [31]. According to *in vitro* studies, essential oils and their components form a part of the group of phytochemicals that are said to have such effects, [32].

CONCLUSION

In this work, it can be concluded that thyme essential oil and honey acted synergistically against some bacterial strains isolated from subclinical goat's mastitis and can be used in alternative therapy. This mixture is natural, easy-to-prepare and it can replace some resistant drugs and medicines.

DISCLAIMER

This paper is based on preliminary dataset. Readers are requested to consider this paper as preliminary research article. Authors are aware that detailed statistical analysis is required to get a scientifically established conclusion. Readers are requested to use the conclusion of this paper judiciously as statistical analysis is absent. Authors also recommend detailed statistical analysis for similar future studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kacaniova M, Vukovic N, Bobkova A, Fikselova M, Rovna K, Hascik P, et al. Antimicrobial and antiradical activity of Slovakian honeydew honey samples. J Microbiol Biotechnol Food Sci. 2011; 1(3):354–360.
2. Basualdo C, Sgroy V, Finola MS, Juam M. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet Microbiol. 2007;124:375–381. DOI: 10.1016/j.vetmic.2007.04.039. [PubMed] [Cross Ref]
3. Mandal MD, Mandal S. Review Honey: Its medicinal property and antibacterial activity. Asian Pac J Trop Biomed. 2011;1(2):154-60.
4. Skiadas PK, Lascaratos JG. Dietetics in ancient Greek philosophy: Plato's concepts of healthy diet. Eur J Clin Nutr. 2001;55:532-7.
5. Yalemwork E, Wossenseged L, Nega B. Synergetic antimicrobial effects of mixtures of Ethiopian Honeys and Ginger Powder Extracts on Standard and Resistant Clinical Bacteria Isolates. Evidence-Based Complementary and Alternative Medicine. 2014;8.
6. Bourabah A, Ayad A, Boukraa L, Hammoudi SM, Benbarek H. Prevalence and Etiology of Subclinical Mastitis in Goats of the Tiaret Region, Algeria. Global Veterinaria. 2013; 11(5):604-608.
7. Kaloustian J, Chevalier J, Mikail C, Martino M, Abou L, Vergnes MF. Étude de six huiles essentielles: Composition chimique et activité antibactérienne. Phytothérapie. 2008; 6:160–164. DOI: 10.1007/s10298-008-0307-1 [Cross Ref]
8. Benjilali B, Ayadi A. Methode d'études des propriétes antiseptiques des huiles essentielles par contact direct en milieu gelose [*Thymus capitatus*, Rosmarinus officinalis, *Eucalyptus globulus*, *Artemisia herba alba*] Plantes Méd. Phytothér. 1986;2:155–167.
9. Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. Int. J. Food Microbiol. 2004;94:223–253. DOI:10.1016/j.ijfoodmicro.2004.03.022. [PubMed] [Cross Ref]
10. Stefanakis MK, Touloupakis E, Anastasopoulos E, Ghanotakis D, Katerinopoulos HE, Makridis P. Antibacterial activity of essential oils from plants of the genus *Origanum*. Food Control. 2013;34:539–546. DOI: 10.1016/j.foodcont.2013.05.024
11. Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus*

flavus in liquid medium and tomato paste. Food Control. 2007;18:1518–1523.
12. Moussa A, Djebli N, Meslem A, Aisset S. Antibacterial activity of various honey types of Algeriaagainst Pathogenic Gram-Negatine Bacilli: *E. coli* and *Pseudomonas aeruginosa*. Asian Pacific Journal of Topical Disease. 2012;211-214.
13. Bourabah A, Ayad A, Hammoudi SM, Boukraa L, Benbarek H. Antimicrobial activity of Algerian honey on subclinical mastitis pathogens isolated from goat's milk. Veterinary World. 2014 ;7(4):248-252.
14. Policegoudra R, Goswami S, Aradhya SM, Chatterjee S, Datta S. Bioactive constituents of Homalomena aromatic essential oil and its antifungal activity against dermatophytes and yeasts. Journal de Mycologie Medicale. 2012; 22:83-87.
15. Balouiri M, Sadiki M, Ibnsouda SA. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Biomed. Anal. 2016;6:71–79. DOI: 10.1016/j.jpha.2015.11.005
16. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically: Approved Standard—8th Edition. Clinical and Laboratory Standards Institute; Wayne, PA, USA: 2009. CLSI Document M07-A8.
17. Dutta BK, Karmakar S, Naglot A, Aich JC, Begam M. Anticandidial activity of some essential oils of a mega biodiversity hotspot in India. Mycoses. 2007;50(2):121-4.
18. Boukraa L, Sulaiman SA. Honey use in Burn management: Potentials and Limitarions, Forschende Komplementarmidizin. 2010;17:74-80.
19. Molan PC. The antibacterial properties of honey. Chem NZ. 1995;59:10-4.
20. Weston RJ, Bronchlebank LK, Lu Y. Identification and quantita-tive levels of antibacterial components of some New Zealand hon-eys. Food Chem. 2000;70:427.
21. Snowdon JA, Cliver D. Micro-organisms in honey. Int J Food Microbiol. 1996;31:1-26.
22. Al-Waili N, Al Ghamdi A, Ansari MJ, Al-Attal Y, Mubarak Al. Differences in composition of honey simples and their impact on the antimicrobial activities against drug multiresistant bacteria and pathogenic fungi. Arch Med Res. 2013;44:307-316.
23. Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. 2000;88:308–316.
DOI: 10.1046/j.1365-2672.2000.00969.x
[PubMed] [Cross Ref]
24. Huang DF, Xu JG, Liu JX, Zhang H, Hu QP. Chemical constituents, antibacterial activity and mechanism of action of the essential oil from Cinnamomum cassia bark against four food related bacteria. Microbiology. 2014;83:357–365.
DOI:10.1134/S0026261714040067
[Cross Ref]
25. Azhdarzadeh F, Hojjati M. Chemical composition and antimicrobial activity of leaf, ripe and unripe peel of bitter orange (*Citrus aurantium*) essential oils. Nutr. Food Sci. Res. 2016;3:43–50.
DOI:10.18869/acadpub.nfsr.3.1.43
[Cross Ref]
26. Zinoviadou KG, Koutsoumanis KP, Biliaderis CG. Physico-chemical properties of whey protein isolate films containing oregano oil and their

antimicrobial action against spoilage flora of fresh beef. Meat Sci. 2009; 82:338–345.
DOI:10.1016/j.meatsci.2009.02.004
[PubMed] [Cross Ref]

27. Ettayebi K, El-Yamani J, Rossi-Hassani BD. Synergistic effects of nisin and Thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. FEMS Microbiol Lett. 2000;183:191-195.
28. Sikkema J, De Bont JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 1995; 59:201–222.
[PMC free article] [PubMed].
29. Iannitelli A, Grande R, Stefano AD, Giulio MD, Sozio P, Bessa LJ, Laserra S, Paolini C, Protasi F, Cellini L. Potential antibacterial activity of carvacrol- loaded poly(DL-lactide-co-glycolide) (PLGA) nanoparticles against microbial biofilm. Int. J. Mol. Sci. 2011;12:5039–5051.
DOI: 10.3390/ijms12085039
[PMC free article]
[PubMed] [Cross Ref]
30. Boukraâ L, Amara K. Synergistic Effect of starch on the antibacte-rial activity of honey. J Med Food. 2008; 11(1):195-8.
31. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008;15:639–652.
DOI: 10.1016/j.phymed.2008.06.008
[PubMed] [Cross Ref]
32. Langeveld WT, Veldhuizen EJ, Burt SA. Synergy between essential oil components and antibiotics: A review. Crit. Rev. Microbiol. 2014;40:76–94.
DOI:10.3109/1040841X.2013.763219.
[PubMed] [Cross Ref].