Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 7, 2033 – 2038

Antimicrobial Effects Of Lemon balm (*Melissa officinalis* L.) Essential Oil Against Pathogenic Bacteria

Salman Heidarian[1], Mehrdad Ataie Kachoie*[2], Sadegh Mousavi-Fard[3], Fariborz Moattar[4]

[1, 2, 3] *Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.*

[2,4] *Medicinal Plants Processing Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.*

[3] *Department of Horticultural Science, Faculty of Agriculture, Lorestan University, Khorramabad, P.O. Box 465, Iran.*

[4] *Professor Emeritus, Department of Pharmacology, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran.*

[4] *R & D Goldaru-co, Pharmaceutical Laboratory, Iran.*

***Corresponding Author:** Dr. Mehrdad Ataie Kachoie

Associate Professor, Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Email address: Drataie@iaushk.ac.ir.

Abstract
Background: Lemon balm (*Melissa officinalis* L.) is a medicinal plant with high antimicrobial properties. The present survey was aimed to assess the antimicrobial effects of *M. officinalis* essential oil against pathogenic bacteria. Methods: The *M. officinalis* aerial parts were prepared and, after approval by experts, dried and powdered and used to prepare the essential oil. The diameter of the growth inhibition zones of bacteria was assessed using disk diffusion. Findings: Dose-dependent antimicrobial effect was reported. The highest diameters of the growth inhibition zones of *P. aeruginosa*, *E. coli*, *S. aureus*, and *A. baumannii* bacteria were obtained against 4 mg/ml *M. officinalis* essential oil (14.21±0.74 mm), 4 mg/ml *M. officinalis* essential oil (13.38±0.36 mm), imipenem (14.61±0.29 mm), and imipenem (10.01±0.31 mm), respectively. Conclusion: Considering the high diameter of the growth inhibition zone of 4 mg/mL *M. officinalis* essential oil compared to antibiotic agents, it can be used as an economical source of antimicrobials.

Keywords: *Melissa officinalis*, Essential oil, Antimicrobial effects, Pathogenic bacteria.

Background

Notwithstanding the cumulative advances in medical sciences, antibiotic resistance remains a challenging issue (1-5). Researches show that if antibiotic resistance continues to develop at the present rate, antibiotic-resistant infections will measure to over 10 million human deaths yearly by 2050, with an assessed worldwide economic cost reaching $100 trillion (6). Thus, extensive research should be addressed to develop new antimicrobials with high efficacy against a wide range of bacteria. Studies have revealed that bacteria have a high ability to develop resistance to a wide range of drugs and also cause severe host infections (7, 8). Nowadays, *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Acinetobacter baumannii* (*A. baumannii*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) have more been involved in epidemics of the infectious diseases globally (9-12). They are mostly associated with the occurrence of urinary and respiratory tract infections (UTIs and RTIs, respectively), blood, soft tissue, wound, burn, gastrointestinal, and reproductive infections, and food-borne diseases (13-17). Several reports showed the high antimicrobial resistance of these bacteria against commonly used antimicrobial agents, including tetracyclines, aminoglycosides, penicillin, macrolides, quinolones, and phenicols (18, 19). Consequently, many efforts have been made to find new antimicrobials sources over the years (20).

Lemon balm (*Melissa officinalis* (*M. officinalis*)) belongs to the Lamiaceae family and is native to Iran, the Middle East, Central Asia, and some parts of Europe. In some countries, *M. officinalis* used to give fragrance to diverse food and beverage products (21). It is also used as an antimicrobial agent in food packaging techniques (22). As a medicinal plant, *M. officinalis* used to treat several diseases and disorders, including headaches, nervousness, colic, indigestion, cardiac failure, depression, and rheumatism (23). It is also used as an antibacterial, antifungal, and antiparasitic agent in different studies (24-26).

Despite the high antimicrobial effects of the *M. officinalis*, the current survey aimed to assess the antimicrobial effects of *M. officinalis* essential oil compared to diverse antibiotic agents against *S. aureus*, *E. coli*, *A. baumannii*, and *P. aeruginosa in vitro* condition.

Methods

Plant materials

The aerial parts of the *M. officinalis* plants were collected in the Shahrekord city, Chaharmahal Va Bakhtiari province, Iran (Southwest of Iran, 32.3282° N, 50.8769° E, and 2,061 m elevation) in June and September 2020. First, the plant species was approved by one of the specialists of the Department of Medicinal and Aromatic Plants, Islamic Azad University, Shahrekord Branch. A voucher specimen (Herbarium No.) was deposited in the Agricultural Research and Training Center and Natural Resources of Chaharmahal Va Bakhtiari Province. The plant material was air-dried at room temperature (20 ± 2 °C).

Extract preparation

Extraction of essential oil in the laboratory was done by distillation with water (UK Pharmacopoeia) and using a Cloninger machine. For this purpose, 50 grams of dried aerial parts of *M. officinalis* plant were weighed and essential oil extraction was carried out for 3 hours. In order to dehydrate the essential oil, sodium sulfate was used. The final essential oil was kept at a temperature of 4°C and in dark glass until use.

Bacteria and growth conditions

Four pathogenic bacteria, including *E. coli* (ATCC 25922), *S. aureus* (ATCC 9144), *A. baumannii* (ATCC 19606), and *P. aeruginosa* (ATCC 25922) were obtained from the Microbiology Research Center of the Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. Pure culture of the bacteria was cultured separately for regeneration in Tryptic Soy Broth (Merck, Germany) and incubated at 37 ° C for 24 h (27).

Disk diffusion

The antimicrobial activity of *M. officinalis* essential oil was investigated using the disk diffusion method. Briefly, after overnight incubation of bacteria, their concentrations were reached to 1×10^6 colonies per milliliter (CFU/mL). The bacteria were then cultured superficially on Müller-Hinton agar medium. Then 6 mm blank discs were placed on Müller-Hinton agar medium, and 1000 µl of *M. officinalis* essential oils with concentrations of 0.5, 1, 2, and 4 mg/mL were gently poured on the blank discs. To compare the antimicrobial effects of the extracts, ceftazidime (30 µg/disc), imipenem (10 µg/disc), gentamicin (10 µg/disc), vancomycin (30 µg/disc), penicillin (10 µg/disc), ciprofloxacin (5 µg/disc), tetracycline (30 µg/disc), erythromycin (15 µg/disc), ampicillin (10 µg/disc), and azithromycin (15 µg/disc) (Oxoid, UK) antibiotic discs were used. Blank and antibiotic discs were placed at regular intervals on plates containing bacteria. The plates containing the bacteria and discs were then incubated at 37 ° C for 24 h. The diameter of the growth inhibition zones around discs was measured and presented in millimeters (28).

Numerical evaluation

Data collected from the experiment were numerically evaluated by the SPSS/21.0 software (SPSS Inc., Chicago, IL) software (29). Qualitative data taken from the tests were examined using the chi-square test and Fisher's exact two-tailed test (30). *P*-value less than 0.05 was determined as a significance level (31).

Results

Findings of the disk diffusion

Table 2 shows the diameter of the growth inhibition zone of examined bacteria toward *M. officinalis* essential oil and antibiotic discs. The mean diameter of the tested bacteria's growth inhibition zone increased with increasing the *M. officinalis* essential oil. *M. officinalis* essential oil with a concentration of 4 mg/ml harbored the highest diameter of the growth inhibition zone against all examined bacteria. The highest diameters of the growth inhibition zones of *P. aeruginosa*, *E. coli*, *S. aureus*, and *A. baumannii* bacteria were obtained against 4 mg/ml *M. officinalis* essential oil (14.21±0.74 mm), 4 mg/ml *M. officinalis* essential oil (13.38±0.36 mm), imipenem (14.61±0.29 mm), and imipenem (10.01±0.31 mm), respectively. Statistically, significant differences were observed for the diameter of the growth inhibition zone of the examined bacteria between some different concentrations of *M. officinalis* essential oil and antibiotic agents (P <0.05).

Table 2. The growth inhibition zone diameter of examined bacteria toward *M. officinalis* essential oil and antibiotic discs.

Essential oil and antibiotics/concentrations		Diameter of the growth inhibition zone (mm)			
		P. aeruginosa	*E. coli*	*S. aureus*	*A. baumannii*
M. officinalis essential oil	4	14.21±0.74 a***	13.38±0.36 a	10.72±0.10 b	9.19±0.14 a
	2	13.70±1.02 a	12.99±0.18 a	9.88±0.15 c	8.20±0.29 b
	1	12.03±0.55 b	11.01±0.29 b	9.22±0.41 c	7.95±0.19 bc
	0.5	11.82±0.33 b	10.17±0.24 b	8.27±0.33 d	7.30±0.22 c
Antibiotics	Cef30**	11.51±0.64 b	12.17±0.35 a	13.42±0.93 a	9.13±0.29 a
	Imp 10	12.15±0.50 b	12.93±0.48 a	14.61±0.29 a	10.01±0.31 a
	G10	10.41±0.60 b	10.53±0.28 b	11.14±0.22 b	7.20±0.35 c
	V30	11.90±0.75 b	12.86±0.63 a	13.97±0.49 a	9.92±0.71 a
	P10	10.65±0.39 b	11.51±0.40 b	12.08±0.27 b	8.23±0.45 b
	Cip5	10.96±0.27 b	11.95±0.57 ab	12.58±0.39 b	8.77±0.41 b
	Tet30	10.37±0.56 b	10.60±0.14 b	11.02±0.18 b	7.07±0.27 c
	Er15	10.89±0.44 b	11.72±0.48 ab	12.39±0.26 b	8.61±0.53 b
	Am 10	10.77±0.42 b	11.25±0.56 b	11.83±0.36 b	8.05±0.20 b
	Az15	11.30±0.55 b	12.26±0.18 a	13.22±0.46 a	9.19±0.14 a

*mg/mL

**Cef30: ceftazidime (30 µg/disc), Imp10: imipenem (10 µg/disc), G10: gentamicin (10 µg/disc), V30: vancomycin (30 µg/disc), P10: penicillin (10 µg/disc), Cip5: ciprofloxacin (5 µg/disc), Tet30: tetracycline (30 µg/disc), Ertt15: erythromycin (15 µg/disc), Am10: ampicillin (10 µg/disc), Az15: azithromycin (15 µg/disc).

***Dissimilar letters in each column show statistically significant differences about $P < 0.05$.

Discussion

Plant antimicrobial agents act by targeting specific sites of bacteria, fungi, and parasites. Among the essential antimicrobial compound's mechanisms, inhibition of protein synthesis, interference with cell wall synthesis, inhibition of metabolic mediated pathways, interference with nucleic acid synthesis, and disruption of cell cytoplasmic membrane are the most important ways of applying antimicrobial effects (32).

The present survey was performed to assess the antimicrobial effects of *M. officinalis* essential oil against bacterial species using disk diffusion. Our findings showed the high antimicrobial effects of *M. officinalis* essential oil against tested bacteria, particularly *P. aeruginosa* and *E. coli*. Findings showed that 4 mg/mL *M. officinalis* essential oil harbored the highest antimicrobial effects against tested bacteria. The diameter of the growth inhibition zone of *P. aeruginosa* treated with the 4 mg/mL *M. officinalis* essential oil was significantly higher than all examined antibiotic agents. ($P < 0.05$). Additionally, the diameter of the growth inhibition zone of *E. coli* treated with the 4 mg/mL *M. officinalis* essential oil was significantly higher than gentamicin, penicillin, tetracycline, and ampicillin antibiotic agents. ($P < 0.05$). A part of the antimicrobial effects of *M. officinalis* essential oil is due to the presence of chemical components with antimicrobial effects, including citronellal, caryophyllene oxide, geranial and neral. High antimicrobial effects of citronellal, caryophyllene oxide, geranial and neral was also reported in previous surveys (33, 34). Despite the findings of other investigations which showed the higher susceptibility of the Gram-positive bacteria (*S. aureus*) than Gram-negatives (*E. coli*, and *P. aeruginosa*) against natural essential oils (35, 36), our findings showed that *M. officinalis* essential oil harbored the higher antimicrobial effects against *P. aeruginosa* and *E. coli*. Similar to our findings, Alizadeh Behbahani and Shahidi (2019) (37) and Klūga et al. (2017) (38) stated the high antimicrobial effects of *M. officinalis* essential oil against *P. aeruginosa*, and Ehsani et al. (2017) (39) and Jafarzadeh et al. (2020) (40) mentioned its high effect against *E. coli*. However, Gram-positive bacteria's cell wall is more susceptible to many antimicrobial agents, chemical compounds, and even herbal medicines but due to the possible presence of alcohol-based chemical compounds, *M. officinalis* essential oil can directly penetrate to the outer membrane lipopolysaccharides of *P. aeruginosa* and *E. coli*. Thus, it will be a good candidate for Gram-negative bacteria, particularly *P. aeruginosa* and *E. coli*. However, determination of the exact reason of this finding need more researches. Literature searches revealed that *M. officinalis* essential oil are reported to be more active against *Shigella sonei* (41), *Vibrio parahaemolyticus* (42), *E. coli* (43), and *S. aureus* (44). Abdellatif et al. (2021) (45) reported that *M. officinalis* essential oil collected from Algeria harbored the high antimicrobial effects against *Bacillus subtilis* (10±1 mm dimeter of the growth inhibition zone), *P. aeruginosa* (50±1 mm), *E. coli* (50±1 mm), *Klebsiella pneumonia* (10±1 mm), and *Salmonella enterica* (50±1 mm) using the disk diffusion technique.

Conclusion

This study showed that *M. officinalis* essential oil, particularly at a 4 mg/mL concentration, has sound antimicrobial effects against all examined bacteria, particularly *P. aeruginosa* and *E. coli*. Its antimicrobial effects were also higher than the majority of tested antibiotic agents, especially against *P. aeruginosa* and *E. coli*. It is recommended to use *M. officinalis* essential oil as an oral antimicrobial compound in the food and medical industries. However, further studies are needed in this area.

Acknowledgments

The authors thank the R & D team of the Goldaru-co, Pharmaceutical Laboratory, Iran and Agricultural Research and Training Center, and Natural Resources of Chaharmahal Va Bakhtiari province, Iran, for the substantial supports.

References

[1]. Dehkordi FS, Barati S, Momtaz H, Ahari SN, Dehkordi SN. Comparison of shedding, and antibiotic resistance properties of Listeria monocytogenes isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo, and camel species in Iran. Jundishapur Journal of Microbiology. 2013b; 6(3):284.

[2]. Dehkordi FS, Yazdani F, Mozafari J, Valizadeh Y. Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC Research Notes. 2014a; 7(1):1-8.

[3]. Dehkordi FS, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial Resistance & Infection Control. 2017a; 6(1):1-1.

[4]. Dehkordi FS, Tavakoli-Far B, Jafariaskari S, Momtaz H, Esmaeilzadeh S, Ranjbar R, Rabiei M. Uropathogenic Escherichia coli in the high vaginal swab samples of fertile and infertile women: virulence factors, O-serogroups, and phenotyping and genotyping characterization of antibiotic resistance. New Microbes and New Infections. 2020; 38:100824.

[5]. Safarpordehkordi F, Yahaghi E, Khodaverdi Darian E. Prevalence of antibiotic resistance in Escherichia coli isolated from poultry meat supply in Isfahan. Iranian Journal of Medical Microbiology. 2014a; 8(2):41-7.

[6]. Goff, D. A., Kullar, R., Goldstein, E. J., Gilchrist, M., Nathwani, D., Cheng, A. C., Cairns, K. A., Escandon-Vargas, K., Villegas, M. V., Brink, A., van den Bergh, D., and Mendelson, M. A global call from five countries to collaborate in antibiotic stewardship: united we succeed, divided we might fail. Lancet Infect. Dis. 2017; 17(2):e56-e63.

[7]. Ranjbar R, Seif A, Dehkordi FS. Prevalence of antibiotic resistance and distribution of virulence factors in the shiga toxigenic Escherichia coli recovered from hospital food. Jundishapur J Microbiol. 2019; 12(5):8.

[8]. Rahi A, Kazemeini H, Jafariaskari S, Seif A, Hosseini S, Dehkordi FS. Genotypic and phenotypic-based assessment of antibiotic resistance and profile of staphylococcal cassette chromosome mec in the methicillin-resistant Staphylococcus aureus recovered from raw milk. Infection Drug Res. 2020; 13:273.

[9]. Abdolmaleki Z, Mashak Z, Dehkordi FS. Molecular and virulence characteristics of methicillin-resistant Staphylococcus aureus bacteria recovered from hospital cockroaches. Jundishapur Journal of Microbiology. 2019 Dec 31; 12(12).

[10]. Dehkordi FS, Yahaghi E, Darian EK. Prevalence of antibiotic resistance in Escherichia coli isolated from poultry meat supply in Isfahan. Iran J Med Microbiol: Volume. 2014 Jul; 8(2).

[11]. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. Acinetobacter baumannii antibiotic resistance mechanisms. Pathogens. 2021 Mar 19; 10(3):373.

[12]. Sindeldecker D, Stoodley P. The many antibiotic resistance and tolerance strategies of Pseudomonas aeruginosa. Biofilm. 2021 Dec 1; 3:100056.

[13]. Ranjbar R, Dehkordi FS, Heiat M. The frequency of resistance genes in Salmonella enteritidis strains isolated from cattle. Iranian Journal of Public Health. 2020 May; 49(5):968.

[14]. Rostami F, Rahimi E, Yahaghi E, Khodaverdi Darian E, Bagheri Moghadam M. Isolation and evaluation virulence factors of Salmonella typhimurium and Salmonella enteritidis in milk and dairy products. Iranian Journal of Medical Microbiology. 2014 Jun 10; 8(1):54-61.

[15]. Momeni Shahraki M, Shakerian A, Rahimi E, Safarpoor Dehkordi F. Study the frequency of enterotoxin encoding genes and antibiotic resistance pattern of methicillin-resistant Staphylococcus aureus isolated from vegetable and salad samples in Chaharmahal Va Bakhtiari province. Journal of Food Microbiology. 2020 Jun 21; 7(2):55-69.

[16]. Hasanpour Dehkordi A, Khaji L, Sakhaei Shahreza MH, Mashak Z, Safarpoor Dehkordi F, Safaee Y, Hosseinzadeh A, Alavi I, Ghasemi E, Rabiei-Faradonbeh M. One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant

Staphylococcus aureus recovered from raw meat. Trop Biomed. 2017; 34(2):396-404.
[17]. Watkins K. Emerging infectious diseases: a review. Current Emergency and Hospital Medicine Reports. 2018 Sep; 6(3):86-93.
[18]. Abdolmaleki Z, Mashak Z, Safarpoor Dehkordi F. Phenotypic and genotypic characterization of antibiotic resistance in the methicillin-resistant Staphylococcus aureus strains isolated from hospital cockroaches. Antimicrobial Resistance & Infection Control. 2019 Dec; 8(1):1-4.
[19]. Dormanesh B, Dehkordi FS, Hosseini S, Momtaz H, Mirnejad R, Hoseini MJ, Yahaghi E, Tarhriz V, Darian EK. Virulence factors and o-serogroups profiles of uropathogenic Escherichia coli isolated from Iranian pediatric patients. Iranian Red Crescent Medical Journal. 2014 Feb; 16(2).
[20]. Dehkordi FS, Tirgir F, Valizadeh Y. Effects of Guajol® ointment synthesized from medicinal smoke condensate of jennet feces on burn wound healing on Wistar rat. Veterinary Research Forum. 2017b; 8(3):215.
[21]. Miraj S, Rafieian-Kopaei, Kiani S. Melissa officinalis L: A Review study with an antioxidant prospective. Journal of evidence-based complementary & alternative medicine. 2017 Jul; 22(3):385-94.
[22]. Carocho M, Barros L, Calhelha RC, Ćirić A, Soković M, Santos-Buelga C, Morales P, Ferreira IC. Melissa officinalis L. decoctions as functional beverages: a bioactive approach and chemical characterization. Food & function. 2015; 6(7):2240-8.
[23]. Shakeri A, Sahebkar A, Javadi B. Melissa officinalis L.–A review of its traditional uses, phytochemistry and pharmacology. Journal of ethnopharmacology. 2016 Jul 21; 188:204-28.
[24]. Ehsani A, Alizadeh O, Hashemi M, Afshari A, Aminzare M. Phytochemical, antioxidant and antibacterial properties of Melissa officinalis and Dracocephalum moldavica essential oils. InVeterinary Research Forum 2017 (Vol. 8, No. 3, p. 223). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
[25]. Abdel-Naime WA, Fahim JR, Fouad MA, Kamel MS. Antibacterial, antifungal, and GC–MS studies of Melissa officinalis. South African Journal of Botany. 2019 Aug 1; 124:228-34.
[26]. Anthony JP, Fyfe L, Smith H. Plant active components–a resource for antiparasitic agents? Trends in parasitology. 2005 Oct 1; 21(10):462-8.
[27]. Dehkordi FS, Yazdani F, Mozafari J, Valizadeh Y. Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC Research Notes. 2014;7(1):1-8.
[28]. Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. Journal of Food Safety. 2019;39(2):e12594.
[29]. Dehkordi FS. Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. American Journal of Animal and Veterinary Sciences. 2011.
[30]. Dehkordi FS, Momtaz H, Doosti A. Application of Real-Time PCR for detection of Aspergillus species in aborted ruminant foetuses. Bulgarian Journal of Veterinary Medicine. 2012 Mar 1; 15(1):30-6.
[31]. Mashak Z, Jafariaskari S, Alavi I, Shahreza MS, Dehkordi FS. Phenotypic and genotypic assessment of antibiotic resistance and genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 alleles of Helicobacter pylori bacteria isolated from raw meat. Infection and drug resistance. 2020; 13:257.
[32]. Khameneh B, Iranshahy M, Soheili V, Bazzaz BS. Review on plant antimicrobials: A mechanistic viewpoint. Antimicrob Res Infect Control. 2019;8:1-28.
[33]. Jalal Z, El Atki Y, Lyoussi B, Abdellaoui A. Phytochemistry of the essential oil of Melissa officinalis L. growing wild in Morocco: Preventive approach against nosocomial infections. Asian Pacific Journal of Tropical Biomedicine. 2015 Jun 1; 5(6):458-61.
[34]. Hamad A, Djalil AD, Dewi DY, Hartanti D. Development of lemon basil essential oil as a natural chicken meat preservative. InIOP Conference Series: Earth and Environmental Science 2021 Jul 1 (Vol. 803, No. 1, p. 012028). IOP Publishing.
[35]. Abers M, Schroeder S, Goelz L, Sulser A, St Rose T, Puchalski K, Langland J. Antimicrobial activity of the volatile substances from essential oils. BMC complementary medicine and therapies. 2021 Dec; 21(1):1-4.
[36]. Man a, Santacroce L, Iacob R, Mare A, Man L. Antimicrobial activity of six essential oils

against a group of human pathogens: A comparative study. Pathogens. 2019 Jan 28; 8(1):15.

[37]. Alizadeh Behbahani B, Shahidi F. Melissa officinalis essential oil: Chemical compositions, antioxidant potential, total phenolic content and antimicrobial activity. Nutrition and Food Sciences Research. 2019 Jan 10;6(1):17-25.

[38]. Klūga A, Terentjeva M, Kántor A, Kluz M, Puchalski C, Kačániová M. Antibacterial activity of Melissa officinalis L., Mentha piperita L., Origanum vulgare L. and Malva mauritiana against bacterial microflora isolated from fish. Advanced Research in Life Sciences. 2017 Jul 26; 1(1):75-80.

[39]. Ehsani A, Alizadeh O, Hashemi M, Afshari A, Aminzare M. Phytochemical, antioxidant and antibacterial properties of Melissa officinalis and Dracocephalum moldavica essential oils. InVeterinary Research Forum 2017 (Vol. 8, No. 3, p. 223). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

[40]. Jafarzadeh MM, Moghaddam MJ, Bakhshi D. Antimicrobial activity of three plant species against multi-drug resistant E. coli causing urinary tract infection. Journal of Herbal Medicine. 2020 Aug 1; 22:100352.

[41]. Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of Melissa officinalis L. (Lamiaceae) essential oil. Journal of agricultural and food chemistry. 2004 May 5; 52(9):2485-9.

[42]. Yu H, Pei J, Qiu W, Mei J, Xie J. The Antimicrobial Effect of Melissa officinalis L. Essential Oil on Vibrio parahaemolyticus: Insights Based on the Cell Membrane and External Structure. Frontiers in Microbiology. 2022; 13.

[43]. Arzhang M, Dakhili M, Farahani F. Investigation of Chemical Compounds and Anti-microbial Activity of Essential Oil of Melissa officinalis L. Qom University of Medical Sciences Journal. 2015 Apr 10; 9(1):7-13.

[44]. JAFARI-SALES A, Pashazadeh M. Study of chemical composition and antimicrobial properties of Rosemary (Rosmarinus officinalis) essential oil on Staphylococcus aureus and Escherichia coli in vitro. International Journal of Life Sciences and Biotechnology. 2020; 3(1):62-9.

[45]. Abdellatif F, Akram M, Begaa S, Messaoudi M, Benarfa A, Egbuna C, Ouakouak H, Hassani A, Sawicka B, Elbossaty WF, Simal-Gandara J. Minerals, essential oils, and biological properties of Melissa officinalis L. Plants. 2021 Jun; 10(6):1066.