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<http://dx.doi.org/10.12739/NWSA.2017.12.2.5A0084>

EVALUATION OF ANTIBACTERIAL ACTIVITY OF MACERATED OIL FROM Lemon Balm (*Melissa officinalis* L, Lamiaceae), Thyme (*Thymus vulgaris* L, Lamiaceae), Mint (*Mentha longifolia* L, Lamiaceae) and Marigold (*Calendula officinalis*, Amaryllidaceae) WITH MODIFIED TUBE AGAR DILUTION METHOD AGAINST *YERSINIA RUCKERI*

ABSTRACT

Aromatic and medicinal plants (AMPs) have been most popular for human and animal in phytotherapy, phytochemistry and pharmacology recently owing to their anti-bacterial, anti-viral, and antiseptic characteristics. Therefore, experiments were designed for determination the antibacterial effects of macerated and distilled oils of lemon balm (*Melissa officinalis*), thyme (*Thymus vulgaris*), mint (*Mentha longifolia*) and marigold (*Calendula officinalis*) against *Yersinia ruckeri* with modified tube agar dilution method. Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) were determined. Our results indicated that number and concentration of bacteria were reduced by macerated and distilled oils of lemon balm, thyme, mint and marigold. Compared all treatments, the best results were obtained by thyme. In conclusion, macerated oils of lemon balm, thyme, mint and marigold showed antibacterial activity against the pathogen although to a lesser extent than in distilled oil. It is recommended that macerated oils could be used as protective to the causative agent.

Keywords: Antibacterial Activity, *Yersinia ruckeri*, Modified Tube Dilution Method, Aromatic plants, Pathogen

Melisa (*Melissa officinalis* L, Lamiaceae), Kekik (*Thymus vulgaris* L, Lamiaceae), Nane (*Mentha longifolia* L, Lamiaceae) ve Nergisten (*Calendula officinalis*, Amaryllidaceae) ELDE EDİLEN MASERE YAĞININ *YERSINIA RUCKERI*'YE KARŞI ANTİBAKTERİYEL ETKİSİNİN TÜP AGAR DİLUSYON METODUYLA BELİRLENMESİ

Öz

Aromatik ve tıbbi bitkilerin (ATB) antibakteriyel, antiviral ve antiseptik özelliklerinden dolayı insan ve hayvanlarda fitoterapi, fitokimya ve farmakoloji açısından son yıllarda önemi dünyada artmıştır. Bu nedenden dolayı, melisa (*Melissa officinalis* L, Lamiaceae), kekik (*Thymus vulgaris* L, Lamiaceae), nane (*Mentha longifolia* L, Lamiaceae) ve nergisten (*Calendula officinalis*, Amaryllidaceae) elde edilen masere yağının *Yersinia ruckeri*'ye karşı antibakteriyel etkisinin tüp agar dilüsyon methoduyla belirlenmesi için deneyler gerçekleştirilmiştir. Minimal inhibitör konsantrasyonu (MİK) ve Minimum bakterisidal konsantrasyonu (MBK) belirlenmiştir. Elde edilen sonuçlar melisa, kekik, nane ve nergisin masere ve distile yağlarının bakteri sayısı ve konsantrasyonunu azalttığını göstermiştir. Bütün denemeler karşılaştırıldığında en iyi sonuçlar kekikten elde edilmiştir. Sonuç olarak, melisa, kekik, nane ve nergisin masere yağlar distile yağlara oranla düşük olmasına rağmen patojene karşı antibakteriyel aktivite göstermiştir ve masere yağlar patojene karşı koruyucu olarak kullanılabilir.

Anahtar kelimeler: Antibakteriyel Aktivite, *Yersinia Ruckeri*, Modifiye Tüp Agar Dilüsyon Method, Aromatik Bitkiler, Patojen

How to Cite:

Altınterim, B. and Kocabaş, M., (2017). Evaluation of Antibacterial Activity of Macerated Oil from Lemon Balm (*Melissa Officinalis* L, Lamiaceae), Thyme (*Thymus Vulgaris* L, Lamiaceae), Mint (*Mentha Longifolia* L, Lamiaceae) and Marigold (*Calendula Officinalis*, Amaryllidaceae) with Modified Tube Agar Dilution Method Against *Yersinia Ruckeri*, **Ecological Life Sciences (NWSAELS)**, 12(2):26-32, DOI: 10.12739/NWSA.2017.12.2.5A0084.



1. INTRODUCTION

Melissa officinalis L. (Labiatae; lemon balm), *Thymus vulgaris* Labiatae; thyme) and *Mentha longifolia* (Labiatae; mint) are aromatic herbs in the mint family Lamiaceae, native to Mediterranean region and the Southern Europe [1 and 6] *Calendula officinalis* is belonging the family Asteraceae, native to Mediterranean region and its flowers are particularly used for ornamental and medicinal purposes [7 and 9]. In particular, these aromatic and medicinal plants (AMPs) have been most popular for human and animal in phytotherapy, phytochemistry and pharmacology recently owing to their anti-bacterial, calmative, mnemonic improvement, anti-viral, anticancer, spasmolytic, antiseptic characteristics, cognitive function and reducing excitability, gastrointestinal disorders, sleep disturbance, stress, and anxiety [3, 6, 10 and 17]. Furthermore, they have been used for treatment of various disorders and diseases such as insomnia, CNS diseases, dyspepsia, vomiting, cardiovascular and bloating, respiratory problems, various cancers, attention deficit-hyperactivity disorder (ADHD), dysmenorrhoeal, headache, toothache, cramps, insect bites, tumours, Alzheimer's disease, hysteria, neurological diseases, colic, melancholia, palpitations, rheumatism, colitis, duodenal ulcers and high blood pressure for more than two millennia due to their high content of bioactive compounds [5, 6, 15, 17, 18 and 24].

2. RESEARCH SIGNIFICANCE

Yersinia ruckeri is the causative agent of yersiniosis or enteric redmouth disease (ERM) being serious septicemic bacterial disease in aquatic animals and, potentially affects fingerlings of all Salmonids [25]. It causes a significant economic problem for aquaculture producers in farms owing to mortality increases following periods of stress as a result of environmental conditions and handling. For these reasons, control of the bacteria is the most important due to cause significant economic losses [26 and 27]. To date, studies have been conducted on the antibacterial effect of lemon balm, thyme, mint and marigold. As in the previous studies, it was determined antioxidant or antimicrobial characteristics of lemon balm. Schnitzler et al. [28] determined a high antiviral activity against herpes simplex virus type 1 (HSV-1) *in vitro* using an aqueous extract of *M. officinalis*. Mazzanti et al. [19] stated that lemon balm could be used for treating Herpes simplex lesions. Astani et al. [29] suggested an inhibition activity against HSV-1 and HSV-2 of *M. officinalis*. Giarratana et al. [30] found that thyme showed a significant activity against *Anisakis* larvae. In previous studies, it was determined that marigold possessed an effect on some microorganisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* [22, 31 and 32]. Altınterim et al. [33] and Yüngül et al. [34] used agar-well method and aromatoqram method for determination of antibacterial activity with lemon balm, thyme, mint and marigold macerated oils against *Y. ruckeri*, respectively. Within this framework, the aim of the study was to determinate the antibacterial effects of macerated and distilled oils of lemon balm (*M. officinalis*), thyme (*T. vulgaris*), mint (*M. longifolia*) and marigold (*C. officinalis*) against *Y. ruckeri* with modified agar dilution method.

3. EXPERIMENTAL METHOD-PROCESS

The tested microorganisms were acquired from ATCC (American Type Culture Collection) or Adolfo Lutz Institute (Instituto Adolfo Lutz - IAL). Culture mediums, hydrogen peroxide, iodine, peracetic acid, and

remaining reagents were acquired from Sigma (St. Louis, MO). The cultures were started from the transference of stock cultures for trypticase soy broth (TSB, Difco, Detroit, MI, USA) at 24°C for *Y. ruckeri* (ATCC29473) for a period of 24 hours. The suspension was adjusted to 0.5 McFarland standards. Trypticase soy broth (TSB, 10 ml) including 1.5×10^8 bacteria to a 0.5 McFarland standards was added to first tube of the 10 tubes. 9 ml of TSB was added other tubes. 1 ml was withdrawn from first tube and transferred for tube 2. This successive transference was repeated until tube 10. In this way, bacteria dilutions were prepared from 1.5×10^8 to 1.5×10^{-1} in all tubes (Figure 1).

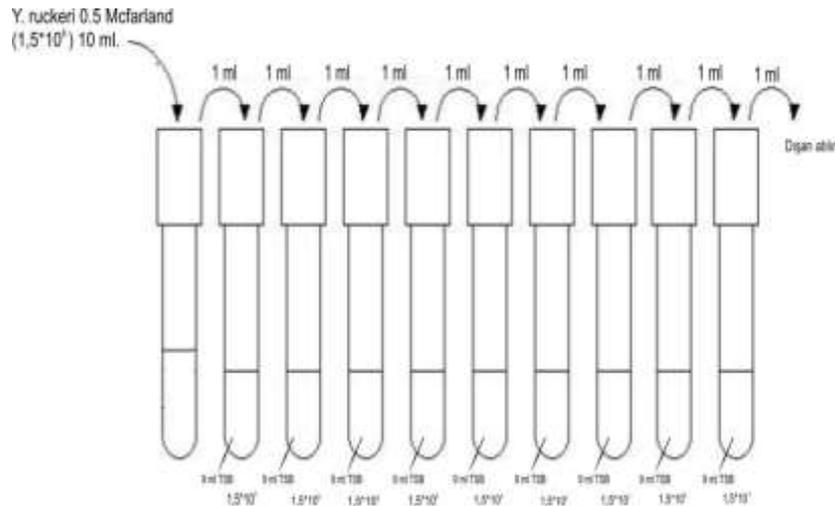


Figure 1. Illustrative scheme of successive dilutions method

Lemon balm (*M. officinalis*), thyme (*T. vulgaris*), mint (*M. longifolia*), marigold (*C. officinalis*) and distilled oil used in experiments were obtained from Defne Doğa Firm, Elazığ, Turkey. For obtaining macerated oil, lemon balm, thyme, mint and marigold was kept in sunflower oil (1/10) during 15 days. 1 ml of the obtained oils was transferred to tubes in two different dilution series. Tubes were shaken and incubated at 24 h. MIC and MBC values were determined according to macrodilution method (Figure 2). At the end of 24 h of incubation, the tubes were read for the MIC and then the MBC was determined by sampling all according to visible turbidity control and spectrophotometrically (550 nm).

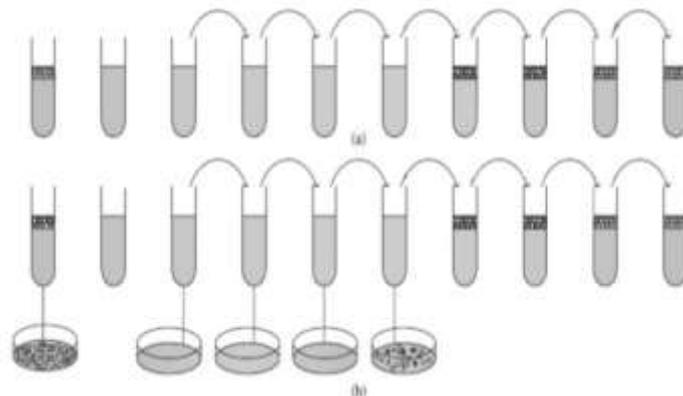


Figure 2. Illustrative scheme of macrodilution method for determination (a) MIC and (b) MBC values



4. FINDINGS AND DISCUSSIONS

Although a great deal of past research has focused on using lemon balm (*M. officinalis*), thyme (*T. vulgaris*), mint (*M. longifolia*) and marigold (*C. officinalis*) for human and animal in phytotherapy, phytochemistry and pharmacology [3, 6, 10 and 17]., there is a lack of information in the literature about the antibacterial effects of macerated and distilled oils of lemon balm, thyme, mint and marigold against *Y. ruckeri*. Nevertheless, only a few studies have been examined related to determination of antibacterial activity with different methods. Altınterim et al. [33] and Yüngül et al. [34] used agar-well method and aromagram method for determination of antibacterial activity with lemon balm, thyme, mint and marigold macerated oils against *Y. ruckeri*, respectively and they reported non-inhibitory activity against number of *Y. ruckeri* (1.5×10^8 and 1.5×10^7) depending on the higher concentration of bacteria. Briefly, successful results were not obtained from these studies with the methods. In light of the above researches, modified agar dilution method was used for examination of antibacterial activity with lemon balm macerated oils against *Y. ruckeri* due to be rapid and simplified method. The results are presented in Table 1. The present results indicated that number and the concentration of bacteria were reduced by macerated and distilled oils of lemon balm, thyme, mint and marigold. The best results were obtained by thyme (*T. vulgaris*). Overall, we demonstrated that macerated oil indicated inhibitory activity against *Y. ruckeri* though distilled oil showed a strong antibacterial activity against the pathogen. The present results may be due to contain a great number of active substances of macerated oils compared to extracts [35].

Table 1. The MICs and MBCs of macerated oils and distilled oils of lemon balm (*Melissa officinalis*), thyme (*Thymus vulgaris*), mint (*Mentha longifolia*) and marigold (*Calendula officinalis*).

Plant oil type	Minimum inhibitory concentrations (cfu/ml)		Minimum bactericidal concentrations (cfu/ml)	
	Distilled oil	Macerated oil	Distilled oil	Macerated oil
Lemon balm (<i>Melissa officinalis</i>)	1.5×10^3	1.5×10^1	1.5×10^7	1.5×10^5
Thyme (<i>Thymus vulgaris</i>)	1.5×10^7	1.5×10^7	1.5×10^6	1.5×10^5
Mint (<i>Mentha longifolia</i>)	1.5×10^3	1.5×10^2	1.5×10^2	1.5×10^{-1}
Marigold (<i>Calendula officinalis</i>)	1.5×10^0	1.5×10^{-1}	1.5×10^5	1.5×10^4
Control (Sunflower oil)	1.5×10^6		1.5×10^5	

5. CONCLUSION AND RECOMMENDATIONS

In conclusion, based on the data obtained within the context of this study, macerated oil of lemon balm (*M. officinalis*), thyme (*T. vulgaris*), mint (*M. longifolia*) and marigold (*C. officinalis*) could be used for treating *Y. ruckeri* lesions and support clinical trials on this medicinal plant.

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