Antimicrobial effect of *Melissa officinalis* extracts on typical food-borne pathogens

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**Abstract**

The present study was designed to evaluate an antibacterial activities of methanolic and ethanolic extracts of *Melissa officinalis* against different pathogenic bacteria using broth micro-dilution and disk-diffusion method. The methanolic extract of *Melissa officinalis* in Disk-diffusion method were active against the gram-positive bacteria, *Staphylococcus aureus* (inhibition zone = 9.31 mm) and *Bacillus cereus* (inhibition zone = 0.76 mm) was more effective than the methanol extract inactive against gram-negative bacteria (*E.coli* and *Salmonella enterica*). The ethanolic extract (inhibition zone = 11.66 mm for *Staphylococcus aureus*) was more effective than the methanol extract. The methanolic extract of this plant in broth micro-dilution assay, showed higher antimicrobial activity compared to ethanolic extracts (MIC= 83.33mg ml⁻¹ for *E. coli* and MIC = 33.33 *S. enterica*). Results showed good antibacterial activity for extracts on *S. aureus*, which suggested its capacity as a natural food preservative, however, it remained to consider the organoleptic properties and probable interactions in the food system.

**Introduction**

Food borne infections have been one of the major public health concerns worldwide and account for considerably high cases of illnesses. Recent studies reported that *Bacillus cereus*, *Campylobactre jejuni*, *Escherichia coli*, *Salmonella*, *Shigella* and *Staphylococcus aureus* are considered to be the most frequent pathogens (Voravuthikunchai et al., 2006). Also the increasing antibiotic resistance of some pathogens that are associated with diseases has increased the interest in the development of new types of effective and non-toxic antimicrobial compounds (Sobhy and El. Feky, 2007). The addition of chemical preservatives has long been an effective method to control microbial contamination and the development of oxidative reactions, although in recent years, popular demand has shown a marked aversion to such synthetic chemical preservatives. This has resulted in a growing demand for natural products, principally, plant extracts, which are, in the consumers mind, safer, functional and provide nutritional and health benefits. This demand has increased the importance of searching for alternative sources of natural preservatives rich in phenolic compounds (Viuda-Martos et al., 2012).

A variety of plant species have been known to synthesize many bioactive secondary metabolites with antimicrobial and biological properties, like alkaloids, terpenoids (triterpenes and steroid saponins), phenolic compounds, glycosides, flavonoids, tannins, and poly-saccharides. In addition, phytomedicines are...
eco-friendly, inexpensive, easily prepared, and mitigate many of the side-effects that are often associated with synthetic antibiotics. Regarding the antibacterial activity of plant extracts / essential oils, the researchers have described several mechanisms of action, including cell membrane damage resulting in increased permeability, changes in intracellular pH and membrane potential, dissipation of cellular components, decrease in the cytoplasmic ATP concentration, which together induce bacterial death. Secondary effects that may be involved seem to be the inhibition of enzymes, loss of turgor pressure, alterations in macromolecules synthesis, and other cellular processes (Bulfon et al., 2014).

*M. officinalis* from Lamiaceae family, with other common names like bee balm, garden balm, melissa, melissengeist, is a perennial herbaceous plant which grows vastly from the central and southern Europe to Iran and central Asia. It is also cultivated worldwide for its edible properties (Chen et al., 2006; Ghayoor et al., 2010). *Melissa officinalis* L. is a perennial edible herb native to the Mediterranean region. The plant is cultivated in various parts of the world and grows especially in western Asia, south-western Serbia and North Africa. In Algeria, it is considered as an important medicinal plant largely used in traditional medicine, for the treatment of headaches, indigestion, colic, nervousness, cardiac failure and depression (Beloued, 2009).

The aim of this study was to determine the antibacterial activity of different extracts of *Melissa officinalis* including ethanolic and methanolic extracts on food-borne pathogens.

**Material and Methods**

**Chemicals and Plant materials**

Gentamicin (Sinadaroo, Iran), methanol, ethanol and Dimethyl Sulfoxide (DMSO), Muller Hinton (MH) Agar and MH Broth (Merck, Germany) were purchased. The leaves of *M. officinalis* were provided from Pharmaceutical Store at Tabriz-Iran during April, 2015. The plant was identified by the Vice Chancellor for Research and Technology, Ferdowsi University of Mashhad (Iran).

**Preparation of methanolic and ethanolic extract**

Plant samples were dried at room temperature under shade, blended into fine powder and stored in the dark in a dry place. 30 g of the powdered leaves of the plant was extracted in 500 ml of methanol and ethanol for 72 h under agitation at room temperature. The extracts were concentrated by rotary evaporation at 40°C and the yield of extraction was determined. All the dried extracts were preserved in the refrigerator at 4°C until further use. Plant extracts were dissolved in the solvent before use in the antimicrobial assay (Bousselsela et al., 2012).

**Organisms and inoculation conditions**

The methanolic and ethanolic extracts of *M. officinalis* were individually tested against four bacterial strains, including *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709) and *Escherichia Coli* (PTCC 1399) which were obtained from the Persian Type Culture Collection, Iranian Research Organization for Science and Technology (PTCC, Iran). Bacterial strains were cultured overnight at 37°C in MH agar (YesilCeliktas et al., 2007).

**Antimicrobial assay**

An antibacterial activity of the extracts and fractions were determined by the disk diffusion and micro-dilution methods. All tests were performed in three replicate.

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Test**

The MIC of the extracts and reference antibiotic
(Gentamicin Sinadaroo, Iran) were determined by microdilution techniques in MH broth (Merck) (Sanches et al., 2005). The 96-well plates were prepared by dispensing into each well where placed 100 L of extract solution in DMSO. Then, 100 L of MH broth was added and 20 L of the inoculum (standardized at 1.5× 106 CFU/ml by adjusting the optical density to 0.1 at 600 nm by Shimadzu UV-120-01 spectro-photometer). One hundred microliter aliquot from the stock solutions of the extract and their serial dilutions were prepared initially that was transferred into wells. The final volume in each well was 220 L. The plates were covered with sterile plate sealer and followed by shaking for 30s and then incubated at 37°C for 24 h. Micro titer plates were then incubated at 37°C for 24 hours. After incubation, the wells were examined for microbial growth. MIC was defined as the lowest concentration of the extract or essential oil in the medium in which there was no visible growth after incubation (Laciar et al., 2009; Lin et al., 2014) the MBC was defined as the lowest concentration in which no growth was noted on MH agar (Bento et al., 2009). For the determination of the MBC, 50 μL of the four last concentrations of the extract were removed, in the microdilution plate with growth, absence, and were inoculated in a plate content (Costa et al., 2009). The same plates were incubated at 37ºC for 24 hour. The reading was made.

Disk-diffusion method

For preparation of bacterial growth condition, the medium culture was poured in a 10 cm petri dish and surface of culture was radiated by ultraviolet ray under the microbial hood that ensures the sterilized condition. Then 100 L of microbial suspension was poured on the surface of culture and stroke lightly by sterile swab in all over the medium. Then a paper disk which was stained by 15 L essential oils or extracts, was placed on the surface of the medium, after that inoculated plates were incubated in an inverted position at 30°C for 24 hour. The zones of inhibition were recorded by ruler. Finally, the diameter of zones of inhibition compared with positive control (Gentamicin antibiotic) and negative control (DMSO) (Koochak et al., 2010).

Results

Results of disc-diffusion test

The diameters of inhibition zones, varied from 0.71 - 9.31 and 26.1 - 33.44 mm for various concentrations of methanolic extract and gentamycin respectively. Among the four bacteria, S. aureus was the most sensitive (the diameter of inhibition zone was 9.31 mm at 200 mg/ml against methanolic extract) and two bacteria (S.enterica and E. coli) were resistant to the extract at all concentrations. The diameters of inhibition zones, varied from 0.81-11.66 and 27.99 –31.1mm for various concentrations of ethanolic extract and gentamycin respectively. Among the bacterial species, S.aureus was the most sensitive (the diameter of inhibition zone was 11.66 mm at 200 mg/ml ethanolic extract) and S.e enterica and E. coli were resistant to the extract at all concentrations.

Results of MIC and MBC

The minimum inhibitory (MIC) and bactericidal (MBC) concentrations, two parameters that respectively quantify the bacteriostatic and bactericidal potential of bioactive compounds, were determined using dry extracts and the serial dilution method for the 2 extracts with sub-stantial antibacterial activity against the S. aureus, B. cereus, S. enterica and E. coli. As is shown in Table - 3, obtained values were in the ranges of 5.2 - 200 mg ml⁻¹ for MIC and 8.33- MBC>200mg ml⁻¹ for MBC, and on the whole were in agreement with the inhibitory activities shown above. The methanolic extract was the strongest bacteriostatic effects on the bacteria test and gram-negative bacteria were the most sensitive to this extract.
Table – 1. Results of disc-diffusion test and inhibition zones (mm) for methanolic and ethanolic extract of *Melissa officinalis* against gram positive bacteria

<table>
<thead>
<tr>
<th>Concentration (mg ml(^{-1}))</th>
<th>Microorganism</th>
<th>Bacillus cereus</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanolic extract</td>
<td>Ethanol extract</td>
<td>Methanolic extract</td>
</tr>
<tr>
<td>50</td>
<td>0.71</td>
<td>6</td>
<td>0.81</td>
</tr>
<tr>
<td>100</td>
<td>0.74</td>
<td>6</td>
<td>0.73</td>
</tr>
<tr>
<td>200</td>
<td>0.76</td>
<td>1.8</td>
<td>9.31</td>
</tr>
<tr>
<td>Positive control Gentamicin</td>
<td>33.44</td>
<td>31.1</td>
<td>29.44</td>
</tr>
<tr>
<td>Negative control DMSO</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table - 2. Results of disc-diffusion test and inhibition zones (mm) for methanolic and ethanolic extract of *Melissa officinalis* against gram negative bacteria

<table>
<thead>
<tr>
<th>Concentration (mg ml(^{-1}))</th>
<th>Microorganism</th>
<th>Escherichia coli</th>
<th>Salmonella enterica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanolic extract</td>
<td>Ethanol extract</td>
<td>Methanolic extract</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>200</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Positive control Gentamicin</td>
<td>28.88</td>
<td>27.99</td>
<td>28.1</td>
</tr>
<tr>
<td>Negative control DMSO</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table – 3. MIC and MBC for methanolic and ethanolic extract of *Melissa officinalis* (mg ml\(^{-1}\))

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>B. cereus</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>S. enterica</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC Methanolic extract</td>
<td>10.41±3.60</td>
<td>5.2±1.80</td>
<td>83.33±28.86</td>
<td>33.33±14.43</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>133±57.73</td>
<td>166.66±57.73</td>
<td>200±0</td>
<td>200±0</td>
</tr>
<tr>
<td>MBC Ethanol extract</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

Discussion

Natural products, such as a plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for control of microbial growth owing to their chemical diversity. Besides antimicrobial, several plants are being used in different areas of human health such as traditional medicine, functional foods, dietary supplements and recombinant protein manufacturing. Phytochemicals, especially flavonoids, polyphenols, anthocyanins and carotenoids, share...
the major market (Negi, 2012). Flavonoids (derivatives of phenylchromone ring) are a large group of compounds naturally occurring in higher and lower plants. Flavonoids have been shown to be able to affect various biological functions: capillary permeability, cellular secretory processes involved in the inflammatory response and inhibition of enzymes, receptors and carriers (Sanches et al., 2005).

Some studies showed that gram-negative bacteria are more resistant to essential oil others claim the same for gram-positive bacteria. In the present study gram-negative bacteria were in both categories (Klein et al., 2013). The internal stability of the bacterial cells depends on the interaction between a series of physiological factors, and the disturbance of this stability, may determine the bacteria’s death or the inhibition of its growth. To provide products, which reduce the toxicity risk and at the same time are obtained from a new natural and renewable source becomes a growing and economically viable option. The use of vegetal extracts for antibacterial activity is a consummated fact (Nogueirasa et al., 2014).

Conclusion

The results presented in this study indicated that extracts obtained from leaves of *M. officinalis* possess antibacterial properties. On the basis of the experimental results, it can be postulated that the extracts of *M. officinalis* have the potent antibacterial properties against some representative food-borne pathogens. Specifically, the methanolic extract was more active against gram-negative bacteria which indicated the presence of active compounds. Therefore, they could be used as possible food antimicrobial preservative in the food industry, but the *in vivo* studies should be done to evaluate the probable adverse effect on food sensory properties.

References


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