THE ANTIMICROBIAL ACTIVITIES OF *Myrtus communis* AND *Micromeria fruticosa* ESSENTIAL OILS

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This study was aimed to investigate the antimicrobial activity of *Myrtus communis* and *Micromeria fruticosa* against four Gram-positive (Methicillin resistant *Staphylococcus aureus*, Methicillin sensitive *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*) and four Gram-negative [(*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter aerogenes*)] bacteria by employing broth microdilution methods. The main components determined by GC-MS for *Myrtus communis* essential oil were Eucalyptol (45,41%), α -Pinene (20,86%), Linalool (9,71%), α -Terpineol (6,11%) and Limonene (5,88%) and for *Micromeria fruticosa* L. essential oil were Pulegone (58,32%), p-Menthan-3-one (12,99%) and Levomenthol (9,85%). Antibacterial activities of *Myrtus communis* and *Micromeria fruticosa* essential oils were searched against Methicillin resistant *Staphylococcus aureus*, Methicillin sensitive *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Enterobacter aerogenes*. In order to evaluate the MIC values of the bacterial strains, Minimal inhibition concentrations (MICs) determined by the National Committee for Clinical Laboratory standards. Amikacin was selected as standard drug for bacterial strains. As a result, it has been found that *Myrtus communis* and *Micromeria fruticosa* essential oils are very promising against these bacteria, which are among the major infectious agents in the world as well as in our country. Essential oils of these plants have been found very promising in the production of new antimicrobials. Further in vivo studies are needed in this regard.

Keywords: *Myrtus communis*, *Micromeria fruticosa*, essential oils, Gram positive, Gram negative, bacteria

INTRODUCTION

Increasing antibiotic resistance in bacteria all over the world has become a big problem. The unauthorized use of antibiotics increases resistance to antibiotics day by day. Gram-positive (methicillin resistant *Staphylococcus aureus*, methicillin sensitive *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter aerogenes*) are among the causes of serious morbidity and mortality. New drug studies against these kinds of infection agents have great prospects. The presence of methicillin-resistant strains, especially in staphylococci, can lead to serious consequences for both hospital infections and community-acquired infections (Utta and Wells, 2016; Wenzel, 2007; Burke, 2003; Doshi *et al.*, 2009; Vasoo *et al.*, 2005; Nam *et al.*, 2011).

In this study, it was aimed to investigate the antimicrobial activity of *Myrtus communis* and *Micromeria fruticosa* against four Gram-positive (methicillin resistant *Staphylococcus aureus* (ATCC 33591), methicillin sensitive *Staphylococcus aureus* [(ATCC 25923), *Staphylococcus epidermidis* (12228), *Enterococcus faecalis* (ATCC 29212)], and four Gram-negative [(*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 13883), *Enterobacter aerogenes* (ATCC 13048)] bacteria by employing broth microdilution methods.

MATERIALS AND METHODS

Plant Materials

Myrtus communis and *Micromeria fruticosa* plants were harvested during the full bloom period, which the amount of active substance was most intense and after being dried in the shade at room temperature.

Preparation of Plant Extracts

Plant material was weighted and placed in a round bottom flask with a volume of distilled water as extraction solvent; the herba-water mixture was refluxed about 2 h, during which the oil was collected in the side arm of the system. The installation was allowed to stand for about half an hour to prevent the oil to reach room temperature. The oil was dried over anhydrous sodium sulphate and then stored in dark color glass bottles and kept to refrigerator (about 4 °C) until use for analysis.

GC/MS Analysis

Analysis of essential oil was performed using the Thermo Scientific Focus gas chromatograph equipped with a DSQ II single quadrupole mass spectrometer, Triplus autosampler and fused-silica capillary column TR-5MS (5% phenyl-polysilphenylene-siloxane, 30 m×0.25 mm inner diameter, film thickness 0.25 µm). The injection volume was 2 µL. The samples were injected with a split ratio of 250:1 by using helium (99.99 %) as carrier gas, at a flow rate of 1 mL/min; ionization energy was 70 eV. The transfer line temperature of the mass spectrometer was 220 °C, while the temperature of orifice injection was of 220 °C. The temperature of oven was programmed in the range 50–220 °C at a rate of 3 °C/min. Data acquisition was made in the scanning mode. Identification was done on full scan mode in the m/z range of 50–650 a.m.u.

RESULTS

GS/MS analysis results of essential oils and their main components were given in Table 1, 2 and Figure 1, 2.

Figure 1. MIC and MFC values of *Myrtus communis* L.

Table 1. The main essential oil components of *Myrtus communis* L.

RT	Compound Name	SI	RSI	Cas #	%
6,86	α -Pinene	987	990	80-56-8	20,86
10,38	Limonene	984	990	138-86-3	5,88
12,47	Eucalyptol	988	988	470-82-6	45,41
21,12	Linalool	994	994	78-70-6	9,71
27,51	α -Terpineol	983	984	98-55-5	6,11

Figure 2. MIC and MFC values of *Micromeria fruticosa* L.

Table 2. The main essential oil components of *Micromeria fruticosa* L.

RT	Compound Name	SI	RSI	Cas #	%
23,79	Levomenthol	987	992	2216-51-5	9,85
24,05	p-Menthan-3-one	988	992	491-07-6	12,99
24,63	trans-Caryophyllene	956	966	87-44-5	1,96
29,04	bicyclogermacrene	944	964	100762-46-7	1,17
29,42	Pulegone	987	987	89-82-7	58,32
33,38	p-Menth-1-en-3-one	968	981	89-81-6	1,61

The main components of *Myrtus communis* essential oil were Eucalyptol (45,41%), α -Pinene (20,86%), Linalool (9,71%), α -Terpineol (6,11%) and Limonene (5,88%) and for *Micromeria fruticosa* L. essential oil were Pulegone (58,32%), p-Menthan-3-one (12,99%) and Levomenthol (9,85%).

In the study, it was found that *Myrtus communis* essential oils showed the highest antibacterial activity of methicillin sensitive *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228) at the 50 μ g/ml concentration among the Gram-positive bacteria. Besides this, *Micromeria fruticosa* essential oils showed the highest antibacterial activity against *S. epidermidis* at the 25 μ g/ml concentrations (Figure 3).

In the combined use of *Myrtus communis* and *Micromeria fruticosa* essential oils, a statistically significant decrease in the MIC values of all tested Gram-positive bacteria was found ($p < 0.01$).

In study, it was found that *Myrtus communis* essential oils showed the highest antibacterial activity against *Klebsiella pneumonia* (ATCC 13883) at the 75 μ g/ml concentration among Gram-negative bacteria. *Micromeria fruticosa* essential oils showed the highest antibacterial activity against *Klebsiella pneumonia* (ATCC 13883) and *Enterobacter aerogenes* (ATCC 13048) at the 50 μ g/ml concentrations (Figure 4).

In the combined use of *Myrtus communis* and *Micromeria fruticosa* essential oils, a statistically significant decrease in the MIC values of all tested Gram-negative bacteria was found ($p < 0.01$). MIC values of standard drugs and essential oils are given in figures 5 and 6 in the study.

Figure 3. MIC values of *Myrtus communis* and *Micromeria fruticosa* essential oils against Gram-positive bacteria

Figure 4. MIC values of *Myrtus communis* and *Micromeria fruticosa* essential oils against Gram-negative bacteria

Figure 5. Compare the MIC values of standard drugs with *Myrtus communis* and *Micromeria fruticosa* essential oils against Gram-positive bacteria

Figure 6. Compare the MIC values of standard drugs with *Myrtus communis* and *Micromeria fruticosa* essential oils against Gram-negative bacteria

DISCUSSION AND CONCLUSION

In our study, it was determined that essential oils of *Myrtus communis* and *Micromeria fruticosa* essential oils have considerable antibacterial activity against both Gram-positive and Gram-negative bacterial agents. *Micromeria fruticosa* essential oils

have been found to be more effective than *Myrtus communis* essential oils against both Gram-positive and Gram-negative infection agents. More importantly in our study, the combined use of *Myrtus communis* and *Micromeria fruticosa* essential oils was found to be significantly more effective against both Gram-positive and Gram-negative bacteria.

As a result, it has been found that *Myrtus communis* and *Micromeria fruticosa* essential oils are very promising against these bacteria, which are among the major infectious agents in the world as well as in our country. Essential oils of these plants have been found very promising in the production of new antimicrobials. Further *in vivo* studies are needed in this regard.

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