Determination of antibacterial activities of different *Thymus praecox subsp. grossheimii var. grossheimii* extracts

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ABSTRACT

In this study, 4 different extracts of *Thymus praecox subsp. grossheimii var. grossheimii* (Ronniger) an endemic species of genus *Thymus* were investigated on gram positive and gram negative test bacteria for the antibacterial activity. The plant extracts were obtained via Soxhlet method, and the antibacterial activities were determined by macrodilution liquid (tube) method (MIC). Results indicated that 4 different extracts at different concentrations showed antibacterial activities on both gram positive and gram negative strains. The highest antibacterial activity was observed in acetone extract against *Bacillus cereus*, whereas the lowest activity was seen in methanol extract against *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella Enteritidis*. In conclusion, it was determined that *Thymus praecox subsp. grossheimii var. grossheimii* (Ronniger) has an antibacterial activity against gram positive and gram negative test bacteria.

Key words: Antibacterial activity, Macrodilution, *Thymus praecox*.

INTRODUCTION

Medicinal plants and their derivatives have since long been used in traditional medicine for prevention and treatment of several pathologies. In various diseases, including infectious diseases were also treated by those plants preparations since ancient times (Fethi et al., 2013).

*Thymus* genus of the family *Labiatae* consists of Turkey’s largest genus (Avci, 2011), and there are 39 species and 59 taxa in this genus with an endemic rate of 53 % (Ozkan et al. 2010). Of the member of this species, *Thymus praecox* is common in Turkey and has a large number of subspecies and varieties (Avci, 2011). It is known that different *Thymus* species are used for medical and non-medical purposes (Laila et al., 2013). Previous studies indicate that *Thymus* species possess a broad spectrum of biological activities including carminative, antispasmodic, antitussive, bactericidal and anthelmintic effects (Atalay et al., 2004, Maria et al. 2008; Boros et al. 2010; Mariam et al. 2012).

*Thymus praecox subsp. grossheimii var. grossheimii* (Ronniger) is an endemic species of *Thymus* family (Ozen et al. 2012). Although there are a large number of studies about different *Thymus* species, the antibacterial activity of *Thymus praecox subsp. grossheimii var. grossheimii* (Ronniger) was not found.

In this study, extracts of *Thymus praecox subsp. grossheimii var. grossheimii* (Ronniger) obtained with different solvents were investigated for *in vitro* antibacterial activity.

MATERIALS AND METHODS

The plant samples were collected during July, 2012 in Kars city, and then they were allowed to dry under a shaded area for 20 days. The samples were ground for extraction. The powdered herb material (20 g) was extracted with 500 ml solvent in soxhlet apparatus for 3 h. It was filtered from Whatman No.1 filter paper. The filtrate extracts were concentrated under reduced pressure at 35°C, using a rotary evaporator (Heidolph, Laborata 4000 series). The dried crude concentrated extracts were stored in a refrigerator (- 4°C), until used for analyses.

Test microorganisms: Bacteria strains were provided by the collection of Istanbul University, Faculty of Veterinary Medicine, Department of Microbiology, and Istanbul, Turkey. *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 4352), *Proteus mirabilis* (CCM 1944), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella Enteritidis* (KUEN 349), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228) strains were used in the study.

Agar media: In macrodilution liquid (tube) method for the quantitative determination of antibacterial activity, Ca²⁺ and Mg²⁺- added cationic Mueller-Hinton Broth (HiMedia M391) (CAMBH) media was used. Seven percent blood added nutrient agar (HiMedia M001) was used to maintain the strains investigated.
Determination of antibacterial activity: Antibacterial activity for 4 different extracts of *Thymus praecox* was screened by macrodilution liquid (tube) method (MIC) according to the standards of Clinical and Laboratory Standards Institute (CLSI). Double serial dilutions of plant extracts within CAMHB as *Thymus praecox* -chloroform 6200 µg/mL – 0.878 µg/mL, *Thymus praecox* - acetone 6000 µg/mL – 1.46 µg/mL, *Thymus praecox* - methanol 14000 µg/mL – 1.7 µg/mL, *Thymus praecox* - n-hexane 5700 µg/mL – 0.69 µg/mL were prepared. 500 µL from each dilution was added to the sterilized tubes. A bacterial suspension equal to a density of 0.5 McFarland turbidity from 24-hour culture in blood agar was prepared in saline. 500 µL from the suspension was loaded to each tube. Positive (without plant extract) and negative (without bacterial inoculum) controls were also used at the end of each serial dilution tested. All tubes were incubated at 37 °C. The lowest concentration of plant extract which fully inhibited the bacterial growth was calculated as minimum inhibitory concentration (MIC). Parallel to plant extract, gentamicin was used as control (Metiner et al. 2012).

RESULTS AND DISCUSSION

Minimum inhibitory concentrations (MIC) of the plant extracts were presented in Table 1.

All extracts obtained from the leaves of *Thymus praecox* at different concentrations showed antibacterial activity against all test bacteria. The highest activity in tested extracts was observed with the acetone extract (93.75 µg/mL) against *Bacillus cereus*, whereas the lowest activity was seen with methanol extract (3500 µg/mL) against *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella Enteritidis*. In terms of the individual test bacterium, *Bacillus cereus*, *Bacillus subtilis cereus*, *Klebsiella pneumonia* and *Salmonella Enteriditis* were found to be sensitive to the acetone extract at 93.75, 375, 375 and 187.5 µg/mL, respectively. *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were sensitive to n-hexane extract at 178.1, 712.5, 178.1 and 178.1 µg/mL, respectively. *Pseudomonas aeruginosa* was sensitive to chloroform extract at 900 µg/mL. The antibacterial activity of extracts from highest to lowest was determined to be as acetone, n-hexane, chloroform and methanol.

In this study, extracts of *Thymus praecox* prepared by 4 different solvents were investigated for the antibacterial activity. All extracts at varying concentrations showed antibacterial activity against both gram positive and gram negative test bacteria. Although a number of studies indicating the antibacterial activity of *Thymus* family are present, there is a lack of enough data about the antibacterial activity of *Thymus praecox*. Sokmen et al. (2004) reported that essential oil of *Thymus spathulifolius* an endemic species of genus *Thymus* has a strong antibacterial activity and its methanol extract has moderate antibacterial activity against the test bacteria and four different fungi species tested. Gram positive bacteria was more sensitive to the methanol extract than gram negative bacteria. Similarly, methanol extract of *Thymus praecox* showed greater antibacterial activity against gram positive bacteria than gram negative bacteria (except for *E. coli*) in the present study. Turker and Turker (2009) studied the antibacterial activity of water and ethanol extract of seven different plant species including *Thymus praecox opiz* against some pathogenic bacteria (*Yersinia ruckeri, Aeromonas salmonicida, Enterococcus seriolicida*) in the trout. Only the ethanolic extracts of all plants showed antibacterial activity, and *Thymus praecox opiz* has an antibacterial activity against *Yersinia ruckeri* only. Furthermore, Sekeroglu et al. (2007) studied the antibacterial activity of essential oil of *Thymus praecox*, subsp. caucasicus var. *Caucasicus* against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Ampicilin was used as a control agent. The authors found no statistical significance between ampicilin and *Thymus praecox*, subsp. caucasicus var. *Caucasicus* in terms of antibacterial activity against the test bacteria. In another study, essential oils of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* were reported to have antibacterial activity against all the test bacteria including *S. enteritidis*, *S. typhimurium*, *E. coli*, *L. monocytogenes*, *Y. enterocolitica*, *S. flexneri*, *S. sonnei*, *S. aureus* (Rota et al. 2008).

One of the mechanisms for antibacterial effect is associated with inhibition of cellular processes following the ion efflux out of the bacterial cell due to the disruption

<table>
<thead>
<tr>
<th>Test Bacteria</th>
<th>Extraction</th>
<th>Bc (µg/mL)</th>
<th>Bs (µg/mL)</th>
<th>Ec (µg/mL)</th>
<th>Kp (µg/mL)</th>
<th>Pm (µg/mL)</th>
<th>Pa (µg/mL)</th>
<th>Sen (µg/mL)</th>
<th>Sa (µg/mL)</th>
<th>Se (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td><em>Bacillus cereus</em></td>
<td>450</td>
<td>450</td>
<td>450</td>
<td>1800</td>
<td>900</td>
<td>1800</td>
<td>450</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td><em>Bacillus cereus</em></td>
<td>93.75</td>
<td>375</td>
<td>375</td>
<td>750</td>
<td>1500</td>
<td>187.5</td>
<td>375</td>
<td>750</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td><em>Bacillus cereus</em></td>
<td>218.75</td>
<td>875</td>
<td>875</td>
<td>3500</td>
<td>3500</td>
<td>3500</td>
<td>875</td>
<td>875</td>
<td></td>
</tr>
<tr>
<td>n-Hexane</td>
<td><em>Bacillus cereus</em></td>
<td>178.1</td>
<td>712.5</td>
<td>712.5</td>
<td>712.5</td>
<td>1425</td>
<td>178.1</td>
<td>178.1</td>
<td>178.1</td>
<td></td>
</tr>
</tbody>
</table>

Bc; *Bacillus cereus*, Bs; *Bacillus subtilis*, Ec; *Escherichia coli*, Kp; *Klebsiella pneumonia*, Pm; *Proteus mirabilis*, Pa; *Pseudomonas aeruginosa*, Sen; *Salmonella Enteritidis*, Sa; *Staphylococcus aureus*, Se; *Staphylococcus epidermidis*
of bacterial cell permeability (Khan et al. 2009). Therefore, differences could be seen between gram positive and gram negative bacteria in terms of the antibacterial activity of the particular agent. As shown in the previous studies, gram positive bacteria are more sensitive to plant extracts than gram negative bacteria. This could be explained by the fact that there are structurally some important differences between gram positive and gram negative bacteria. Gram negative bacteria have an extra outer layer and periplasmic space which are absent in gram positive bacteria. Hydrophilic surface of polysaccharide rich outer membrane in gram negative bacteria was attributed to resistance to the antibacterial molecules. The outer membrane functions as a barrier against the penetration of a number of different antibiotic molecules. In addition, some enzymes capable of inactivating external molecules are found in periplasmic space. Since gram positive bacteria have no such a structural cell wall and membrane configuration, antibacterial agents could easily damage cell wall and cytoplasmic membrane (Shan et al. 2007).

Results of the current study indicated that extracts of Thymus praecox prepared by 4 different solvents have antibacterial activity on selected gram positive and gram negative bacteria. Parallel to the previous studies, the lowest activity was observed in methanol extract (3500 µg/mL) against gram negative strains Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa and Salmonella Enteritidis. In addition, MIC values of 375 µg/mL and 187.5 µg/mL in acetone extract were obtained against Klebsiella pneumonia and Salmonella Enteritidis, respectively. MIC value against Salmonella enteritidis was lower than gram positive strains (except for Bacillus cereus). MIC value against Klebsiella pneumonia was similar to the MIC values against Staphylococcus aureus and Bacillus subtilis, but lower than that of Staphylococcus epidermidis. Considering the general conclusion that plant extracts are more effective to the gram positive bacteria than the gram negative bacteria, this general conclusion is only compatible for methanol extract in our study. Results obtained from other extracts of Thymus praecox subsp. grossheimii var. grossheimii (Ronniger) in the present study also are not fully in accordance with the theory.

The antibacterial activity of Thymus genus has been shown in the previous and in the present study. In this study, it was shown that Thymus praecox subsp. grossheimii var grossheimii (Ronniger) an endemic species of Thymus genus has an antibacterial activity against 9 different bacterial strains tested. As a result, the extracts of this plant have the potential to be used in the preservation of food in food industry and in the treatment of bacterial infections.

REFERENCES


