Antimicrobial activities of aqueous extracts and essential oils of two endemic species from Turkey

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Salvia and Ballota species of Lamiaceae have been used in the traditional medicine. Salvia heldreichiana Boiss. Ex Bentham, Ballota saxatilis subsp. brachyodonta Boiss. P. H. Davis & Doroszenko. are endemics, found in Mersin-Hatay-Adana region and to our knowledge their antimicrobial activities never been researched before. Antimicrobial activities of the aqueous extracts (herbal extract) (AE) and essential oils (EO) of two endemic plants were screened against nine human pathogenic bacteria and yeast. Aqueous extracts (AE) and essential oils (EO) of these plants were investigated against four Gram-positive bacteria, three Gram-negative bacteria and two yeast species. Antimicrobial activity were determined by using macrodilution method. The aqueous extracts of the plants showed rather low antimicrobial activity against the studied microorganisms than their essential oils. Minimum inhibitory concentration (MIC) values have been changed in the range of 0.78-50 µg/ml. The aqueous extracts and essential oils of Ballota saxatilis subsp. brachyodonta and Salvia heldreichiana possess compounds with antimicrobial effect against pathogen microorganisms. Further studies should be carried out in order to reveal their potential.

Keywords: Ballota saxatilis subsp. brachyodonta, Salvia heldreichiana, Aqueous extract, Essential oil, Antimicrobial activity

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For thousands years, herbal drugs have been used in traditional medicine all over the world. Especially herbal medicines and many compounds extracted from plants have a great potential in the pharmaceutical industry1. There are several reports on the biological activity of herbal medicines such as remedies for many infectious diseases2. Also, some studies indicated traditional antimicrobial therapy represent a potential source of new antimicrobial agents3.

In recent years, there has been a rising necessity in the discovery of new antimicrobial agent, due to increase antibiotic-resistant microorganisms4. It is well-known that some plants, especially those belonging to the Lamiaceae family, own a wide range of biological activities, such as antimicrobial, carminative, antiviral, etc. effects 5. Lamiaceae family, is known as potentiality of aromatic plants, have economic importance for food and pharmacological industry6. A number of researcher have revealed the antimicrobial properties of a wide variety of essential oils7,8. A number of studies demonstrated the essential oil of some Lamiaceae species have strong biological activities9,10.

Ballota genus belongs to Lamiaceae family and they have 33 species that are mainly distributed around the Mediterranean and Eurasia11. According to the literature the endemism ratio in Lamiaceae family for Turkey is 42.2%12. Several compounds have been investigated and identified from the genus Ballota, among them, diterpenes, phenylpropanoid glycosides, flavonoids and essential oils are major. Sixteen species of Ballota genus originate from Turkey. Plants of the genus have been used in for nausea, vomiting, nervous dyspepsia, antulcer, antispasmodic, diuretic, choleric, antihemorrhoidal and sedative agent. It has been recently reported antimicrobial and antioxidant activities of most Ballota species., Ballota saxatilis subsp. brachyodonta, is an important endemic for our country, was shown that it has a strong antioxidant agent by Citoglu13,14.
Salvia genus, has 900 species throughout the world and 88 species in Turkey, are rather important in Turkish folk medicine. Salvia officinalis, grow in parks and gardens as ornamental plants, used to treat chill, sniffles, abdominal pain and stomach disorders. Some aqueous extracts of this genus, especially S. officinalis, S. viridis, S. multicaulis, are known that have antioxidant activity. Salvia heldreichiana, is an endemic plant, is known that its essential oil has antimicrobial activity on pathogen Staphylococcus aureus, Sarcina lutea, Escherichia coli, Salmonella typhimurium and Pseudomonas aeruginosa. Its aqueous extract hasn’t been yet researched. We presented data on antimicrobial activity of herbal extract (aqueous extract) and essential oil of two endemic Salvia heldreichiana and Ballota saxatilis subsp. brachyodonta.

Methodology

Aerial parts of Salvia heldreichiana, Ballota saxatilis subsp. brachyodonta were collected in the month of May 2011 from Mersin, Turkey and are listed in Table 1. The botanical identification of the plants were done by Biologist Hikmet Çelik and Ersin Öztürk and confirmed by Assoc. Prof. Ayşe Everest at Mersin University Biology Department. The dried plant samples were first ground to fine powder. For aqueous extraction, 0.5 gm of the fine powder was extracted with 10 ml of ultra-filtered water at 100°C for 30 min in a water bath. The samples were then cooled down to room temperature and sterilized by filtration 0.22 µm milipore filters. Final concentration of 50 µg/ml were stored at -20°C and used for antimicrobial tests.

Essential oil extraction (EO): The essential oil of Salvia heldreichiana and Ballota saxatilis subsp. brachyodonta were extracted by hydrodistillation for 4hrs using the Clevenger, with yield 0.75% and 0.05%, respectively. The essential oil was collected over anhydrous sodium sulphate and refrigerated until time of analysis. The antimicrobial effects of aqueous extracts were assessed on several pathogens, namely Escherichia coli (ATCC 25293), Enterococcus faecalis (ATCC 29212), Bacillus subtilis (ATCC 6633), Salmonella typhimurium, Staphylococcus aureus (ATCC 25925), Staphylococcus epidermidis (ATCC 12228), Klebsiella pneumoniae (10031), Candida albicans (clinical strain), Candida parapsilosis (ATCC 22019).

MIC of AE and EO were determined by macrodilution technique as described by the National Committee for Clinical Laboratory Standards. The bacteria inoculum was prepared in 4 ml triptic soy broth medium and incubated at 37°C, over night. The yeast culture was grown in Sabouraud Dextrose Broth medium at 28°C for 24 hrs before being used. The cultures were grown nutrient agar medium and then the inocula of microbial strains were prepared from 12 hrs old culture and suspensions were adjusted to 0.5 McFarland Standard Turbidity (~ 10^8 for bacteria and ~ 10^3 for yeast colony forming unit (CFU) per mililiter).

Ampiciline and fluconazole antibiotics were used as positive reference standards for bacteria and fungal strains, respectively (Table 2). These standards inhibited microorganisms at all concentration more than 0.48 µl/mL. Final concentration of aqueous extracts (50 µg/ml) were direct used for the test. EO of Salvia heldreichiana, Ballota saxatilis subsp. brachyodonta were dissolved at 2 µg/ml with 25% dimethyl sulfoxide (DMSO) and finally diluted concentration of master was 0.02 µl/mL. The extract’s serial two-fold dilution were performed in a concentration range from 0.098 µg/ml to 50 µg/ml in tubes containing Mueller-Hinton broth medium. The results were obtained according to visible turbidity.

Results

In the world, unbelievable agent and plants have discovered yet. The antimicrobial activity of our plants and standard antibiotics have been shown in Table 2. Traditionally, Salvia heldreichiana and Ballota saxatilis subsp. brachyodonta are used to treat many diseases. According to the Table 2, the AE of Ballota saxatilis subsp. brachyodonta showed lower antimicrobial activity than the AE of Salvia heldreichiana. However, the antimicrobial effect of EO of Salvia heldreichiana was considerable low on microorganisms.

The AE of Ballota saxatilis subsp. brachyodonta showed lower antimicrobial activity than its EO. Although there was no effect of Ballota saxatilis...
heldreichiana were found to be more susceptible to the Salvia (MIC: 25 µg/ml) species. Staphylococcus aureus, Klebsiella pneumoniae was active against Gram negative bacteria except for Enterococcus faecalis (MIC: 50 µg/ml). The EO of Salvia heldreichiana had effect over some of the tested microorganisms; Klebsiella pneumoniae (MIC: 50 µg/ml), Salmonella thphimurium (MIC: 50 µg/ml), Candida parapsilosis (MIC: 50 µg/ml), Candida albicans (MIC: 50 µg/ml) where as it has not antibacterial effect on the other tested microorganisms Escherichia coli and Gram positive bacteria such as Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis.

### Discussion

In the present study, the aqueous extracts and the essential oils of two endemic Lamiaceae species were extracted and screened their antimicrobial properties. It was shown that Salvia heldreichiana have strong antimicrobial effect on E. coli as disc diffusion method by Akin et al.\textsuperscript{16}, whereas Salvia heldreichiana EO was not active against E. coli in our study. The differences of biological activities may be due to different antibacterial procedure and ecological factor of plants. Staphylococcus aureus had similar susceptible to the Salvia heldreichiana EO in reference to disc diffusion method\textsuperscript{16} and macrodilution method in our study. We demonstrated EO of Salvia heldreichiana was not a strong antimicrobial agent. However, showing the antimicrobial effect on some microorganisms of AE of Salvia heldreichiana affirmed using in preventive and curative treatments.

The presence of merely antimicrobial potency of Ballota saxatilis subsp. brachyodonta EO may be due to content of essential oil or flavonoids. Further investigations on EO and AE compounds of Ballota saxatilis subsp. brachyodonta could be attributed to solve the antimicrobial mechanism. This is the first study to demonstrate that the antimicrobial effect of

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ballota saxatilis subsp. brachyodonta AE</th>
<th>Salvia heldreichiana AE</th>
<th>Amp/Flu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli g(-)</td>
<td>&gt;50 50</td>
<td>&gt;50 50</td>
<td>&lt;0.48/-</td>
</tr>
<tr>
<td>Klebsiella pneumoniae g(-)</td>
<td>&gt;50 50</td>
<td>50 50</td>
<td>&lt;0.48/-</td>
</tr>
<tr>
<td>Salmonella thphimurium g(-)</td>
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<td>&gt;50 50</td>
<td>&lt;0.48/-</td>
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<tr>
<td>Enterococcus faecalis g(+)</td>
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<td>25 &gt;50</td>
<td>&lt;0.48/-</td>
</tr>
<tr>
<td>Staphylococcus aureus g(+)</td>
<td>&gt;50 50</td>
<td>12.5 &gt;50</td>
<td>&lt;0.48/-</td>
</tr>
<tr>
<td>Staphylococcus epidermidis g(+)</td>
<td>&gt;50 25</td>
<td>25 &gt;50</td>
<td>&lt;0.48/-</td>
</tr>
<tr>
<td>Bacillus subtilis g(+)</td>
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<td>Candida albicans</td>
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</tr>
<tr>
<td>Candida parapsilosis</td>
<td>0.78 50</td>
<td>&gt;50 50</td>
<td>-/0.48</td>
</tr>
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subsp.brachyodonta AE on the Gram negative bacteria, S. heldreichiana AE was active against Kebssiea pneumoniae (MIC: 50 µg/ml). The Ballota saxatilis subsp. brachyodonta AE was considerable active against Candida parapsilosis, MIC: 0.78 µg/ml. Ballota saxatilis subsp.brachyodonta AE did not show any activity to the other test microorganisms such as Escherichia coli, Enterococcus feacalis, Bacillus subtilis, Salmonella thphimurium, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Candida albicans.

All microorganisms except for Bacillus subtilis demonstrated some degree of sensitivity to EO of Ballota saxatilis subsp. brachyodonta. C. albicans and C. parapsilosis had similar susceptible to the Ballota saxatilis subsp.brachyodonta EO at 12.5 µg/ml concentration. EO of Ballota saxatilis subsp. brachyodonta was showed activity against to Staphylococcus epidermidis (MIC: 25 µg/ml), Candida parapsilosis (MIC: 25 µg/ml), Candida albicans (MIC: 25 µg/ml), Escherichia coli (MIC: 50 µg/ml), Klebsiella pneumoniae (MIC: 50 µg/ml), Salmonella thphimurium (MIC: 50 µg/ml), Staphylococcus aureus MIC: 50 µg/ml), Enterococcus faecalis (MIC: 50 µg/ml).

As shown in Table 2, the AE of Salvia heldreichiana have better antimicrobial activity against Gram positive bacteria than the EO of its, whereas The AE of Salvia heldreichiana was not active against Gram negative bacteria except for Klebsiella pneumoniae (MIC: 50 µg/ml) and yeast. Staphylococcus aureus (MIC: 12.5 µg/ml), Enterococcus faecalis (MIC: 25 µg/ml), Staphylococcus epidermidis (MIC: 25 µg/ml) and Bacillus subtilis (MIC: 50 µg/ml) were found to be more susceptible to the Salvia heldreichiana AE.
aqueous extracts and essential oils of Ballota saxatilis subsp. bachyodonta and Salvia heldreichiana have been researched. Hence, further studies should be carried out in order to confirm and present findings, working with a broader range of microbial species and extracts such as methanol, ethanol, hexane. We may suggest these plants can be used medicine for the treatment of infectious diseases and as new sources for pharmacological studies.

It is important to evaluate the biological activity of members of Ballota and Salvia due to the fact that well-known the using in the traditional medical. Our findings are preliminary scientific validation for the traditional using of Ballota saxatilis subsp. bachyodonta and Salvia heldreichiana for the antimicrobial activity. To sustainable use of such plants and awareness of local communities should be enhanced incorporating the traditional using with scientific findings.

**Conclusion**

We reported for the first time the antimicrobial activity of the essential oil and aqueous extracts of Ballota saxatilis subsp. bachyodonta and Salvia heldreichiana endemic plants, growing naturally in Turkey. Datas on cytotoxic activity in our study could be explain the importance of volatile compounds and aqueous extract of those plants as possible medical plants in the development of new antimicrobial products.

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