Antimicrobial Activity of Endemic *Ziziphora taurica* subsp. *cleonioides* (Boiss) P. H. Davis Essential Oil

Endemik *Ziziphora taurica* subsp. *cleonioides* (Boiss) P. H. Davis Bitkisinin Uçucu Yağının Antimikrobiyel Aktivitesi

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Abstract

The aim of the present study is to investigate the antimicrobial activity of the essential oil prepared from aerial parts of the Turkish endemic *Ziziphora taurica* subsp. *cleonioides* (Boiss) P. H. Davis (Lamiaceae). The dried-in-shade at ambient temperature aerial parts of the plant were hydro-distilled for 3 h, using a modified Clevenger-type apparatus. In vitro antibacterial studies were carried out against eight bacteria strains and *Candida albicans*. The antimicrobial activity of the essential oil was carried out with the disc diffusion method. The results indicate that the essential oil remarkably inhibited the growth of tested microorganisms except *Candida albicans*.

Key Words: *Ziziphora*, essential oil, antimicrobial activity, pulegone.

Introduction

Plants have been used as bioactive compound resources for centuries. Turkish Flora has a great potential on biodiversity and medicinal plants with more than 10,000 taxa and 30 % of endemicism. The six different taxa of five species of the genus *Ziziphora* L. (Lamiaceae) are wildly spread in the Turkish Flora and one of them is endemic. Annual *Z. taurica* Bieb. with 5-35 cm height is well known for its strong aromatic scent, with reddish-violet, lilac or white colored flowers. There are two subspecies; *Z. taurica* M. Bieb. subsp. *taurica* J.R. Edmondson and endemic *Z. taurica* M. Bieb. subsp. *cleonioides* (Boiss) P.H. Davis. Both of them are distributed throughout the Western Anatolian Region (Davis, 1982). Some of *Ziziphora* species are used for their aperitif, carminative and antiseptic effects in treatment of various diseases in Anatolian folk medicine. Especially infusions of *Z. taurica* subsp. *cleonioides* named as “Filiskin otu” and *Z. taurica* subsp *taurica* named as “Nane ruhu”

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(Mint spirit) have been used internally, to treat gastrointestinal symptoms like stomach pains and also Z. taurica subsp. taurica has been used externally for its wound healing effect (Başer et. al., 1992; Baytop, 1999).

The essential oils have been respectively used in history in the pharmaceutical, food and perfume industries because of their antibacterial, culinary and fragrant properties (Salehi et. al., 2005). It has been known that terpenic compounds of essential oils such as camphor and 1,8-cineole showed the strongest antimicrobial activity (Tzakou et. al., 2001; Carson et. al., 1995). The antimicrobial activity of borneol has also been previously reported (Tabanca et. al., 2001). Similarly, pulegone has been reported as having antibacterial and antifungal activity (Duru et. al., 2004). There are some reports on the antibacterial and antifungal activities of pulegone rich plants (Tepe et. al., 2005; Gata-Gonçalves et. al., 2003; Oumzil et. al., 2002; Goren et. al., 2002). Besides, antiviral activity of a pulegone rich plant has been studied (Primo et. al., 2001). There are only a few papers on the biological activities of Ziziphus species. Salehi et al. have published the antibacterial and antioxidant activities of the essential oil of Z. clinopodioides subsp. rigida with 45.8 % pulegone content (Salehi et. al., 2005). Methanolic extract of the Z. tenuior has exhibited significant antimicrobial effect on Morganella morganii and Candida albicans (Bazzaz et. al., 2003). Konyalioglu et al. have also established the antioxidant enzyme activities of endemic Z. taurica subsp. cleonioides and Z. taurica subsp. taurica essential oils depending on LPO and GSH levels in human erythrocytes (Konyalioglu et. al., 2006).

All Ziziphus species, except Z. capitata, are known to contain essential oils in Turkish Flora. (Baser et. al., 1992). Chemical compositions of the essential oils of Z. taurica subsp. taurica and Z. taurica subsp. cleonioides have been studied with GLC (Sezik et. al., 1986; Sezik et. al. 1988; Sezik et. al. 1990). Ozel et al. also studied the essential oil composition of Z. taurica subsp. taurica with GC and GC-TOFMS (Ozel et. al., 2005). Characterisation of Z.taurica subsp. Clinopodioides’ essential oil has exhibited the main component to be pulegone (33.0 %) (Schulz et. al., 2005). The exact compositions of the essential oils of all Turkish Ziziphus taxa have been identified and compared with GC and GC-MS. The variable % of the major components of the essential oils of Z. taurica subsp. cleonioides from three different localities, have been reported as pulegone (69.24-81.86 %), piperitenone (1.28-6.47 %) and limonene (3.59-4.48 %) (Baser et. al., 1992; Konyalioglu et. al., 2006, Elgin Meral et. al., 2002).

The aim of the present study is to investigate the antimicrobial activity of the essential oil prepared from endemic Z. taurica subsp. cleonioides which is traditionally used as a folk medicine and dietary supplement in Anatolia.
Materials and Methods

Plant Material: Aerial parts of fresh plant materials were collected at the beginning of the flowering season, from the Gume Mountain at an altitude of 800 m., Tire, Izmir, Turkey [Z. taurica Bieb. subsp. cleonoides (Boiss) P. H. Davis]. The plants were identified at IZEF Herbarium (http://www.izef.ege.edu.tr), Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Izmir, Turkey and the voucher specimens were deposited at IZEF Herbarium (IZEF No: 5752).

Isolation of the Essential Oil: The dried-in-shade at ambient temperature aerial parts of the plant were hydro-distilled for 3 h, using a modified Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and stored at 4°C before the tests.

Test Microorganisms: In vitro antibacterial studies were carried out against eight bacteria strains (Staphylococcus aureus ATCC 6538/P, Staphylococcus epidermidis ATCC 12228, Enterococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 27853, Enterobacter cloacae ATCC 13047, Escherichia coli ATCC 29998, Proteus vulgaris ATCC 6897, Salmonella thyphimurium CMM 5445) and Candida albicans ATCC 10239. All microorganism cultures were obtained from ATCC, American Type Culture Collection, USA and Microbiology Department Culture Collection of Ege University, Faculty of Science, Izmir, Turkey.

The bacteria strains were inoculated on nutrient broth (Oxoid) and incubated for 24 h at 37±0.1°C, while the yeast strain was inoculated on malt extract broth (Oxoid) and incubated at 30°C for 48 h. Adequate amounts of Muller Hinton Agar (Oxoid) and Malt Extract Agar were dispensed into disposable sterile plates, and allowed to solidify under aseptic conditions. The counts of bacteria strains and yeast strain were adjusted to yield approximately 1.0x10^7-1.0x10^8 ml^-1 and 1.0x10^4-1.0x10^6 ml^-1, respectively. Test organisms (0.1 ml) were inoculated with a sterile swab on the surface of appropriate solid medium in plates. The plates containing the bacteria and fungus cultures were incubated for 1 h at 37°C except the E. cloacae ATCC 13047 and C. albicans ATCC 10239 which were incubated for 1 h at 30°C.

Evaluation of Antimicrobial Activity: The antimicrobial activity of the essential oil was carried out with the disc diffusion method (Collins and Lyne, 1989; Bradshaw, 1992; NCCLS, 1997).
Briefly, sterile, 6 mm diameter filter paper discs (Schleicher & Schül, Nr 2668, Germany) were impregnated with 20-30 μl of the essential oil and dried in an oven at 50±1°C. The agar plates inoculated with the test organisms were incubated for 1 hour before placing the oil impregnated discs on the plates. Then, the sterile discs impregnated with the oil was placed on the agar plates. The plates containing the bacteria and fungus cultures were incubated for 24 h at 37 °C except the E. cloaca ATCC 13047 and C. albicans ATCC 10239 which were incubated for 24 h at 30 °C. Sulbactam/Ampicillin discs (10/10 μg/disc, Oxoid), Amoxicilin discs (20 μg/disc, Oxoid) and Ketokanazole discs (20μg/disc) were used as positive controls. After incubation, all plates were observed for zones of growth inhibition, and the diameter of the zones were measured in millimeters. All tests were performed in triplicate and repeated three times. The results were expressed as average values.

Results and Discussion

Antimicrobial activity results are shown in Table 1. The essential oil of aerial parts of Z. taurica subsp. cleoniioides has a broad spectrum of antimicrobial activity. Although this essential oil has remarkably inhibited the growth of all tested bacteria including medically important pathogen Staphylococcus aureus ATCC 6538/P (inhibition zone is 15 mm), it has not inhibited the growth of Candida albicans ATCC 10239. Our results are consistent with the other antibacterial study results of Ziziphus species and other pulegone rich plants. However, it has been reported that the essential oils of pulegone rich plants such as Micromeria silicica and Mentha suaveolens inhibited Candida albicans.

Essential oil components are probably responsible for the antimicrobial activity of plants. It should be considered that minor components as well as possible interactions between the substances could also affect the microbiological properties of essential oils in some metabolic ways. In this study, antimicrobial activity of the essential oil was related to the presence of high amount of pulegone and low amount of minor components such as piperitenone and limonene which constitute 88.64 % of the essential oil (Cimanga et al., 2002). These results may partially support the use of this medicinal plant as a traditional remedy for wound treatment.

In conclusion, the essential oil of the aerial parts of Z. taurica subsp. cleoniioides, remarkably inhibited the growth of all tested gram positive and gram negative bacteria but not the fungus
Candida albicans. The essential oil with a composition of (+)-pulegone (81.86%), limonene (4.48%) and piperitenone (2.30%) and its observed antibacterial properties show that the essential oil could be evaluated in the pharmaceutical industry as a possible new pulegone resource.

Table 1. Antimicrobial activity of Zizipora taurica subsp. cleonioides essential oil.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Z. taurica subsp. cleonioides</th>
<th>Standards</th>
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<tbody>
<tr>
<td>Staphylococcus aureus ATCC 6538/P</td>
<td>15</td>
<td>19 17 NT</td>
</tr>
<tr>
<td>Staphylococcus epidermidis ATCC 12228</td>
<td>12</td>
<td>18 13 NT</td>
</tr>
<tr>
<td>Escheria coli ATCC 29998</td>
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<td>12 12 NT</td>
</tr>
<tr>
<td>Pseudomonas aeroginosa ATCC 27853</td>
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<td>10 10 NT</td>
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<td>Enterococcus faecalis ATCC 29212</td>
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<td>8 8 NT</td>
</tr>
<tr>
<td>Enterobacter cloacae ATCC 13047</td>
<td>11</td>
<td>19 15 NT</td>
</tr>
<tr>
<td>Proteus vulgaris ATCC 6897</td>
<td>18</td>
<td>17 14 NT</td>
</tr>
<tr>
<td>Salmonella typhimurium CCM 5445</td>
<td>12</td>
<td>18 16 NT</td>
</tr>
<tr>
<td>Candida albicans ATCC 10239</td>
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<td>NT NT 34</td>
</tr>
</tbody>
</table>

A₁: Sulbactam/Ampicillin (10/10μl/disc)  
A₂: Amoxicillin (20 μl/disc)  
A₃: Ketoconazole (20 μl/disc)  
NA: No activity  
NT: Not tested

Özet

Acknowledgements

This study was designed and performed at Ege University, Center for Drug R&D and Pharmacokinetic Applications Center. The authors would like to thank to Prof. Dr. Isik Tuglular, Prof. Dr. Ulvi Zeybek for their valuable support.

References


*Received: 20.02.2006
Accepted: 28.04.2006*