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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 Antimikrobiyal 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 endemism. 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 aperitife, carminative and antiseptic effects in treatment of various diseases in Anatolian folk medicine. Esp cially 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 *Ziziphora* 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 *Ziziphora* 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 *Ziziphora* 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. *cleonioides* (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 30^0C 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 $1.0\text{x}10^7$-$1.0\text{x}10^8$ ml^{-1} and $1.0\text{x}10^5$-$1.0\text{x}10^6$ 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. cloacae* 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. *cleonioides* 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 *Ziziphora* 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. *cleonioides*, 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 *Ziziphora taurica* subsp. *cleonioides* essential oil.

Microorganisms	*Z. taurica* subsp. *cleonioides*	Standarts		
		A_1	A_2	A_3
Staphylococcus aureus ATCC 6538/P	15	19	17	NT
Staphylococcus epidermidis ATCC 12228	12	18	13	NT
Escheria coli ATCC 29998	12	12	12	NT
Pseudomonas aeroginosa ATCC 27853	9	10	10	NT
Enterococcus faecalis ATCC 29212	8	8	8	NT
Enterobacter cloacae ATTC 13047	11	19	15	NT
Proteus vulgaris ATCC 6897	18	17	14	NT
Salmonella typhimurium CCM 5445	12	18	16	NT
Candida albicans ATCC 10239	NA	NT	NT	34

A_1: Sulbactam/Ampicillin (10/10µl/disc)
A_2: Amoxicillin (20 µl/disc)
A_3: Ketoconazole (20 µl/disc)
NA: No activity
NT: Not tested

Özet

Bu çalışmanın amacı Türkiye endemiği *Ziziphora taurica* subsp. *cleonioides* (Boiss) P. H. Davis (Lamiaceae) bitkisinin topraküstü kısımlarından hazırlanan uçucu yağın antimikrobiyal etkisinin araştırılmasıdır.
Bitkinin, gölgede kurutulmuş topraküstü kısımları, Clevenger apareyinde 3 saat süreyle hidrodistilasyona tabi tutulmuştur. In vitro antimikrobiyal aktivite sekiz bakteri cinsi ve *Candida albicans* üzerinde ve disk difüzyon metoduyla gerçekleştirilmiştir.
Sonuçlar, *Z. taurica* subsp. *cleonioides* uçucu yağının *Candida albicans* hariç denenen tüm bakteri suşları üzerine belirgin inhibe edici etkisi olduğunu göstermiştir.

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