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The Antimicrobial Activities of Methanolic Extracts of Some *Lamiaceae* Members Collected from Turkey

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Abstract

In this study, the antimicrobial activities of methanolic extracts of *Satureja hortensis* L., *Stachys woronowii* (Schischkin ex Grossh.) R. Mill, *Ziziphora clinopodioides* Lam. *Origanum vulgare* L. subsp. *gracile* (C. Koch) Letswaart and *Sideritis montana* L. subsp. *montana* growing as naturally in Turkey were investigated. The disk diffusion methods have been used to indicate the antimicrobial activities of these extracts on the test microorganisms as follows: *Pseudomonas aeruginosa* DSM 50071, *Klebsiella pneumonia* FMC 5, *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Epidermophyton* sp. and *Trichophyton* sp. Results indicated that plant extracts inhibited the growth of tested microorganisms (9-20 mm) in the different ratio, However some plant extracts had no effect against to tested microorganisms.

Key words: Antimicrobial activity, Disk diffusion methods, Medicinal plants, Pathogen microorganisms

Türkiye'den Toplanan Bazı *Lamiaceae* Üyelerinin Metanolik Ekstraktlarının Antimikrobiyal Aktiviteleri

Özet

Bu çalışmada; Türkiyede doğal olarak yetişen *Satureja hortensis* L., *Stachys woronowii* (Schischkin ex Grossh.) R. Mill, *Ziziphora clinopodioides* Lam., *Origanum vulgare* L. subsp. *gracile* (C. Koch) Letswaart, *Sideritis montana* L. subsp. *montana*'nın antimikrobiyal aktiviteleri araştırılmıştır. Bu bitki ekstraktlarının *Pseudomonas aeruginosa* DSM 50071, *Klebsiella pneumonia* FMC 5, *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Trichophyton* sp. and *Epidermophyton* sp.'ye karşı antimikrobiyal aktiviteleri belirtmek için disk difüzyon metodu kullanıldı. Sonuçlar bitki ekstraktlarının denenen mikroorganizmaların gelişmelerini farklı oranlarda engellediğini göstermiştir (9-20 mm). Fakat bazı bitki ekstraktlarının denenen mikroorganizmalara karşı etkisi olmadığı belirlendi.

Anahtar Kelimeler: Antimikrobiyal aktivite, Tıbbi bitkiler, Disk diffizyon metodu, Patojen mikroorganizmalar

1.Introduction

Many plant species among the flora of Turkey play an important role in traditional and conventional medicine. Based on the literature, there are approximately 9000 plant species in Turkeys flora, of which more than 500 are widely used in folkloric medicine due to their antimicrobial and anticarcinogenic properties [1]. Turkish people have a tradition of using a number of plant species for the treatment of infectious diseases and various ailments [2].

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking
for new leads to develop beter drugs against microbial infections [3]. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant - derived drugs is mainly due to the current

widespread belief that ‘’green medicine’’ is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects [4]. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants [5].

The effects of different plants extracts on the pathogens microorganism have been numerious studies of researchers [6-15].

These plants used in this study, are used widely because of different substantial features. For instance; the dried leaves of *Satureja hortensis* are used for diuretic, gastric, sudorific, appetizer effects. Species of some *Stachys* and *Siteridis* are used for diuretic, gastric effects. *Ziziphora clinopodioides* is an edible medicinal plant, which is used as antiseptic, appetizer. Further more, *Origanum vulgare* is traditionally used as diuretic, sudorific, sedative [16].

The purpose of this study is to evaluate the potential antimicrobial activities of *S. hortensis*, *S. woronowii*, *Z. clinopodioides*, *O. vulgare* subsp. *gracile*, *S. montana* subsp. *montana* on the some bacteria, yeasts and dermatophyta.

2.Materials and Methods

2.1. Plant materials and extraction procedure

S. hortensis, *S. woronowii*, *Z. clinopodioides*, *O. vulgare* subsp. *gracile* and *S. montana* subsp. *montana* were collected from Elazig province in the Eastern Anatolia of Turkey. The taxonomic identification of plant materials were determined using by Flora of Turkey [17]. The collected plant materials were dried and powdered under steril conditions. Each of the dried and powdered plant materials (20g) was extracted in 400 mL methanol (98.1 %) solvent by keeping on a rotary shaker (100 rpm) for 24h. The aqueous extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuom at 37°C using a Rotary evaporator. The extracts were dissolved in methanol and stored at 4°C for further studies. Then, 20 mg extracts were injected into empty antibiotic paper discs having a diameter of 6 mm (Schleicher&Shüll No: 2668, Germany). Discs injected with methanol served as negative controls.

2.1.2. Test microorganisms

A total 4 bacteria (*Pseudomonas aeruginosa* DSM 50071, *Klebsiella pneumoniae* FMC 5, *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32), 2 yeasts (*Candida albicans* FMC 17, *Candida glabrata* ATCC 66032) and 2 dermatophyte species (*Trichophyton* sp., *Epidermophyton* sp.) were used in the present investigation. Microorganisms were provided from the Department of Biology, Faculty of Science and Arts, Firat University, Microbiology Laboratory, Elazig-Turkey.

2. 1.3. Antimicrobial activity

Antimicrobial tests were carried out by disc diffusion method using 100 µL of suspension containing 10^6 cells / mL of bacteria, 10^4 cells / mL yeast and cells / mL dermatophyta fungi as per McFarland standard, inoculated into Nutrient Broth (Difco), Malt Extract Broth (Difco), and Sabouroud Dextrose Broth (Oxoid), respectively. The discs (6 mm diameter) were impregnated with 400 µL placed on the inoculated mueller hinton agar (Difco), malt extract agar (Difco) and Glukoz Sabouroud agar (Oxoid), respectively. Steril petri dishes (9cm diameter) were placed at 4 °C for 2h. Then, the inoculated plates were incubated at 37±0.1°C at 24 h for bacterial strains and also at 25±0.1°C at 72 h for yeast and dermatophyta fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms [18]. The experimental studies were replicated in three times.

3. Results and Discussions

The antimicrobial activities of plant extracts, negative control group and standart antibiotics have been showed in Table 1. It has been found that the methanol extracts of *S. hortensis*, *S. woronowii*, *Z. clinopodioides*, *O. vulgare* L. subsp. *gracile*, *S. montana* subsp. *montana* have antibacterial and antifungal activity to the microorganism tested and it seems that the antimicrobial activity of those

plants extract are changeable as seen in Table 1 and also some plants extracts have a higher activity usually as to comparison antibiotics.

The extracts of *S. hortensis* have antimicrobial activity on the tested microorganisms except for *C. glabrata,* from high to low respectivelly; *K. pneumoniae* (20 mm), *Epidermophyton sp.* (20 mm), *P. aeruginosa* (18 mm), *B. megaterium* (16 mm), *S. aureus* (15 mm), *C.albicans* (15 mm), *Trichophyton sp.* (14 mm) (showed in Table 1).

Table 1 shows that, The extracts of *S. woronowii* have activity on *P. aeruginosa, K. pneumoniae*, *S. aureus*, *B. megaterium*, *C. albicans, Epidermophyton* sp. and *Trichophyton sp.* (9 mm, 15mm,11 mm, 12 mm, 13 mm, 9 mm *and* 9 mm inhibition zone respectivelly) while it has not antimicrobial activity on *C. glabrata*. In the previous study was demonstrated that the essential oils from diverse species of *Stachys* showed better activity against bacterial species than fungi and also none of the essential oils was active against *Pseudomonas aeruginosa* [19].

It can be seen in the Table 1, The extracts of *Z. clinopodioides* did not show any antimicrobial activity against to *P. aeruginosa, C. albicans and C. glabrata*, However; it has been found that showed different antimicrobial activity on the some microorganisms from high to low; *K. pneumoniae* (17 mm), *B. megaterium* (15 mm), *S. aureus* (13 mm), *Epidermophyton* sp. (13 mm) and *Trichophyton sp.* (12 mm) respectivelly. The previous study supports our results. The essential oil and methanol extract obtained from aerial parts of *Z. clinopodioides* were evaluated for their chemical composition and antibacterial activity against Gram (+) and Gram (-) bacteria. Maximum activity of essential oil (>22mm) and methanol extract (>11mm) was observed against *Acidovorax facilis*, *Bacillus xexus*, *Bacillus* spp, *Bacillus sphaericus*, *Brevibacillus brevis*, *Corynebacterium ammoniagenes*, *Enterobacter sakazakii*, *Erwinia carotovora* subsp. *carotovora*, *Moraxella catarrhalis* and *Xanthomonas arboricola* as reported by Ozturk and Ercisli [10].

The extract of *O. vulgare* have less antimicrobial activity against *S. aureus* (9 mm), *B. megaterium* (9 mm), whereas more active against *K. pneumoniae* and *Epidermophyton* sp. (17 mm, 11 mm inhibition zone respectivelly). This plant extract did not show any activity to the other tested microorganisms such as *P. aeruginosa, C. albicans, C. glabrata* and *Trichophyton* sp. Essential oils from aerial parts of *O. vulgare* were showed activity against to *Staphylococcus aureus, Salmonella choleraesuis*, *Bacillus subtilis, Micrococcus luteus* (Schroeter) and *Enterococcus faecium* (registered at ATCC as *Streptococcus faecium*), while did not observed any activity to *Pseudomonas aeruginosa*, *Enterococcus faecium S. epidermidis*, *Rhodococcus equi*, *Escherichia coli* and *Candida albicans* [13].

The extract of *S. montana* strongly has effect over some of the tested microorganisms; *S. aureus* (8 mm), *B. megaterium* (11 mm), *C. albicans* (13 mm), where as it has not antibacterial and antifungal effect on the other tested microorganisms: *P. aeruginosa, K. pneumoniae, C. glabrata*, *Epidermophyton* sp. and *Trichophyton* sp. In the previous study was supported that the extracts of *S. ozturkii* and *S. caesarea* belongs to *Lamiaceae* could be used as natural antimicrobial and antioxidant agents in the food preservation and treatment of some diseases for human health [20].

As shown in Table 1, the control disks injected with 80 µL of methyl alcohol showed no inhibitory effect against the test microorganisms.

The antimicrobial activity of plant extracts are changeable acording to the other researchers findings [6-15, 19,20]. These differnces be due to the genetic structure of plant species, ecological factors, biochemical constituents of plant extract, extraction solvents and tested microorganisms.

This study indicated that there are differences in the antimicrobial effects of plant species, due to the phytochemical differences between species. To better evaluate the plants growing naturally in Turkey that are potentially useful resources, additional studies

are necessary from both the medicinal and economic stand points. The differential activities of these plant extracts supported developing a new broad spectrum of antimicrobial drugs in future.

In the end of studies, we have found the extract of *S. hortensis*, *S. woronowii*, *Z. clinopodioides*, *O. vulgare* L. subsp. *gracile* and *S. montana* subsp. *montana* revealed antimicrobial activities against to bacteria, yeasts and dermatophyta.

However; there was no effect of this extracts on the some bacteria, yeasts and dermatophyta.

The results in the study suggest that plant extracts may be possess some of the compounds with antibacterial and antifungal properties that can be used traditional medicine for the treatment of infectious diseases.

Table 1. Antimicrobial activities of methanolic extracts of some *Lamiaceae* members collected from Turkey

Materials	**S. h**	**S.w**	**Z.c**	**O.v**	**S.m**	**Control**	**Standart**
Microorganisms	**İnhibition zone (mm)**						
P. aeruginosa	18	9	-	-	-		11**
K.pneumoniae	20	15	17	15	-	-	9**
S. aureus	15	11	13	9	8	-	13**
B. megaterium	16	12	15	9	11	-	9**
C.albicans	15	13	-	-	13	-	18*
C. glabrata	-	-	-	-	-	-	12*
Epidermophyton sp.	20	9	13	11	-	-	NT
Trichophyton sp.	14	9	12	-	-	-	NT

S.h : *S. hortensis*, **S. w:** *S. woronowii*, **Z. c:** *Z. clinopodioides*, **O. v:** *O. vulgare*, **S. m:** *S. montana*, *:Nystatin (30 µg/disc), **: Streptomycin sulphate (10 µg /disc), Control: methanol NT: not tested

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