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Antimicrobial Effect of *Sideritis öztürkii* Aytaç & Aksoy Species

Sideritis öztürkii Aytaç & Aksoy Türünün Antimikrobiyal Etkisi

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ABSTRACT

Introduction: *As an alternative to antibiotics, medical plants have been utilized and some plants are being used as antimicrobial agents. It is aimed in this study to extract the kind Sideritis öztürkii Aytaç & Aksoy, which is endemic to Konya Province, and to determine antimicrobial effects of the kind by use of liquid microdilution technique.*

Materials and Methods: *The plant material was collected from its natural habitat. The antimicrobial effect of the extract against standard isolates such as Escherichia coli ATCC 25922, E. coli ATCC 35218, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213 and Enterococcus faecalis ATCC 29212 was investigated by liquid microdilution method, and minimum inhibitory concentrations (MIC) were determined. Since the highest antimicrobial effect of the extract was detected against S. aureus ATCC 29213, the antimicrobial effect against S. aureus isolates isolated from clinical specimens in Selcuk University Medical Faculty Microbiology Laboratory was also investigated by the same method.*

Results: *The leaf extract of Sideritis öztürkii was more effective than the flower extract against standart isolates. In particular, it had the highest antimicrobial activity against S. aureus ATCC 29213 strain. The application of leaf extract to 111 clinical isolates of S. aureus revealed that the highest antimicrobial effect was observed in 1.56 mg/mL and 3.125 mg/mL.*

Conclusion: *It is thought that different antimicrobial agents may be obtained from endemic plant species such as Sideritis öztürkii Aytaç & Aksoy. It is thought that plant extracts can be used as antimicrobial agents that are more effective in the fight against infections, natural, environment friendly, economic and health-free.*

Key Words: *Antimicrobial; Plant extract; Staphylococcus aureus; Sideritis*

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ÖZ

Sideritis öztürkii Aytaç & Aksoy Türünün Antimikrobiyal Etkisi

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Giriş: *Antibiyotiklere alternatif olarak tıbbi bitkilerden yararlanılma yoluna gidilmiştir ve bazı tıbbi bitkiler günümüzde antimikrobiyal ajan olarak kullanılmaktadır. Bu çalışmada Konya ili çevresinde endemik olan Sideritis öztürkii Aytaç & Aksoy türünün ekstraktının çıkarılması ve sıvı mikrodilüsyon yöntemi ile antimikrobiyal etkisinin belirlenmesi amaçlanmıştır.*

Materyal ve Metod: *Bitki materyali doğal yaşam alanlarından toplandı. Daha sonra elde edilen ekstraktın Escherichia coli ATCC 25922, E. coli ATCC 35218, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213 ve Enterococcus faecalis ATCC 29212 standart suşları üzerine antimikrobiyal etkisi sıvı mikrodilüsyon yöntemiyle araştırıldı ve minimum inhibitör konsantrasyonları (MİK) belirlendi. Ekstraktın en yüksek antimikrobiyal etkisi S. aureus ATCC 29213 suşunda tespit edildiği için Selçuk Üniversitesi Tıp Fakültesi Mikrobiyoloji Laboratuvarında klinik örneklerden izole edilen S. aureus suşlarına antimikrobiyal etkisi de aynı yöntemle araştırıldı.*

Bulgular: *Sideritis öztürkii'nin yaprak ekstraktının, çiçek ekstraktından standart suşlar üzerine daha etkili olduğu saptanmıştır. Özellikle S. aureus ATCC 29213 suşu üzerinde en yüksek antimikrobiyal etkiye sahip olduğu tespit edilmiştir. Yaprak ekstraktının 111 klinik S. aureus suşunda elde edilen verilerde en yüksek antimikrobiyal etki görülen dozların 1.56 mg/mL ve 3.125 mg/mL olduğu tespit edilmiştir.*

Sonuç: *Sideritis öztürkii Aytaç & Aksoy gibi endemik bitki türlerinden farklı antimikrobiyal ajanlar elde edilebileceği düşünülmektedir. Bitki ekstrelerinin, infeksiyonlarla mücadelede daha etkili, doğal, çevre dostu, ekonomik ve sağlık açısından herhangi bir risk taşımayan, antimikrobiyal ajanlar olarak kullanılabileceği düşünülmektedir.*

Anahtar Kelimeler: *Sideritis; Antimikrobiyal; Bitki özütü; Staphylococcus aureus*

INTRODUCTION

Throughout the history of humanity, plants have been used in the treatment of diseases. New species of these plants still continue to be used in traditional treatment[1]. In recent years, especially after the 1980s, synthetic (chemical) drugs have begun to take the place of herbal medicines as a result of the rapid progress of the chemical industry. However, due to the emergence of many side effects of these synthetic drugs and resistance problem, people have turned to prefer treatment with herbal remedies[2,3].

The active ingredients of medicinal plants and their usage areas are very common. The demand for natural products increases every day[4]. As a single plant contains a wide range of phytochemical substances, the effects of using a whole plant as medicine are unclear. Phytochemical content, pharmacological effects and safety of the plants, which are thought to have medical potential, should be determined by rigorous biological studies. Although there were hundreds of applications for new drug status during the period 1999-2012 in the United States of America, only two herbal remedies were approved by the Food and Drug Administration due to the fact that they have medical value[5].

Approximately one-fourth of the medicines given to patients in modern medicine are produced from medical plants and tested rigorously[6]. In developed countries, it is believed that the use natural health products and herbal medicines with herbal origin beneficial for health has increased due to the side effects and costs of drugs of chemical origin. However, despite the trust of people in herbal medicines, toxicity and side effects are not detected precisely[7].

Only 15% of all flowering plants have been chemically and pharmacologically investigated[8]. This ratio is extremely low when considering all

flowering plant species on earth. This shows us that there are still too many plants to be researched from the medical point of view, and they are a great source for the discovery of new drugs. In the light of this information, it is seen that our country has an important working potential in the field of medicinal plants[9].

In general, almost half of the deaths in tropical countries are caused by infections. This has made it necessary to develop new methods in the prevention and treatment of infectious diseases. Therefore, pharmacologists and microbiologists in particular have begun to resort to more medicinal plants in the search for new antimicrobial agents[10]. In time, microorganisms gain resistance against drugs and transfer them to new members. As a result, the use of antibiotics is limited. In this way, medical plants are an important source for microbiologists in the search for new antimicrobial agents.

Sideritis öztürkii Aytac & Aksoy species, are seen in the rugged rocky regions of the forests of Kizildag (Derebucak, located three kilometers north of the Camlik district of Konya). It is a type of Sideritis (L) whose body is 40-90 cm in size, simple or slightly branched, sometimes covered with feathers, with purplish white flowers and usually blooms in July-August[11]. In this study, antimicrobial activity of *Sideritis öztürkii* Aytac & Aksoy species' leaves and flower extracts on some standard bacteria isolates was investigated by broth microdilution test.

MATERIALS and METHODS

This research was conducted with the approval of the Local Ethics Committee of Selcuk University Faculty of Medicine (Date: 07.09.2016; Number of decisions: 2016/13).

Plant Collection and Drying

The endemic *Sideritis öztürkii*, which is used in this study, was collected from the 1450-1700 m high mountainous region located three kilometers north of Camlik district of Konya in July 2016. Species definition of the plant samples was made in Selcuk University Faculty of Science, Department of Botany. The plants collected were dried and stored in a room with no light. At the end of two months, the flowers and leaves were completely separated from each other. Separated parts of the plant were pulverized separately in mechanical mill and extracted after sieving.

Preparation of Plant Extracts

In this study, Soxhlet device was used for the extraction process. The following steps were applied in the process[12].

1. The powdered leaves and flower parts of the plant were kept in the pasteur oven at 110°C for five hours and their essential oils were removed.
2. 30 grams of each part of the leaves and flowers of the plant were weighed each time placed in the extraction arm of the soxhlet device.
3. 200 mL of methanol was added to the glass flask part of the device as solvent.
4. The extraction was continued until the active substance was obtained for 6-8 hours.
5. The extracts obtained at the end of the process were kept in the refrigerator at +4°C overnight.
6. The extracts were removed by evaporation at 40°C in a Rotary Evaporator apparatus to remove the solvent.
7. Raw extracts obtained were stored at -20°C to prevent any loss of activity until use.

Determination of Antimicrobial Effects of Extracts

Standard bacterial isolates were used to investigate the antibacterial effects of the extracts obtained from *Sideritis öztürkii* Aytac & Aksoy (*Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213).

In this study, the first stock solution of the extracts obtained from *Sideritis öztürkii* Aytac & Aksoy was determined as 25 mg/mL and serial dilution was applied. Active substance concentrations in microplate wells as a result of serial dilution were as follows: The first well was 6.25 mg/mL, the second 3.125 mg/mL, the third 1.56 mg/mL,

the fourth 0.78 mg/mL, the fifth 0.39 mg/mL, the sixth 0.19 mg/mL, the seventh 0.097 mg/mL, the eighth 0.048 mg/mL, the ninth 0.024 mg/mL and the tenth 0.012 mg/mL.

The antimicrobial effect of the extracts on standard isolates was investigated by broth microdilution method. As a result of this procedure, the first well without visible growth was accepted as minimum inhibitor concentration (MIC) value.

It was determined that the leaf extract of *Sideritis öztürkii* Aytac & Aksoy had a higher antimicrobial effect than the flower extract. Since the leaf extract showed the highest antimicrobial effect against *S. aureus* ATCC 29213 strain, the antimicrobial effect of the extract on 111 *S. aureus* clinical isolates was performed in the same manner as described above by the broth microdilution method.

RESULTS

Effect of the extracts obtained from the leaves and flower parts of the *Sideritis öztürkii* Aytac & Aksoy species on standard bacterial isolates by liquid microdilution method are shown in Table 1.

According to the findings, it was determined that the leaf extract of *Sideritis öztürkii* Aytac & Aksoy species showed an antimicrobial effect against *S. aureus* ATCC 29213 standard strain at a concentration of 0.39 mg/mL while the extract of flowers showed an antimicrobial effect at a concentration of 3.125 mg/mL but did not show any antimicrobial effect against other used bacteria isolates. *Sideritis öztürkii*'s leaf extract was found to have higher antimicrobial activity on *S. aureus* ATCC 29213 than other standard bacterial isolates.

According to these results, the antimicrobial effect of leaf extract of *Sideritis öztürkii* Aytac & Aksoy species on *S. aureus* clinical isolates was investigated. 111 *S. aureus* isolate that have been sent to Selcuk University Medical Faculty, Medical Microbiology Laboratory from various clinics was studied by liquid microdilution method. 111 clinical *S. aureus* isolates were included in the study (36 wounds, 24 bloods, 13 drainage fluids, 12 bronchoalveolar lavages, six sputums, six abscess, four urines, four CSFs, two tissue biopsies, two pleural fluids, one peritoneal fluid and one vaginal discharge). The clinical samples included into the study were mostly from internal diseases polycilinics, orthopedics and traumatology service, pediatric health and diseases service and neurology service.

The highest susceptibility of *S. aureus* clinical isolates was found to be vancomycin, teicoplanin, tigecycline, linezolid and mupirocin, while the highest resistance was found to be against penicillin, tetracycline and oxacillin, respectively. Sensitivity and resistance rates of *S. aureus* clinical isolates determined by disc diffusion method are shown in Table 2.

The MIC values of the extracts of *Sideritis öztürkii* were found to be 1.56 mg/mL in 46 of *S. aureus* clinical isolates, 3.125 mg/mL in 43, 0.78 mg/mL in 12, 0.39 mg/mL in five and > 6.25 mg/mL in five. As a result of these findings, the lowest MIC of *Sideritis öztürkii* Aytac & Aksoy species on the *S. aureus* strain was found to be 0.39 mg/mL and the highest MIC value was 6.25 mg/mL.

Table 1. The minimum inhibitor concentration (MIC) values of the leaves and flower extracts against standard bacterial isolates

Microorganisms	MIC values of leaf extract	MIC values of flower extract
Escherichia coli ATCC 25922	> 6.25 mg/mL	> 6.25 mg/mL
Escherichia coli ATCC 35218	> 6.25 mg/mL	> 6.25 mg/mL
Pseudomonas aeruginosa ATCC 27853	3.125 mg/mL	> 6.25 mg/mL
Enterococcus faecalis ATCC 29212	3.125 mg/mL	> 6.25 mg/mL
Klebsiella pneumoniae ATCC 700603	3.125 mg/mL	> 6.25 mg/mL
Staphylococcus aureus ATCC 29213	0.39 mg/mL	3.125 mg/mL

Table 2. Sensitivity and resistance rates of antibiotics on *Staphylococcus aureus* clinical isolates

Antibiotics	S	I	R	Sensitivity (%)	Resistance (%)
Erythromycin	85	1	25	76.6	23.4
Clindamycin	105	-	6	94.6	5.4
Fucidic acid	96	4	3	93.2	6.8
Gentamicin	100	-	11	90.1	9.9
Levofloxacin	92	5	14	82.9	17.1
Linezolid	110	-	1	99.1	0.9
Moxifloxacin	96	-	7	93.2	6.8
Mupirocin	98	-	1	98.1	1.9
Oxacillin	75	-	36	67.6	32.4
Penicillin	10	-	101	9.0	91
Cefoxitin	75	-	36	67.6	32.4
Tetracycline	72	1	38	64.9	35.1
Trimethoprim-sulfamethoxazole	105	-	5	94.6	5.4
Vancomycin	111	-	-	100	-
Teicoplanin	111	-	-	100	-
Tigecycline	111	-	-	100	-

S: Sensitive, I: Intermediate, R: Resistance.

DISCUSSION

Antibiotics have a very important place in the fight against diseases that develop due to microorganisms. However, unconscious and overuse of antibiotics can lead to secondary infections and cause serious problems in treatment. For this reason, new antimicrobial agents should be investigated in order to prevent bacterial resistance problems and reduce the side effects and high costs of the antibiotics used[13]. However, due to the problems and side effects associated with the use of synthetic drugs, the interest in medicines with herbal origin is increasing again[14-16].

In many studies, antimicrobial compounds in plants have been found to be more effective against bacterial growth and resistant to bacterial isolates, unlike synthetic antimicrobial agents currently used. For this reason, studies on medicinal plants in the search for new antimicrobial agent have been revived[17].

Copuroglu et al. have examined the antimicrobial effect of the leaves and flowers of *Sideritis phlomoides* plant growing endemic in Nigde region by disk diffusion and microdilution methods and determined that the extracts have similar antimicrobial effect[18]. *Sideritis phlomoides* used in the study has shown that the highest antimicrobial effect against *Proteus mirabilis* (Pasteur Institute 235) strain. At the end of the study, the extracts obtained in 2, 3 and 4 hours with ethanol formed the inhibition of 7.7 mm, 6.7 mm, 7.13 mm on the isolates *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, respectively.

Ayaz has investigated the antimicrobial activity of acetone, ethanol and chloroform extracts of *Sideritis libanotica Labill.* subsp. *violascens* (P.H.Davis) P.H.Davis and *Sideritis hololeuca* Boiss. & Heldr. Apud Bentham species on eight gram-positive (*Bacillus cereus* ATCC 14579, *Bacillus subtilis* B RSHMB, *Listeria monocytogenes* tip 2 NCTC 5348, *Micrococcus luteus* LA 2971, *S. aureus* ATCC 29740, *Streptococcus mutans* NCTC 10449, *Streptococcus pneumoniae* ATCC 6305 and *Streptococcus pyogenes* ATCC 19615) and six gram-negative bacterial isolates (*E. coli* ATCC 35218, *K. pneumoniae* ATCC 10031, P. mirabilis ATCC 15146, *Proteus vulgaris* ATCC 7829, *P. aeruginosa* ATCC 15442 ve *Salmonella enteritidis*) by disk diffusion method. At the end

of the study, none of the extracts has shown antimicrobial activity against *B. cereus*, *S. aureus*, *S. pneumoniae*, *E. coli*, *P. aeruginosa* and *S. enteritidis*. It was determined that at least one extract of both *Sideritis L.* species had 7-8 mm inhibition zone against other bacterial isolates used in the study and had weak antibacterial activity which can be called insignificant[19].

Dulger et al. have investigated antimicrobial effects of *Sideritis (L.)* species of *S. albiflora*, *S. brevibracteata* and *S. pisidica* on *E. coli* ATCC 11230, *S. aureus* ATCC 6538P, *K. pneumoniae* UC57, *M. luteus* La 2971, *Micrococcus flavus* ATCC 14452, *P. vulgaris* ATCC 8427, *P. aeruginosa* ATCC 27853, *Corynebacterium xerosis* CCM 7064, *Mycobacterium smegmatis* CCM 2067, *B. subtilis* ATCC 6633, *B. cereus* ATCC 9730, *Kluyveromyces fragilis* NRRL 24115 and *Rhodotorula rubra* CCY by disk diffusion and microdilution method. *S. pisidica*'s methanol extract has been shown to demonstrate high antimicrobial activity against *M. flavus* ATCC 14452, *P. vulgaris* ATCC 8427, *P. aeruginosa* ATCC 27853, *C. xerosis* CCM 7064, *M. smegmatis* CCM 2067, *B. subtilis* ATCC 6633, *B. cereus* ATCC 9730, *K. fragilis* NRRL 2415 and *R. rubra* CCY bacteria and yeasts with growth inhibition site diameters in the range of 10-20 mm and MIC values of 0.03-0.38 g/mL[20].

Sagdic et al. have examined the biological activity of extracts of two *Sideritis* species endemic in Turkey, including the plant which we used in our study. Antimicrobial activities were evaluated by agar diffusion method against 15 microorganisms (*Aeromonas hydrophila* ATCC 7965, *Bacillus brevis* FMC 3, *B. cereus* FMC 19, *B. subtilis* ATCC 6630, *B. subtilis* var. niger ATCC 10, *E. coli* ATCC 25922, *K. pneumoniae* FMC 5, *Morganella morganii*, *M. smegmatis* RUT, *P. mirabilis* BC 3624, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *Yersinia enterocolitica* ATCC 1501, *C. albicans* ATCC 1223 ve *S. cerevisiae* BC 5461). It has been determined that *Sideritis öztürkii* Aytac & Aksoy and *Sideritis caesarea* Duman, Aytac et Baser extracts can be used as natural antimicrobials for human health and for protection of foods[21].

In our study, the effect of methanol extracts obtained from the leaves and flowers of the endemic *Sideritis öztürkii* Aytac & Aksoy species on standard isolates such as *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 was investigated by broth microdilution method. As a result of the study, it was found that the flower extract had a weak antimicrobial effect on *S. aureus* ATCC 29213 strain and no antimicrobial effect on other isolates. Leaf extract had no antimicrobial effect on other bacterial isolates which had antibacterial effects on *S. aureus* ATCC 29213 strain.

In accordance with this result, the antimicrobial effect of *Sideritis öztürkii* Aytaç & Aksoy leaf extract, which has the highest antimicrobial effect on *S. aureus* strain, was studied with liquid microdilution method. It was determined that 46 of 111 clinical isolates of *S. aureus*, which was applied leaf extract of *Sideritis öztürkii* Aytac & Aksoy species, had MICs of 1.56 mg/mL and 43 had 3.125 mg/mL. These results mean that the leaf extract of *Sideritis öztürkii* Aytac & Aksoy species showed antimicrobial activity.

Researches show that plants showing antimicrobial activity may be an alternative to synthetic antibiotics[22-24]. Due to the fact that microorganism species have been mutated and gained resistance to existing antimicrobial agents, it will be useful to detect, produce and investigate such plants grown in nature in order to contribute to the pharmaceutical industry in our country. For this reason, further investigation of the antimicrobial effect should be done in more detail and in depth with the study of *Sideritis öztürkii* Aytac & Aksoy. Also, phenolic substance, cytotoxic effect, antimutagenic effect of this species should be examined. In addition, it is thought that different antimicrobial agents can be obtained from endemic plant species such as *Sideritis öztürkii* Aytac & Aksoy. Therefore, it can be suggested that these new agents can be used more effectively, more naturally, economically, environmentally friendly and without any risk in terms of health, and have a lower rate of adverse effects as antimicrobial agents, especially against resistant isolates.

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CONFLICT of INTEREST

Authors have no competing interests to disclose.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: EG, SM, HD, HTD

Analysis/Interpretation: EG, SM, HD, HTD

Data Acquisition: EG, HTD

Writting: EG, SM, HD, HTD

Critical Revision: EG, SM, HD, HTD

Final Approval: EG, SM, HD, HTD

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