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Antibacterial Activity of *Stachys sylvatica* Against Some Human Eye Pathogens

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Abstract

The ethanol extracts obtained from the leaves of *Stachys sylvatica* L. (Lamiaceae) were evaluated for their antibacterial activity against some human eye pathogens by disc diffusion method against hospital isolates of *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus capitis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Moraxella nonliquefaciens* and *Cutibacterium acnes*. Besides, Penicillin, Ampicillin and Tobramycin as antibacterial antibiotics were used as a positive reference standard to determine the sensitivity of the isolates. The extracts showed a potential antibacterial effect against *Staphylococcus capitis*, *Moraxella nonliquefaciens* and *Cutibacterium acnes*, with inhibition zones of 22.4 mm, 21.6 mm and 20.2 mm, respectively. The extracts also exhibited a moderate effect against the other bacterial isolates. The plant may be useful in the treatment of eye infections.

Keywords:
Stachys sylvatica, antibacterial activity, eye pathogens

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Introduction

The genus *Stachys* L. (Lamiaceae) is represented by 93 species and 57 of the 118 taxa are endemic in Turkey. This genus is distributed all over the world, especially in warm temperate regions of the Mediterranean and Southwestern Asia, North America, South Amerika and Southern Africa (Bhattacharjee, 1982; Davis et al., 1988; Duman, 2000).

Plants of this genus have been used for the treatment of cold, cough, diarrhea, urinary system disorders, hypertension, headache, throat pain and as an antipyretic or stomachic in folk medicine (Altundag & Ozturk; 2011; Mukemre et al., 2015). The infusion prepared from the aerial

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parts of *S. sylvatica* is used against cardiac disorders in Turkey (Polat et al., 2015). Stachys species are also used for the problem of skin and for the treatment of wounds at veterinary medicine (Manganelli et al., 2001; Viegi et al., 2003; Cornaro et al., 2014).

During routine excursions, it was determined that Stachys sylvatica is used for the treatment of wounds and as eye disinfections. Hence, here the purpose was to determine the antibacterial effects of the ethanolic leaf extract of *S. sylvatica* collected from Turkey, which is used by the local people to cure some illness.

Materials and Method

The Plant Material

The plant material was collected from Sinekli Yaylası, Duzce, Turkey in July and August 2019. Voucher specimens (GD 121-2) of the plant were deposited in the Department of Medical Biology of Duzce University in the author's personal collection.

Preparation of Extract

The leaves of the plant were dried in an oven at 40 ℃ (12 hrs) and powdered. Each dry powdered plant material (20 g) was extracted with 150 mL of 95 % ethanol (Merck, Darmstadt, Germany) for 24 hrs using Soxhlet equipment. The extract was filtered using Whatman filter paper Nº 1 and the filtrate solvent was evaporated under vacuum using a rotary evaporator at 55 ℃ (yield 12.4 % for ethanol). The resulting dried extract was stored in labelled sterile screw-capped bottles at -20 ℃. The extract (in the form of sticky black substances) was dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 1 g/mL for preliminary screening.

Microorganisms

Eye infected pathogens (*Escherichia coli*, *Enterobacter aerogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus capitis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Moraxella nonliquefaciens* and *Cutibacterium acnes*) used in this study were kindly provided from Research Hospital of Medical Faculty of Canakkale Onsekiz Mart University, Turkey, and from Research Hospital of Medical Faculty of Duzce University, Duzce, Turkey.

Disc Diffusion Method

The paper disc diffusion method was employed (Collins et al., 1995). Sterile 6 mm disc filter paper disc (Schleicher and Schull, Nº 2668, Dassel, Germany) were impregnated with 50 µL of the plant extracts. The bacterial cultures were inoculated on Nutrient Broth (Oxoid) and incubated for 24 h at 37±0,1 ℃. The development of Moraxella nonliquefaciens and Cutibacterium acnes were used %5-10 CO2 incubator. Adequate amounts of Mueller Hinton Agar (Oxoid) were dispensed into

sterile plates and allowed to solidify under aseptic conditions. The counts of bacterial cultures were adjusted to yield ca. 1.0×107-1.0×108 mL^{-1}, using the standard McFarland counting method. The test microorganisms (0,1 mL) were inoculated with a sterile swab on the surface of appropriate solid medium in plates. The agar plates inoculated with the test microorganisms were incubated for 1 h before placing the extract impregnated paper disc on the plates. The bacterial plates were incubated at 37±0,1 ℃ for 24 h. After incubation all plates were observed for zones of growth inhibition and the parameters of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times. Penicillin (10 µg/ disc), tobramycin (10 µg/ disc) and ampicillin (20 µg/ disc) were used as positive controls.

Results and Discussion

The significant antibacterial activity of *Stachys sylvatica* extract and the standard antibiotics assessed by inhibition zones are given in Table 1. The ethanolic extracts of the plant shown potential antibacterial effects against the eye pathogens, with inhibition zones at 12.8-22.4 mm. Notably, *Staphylococcus capitis*, *Moraxella nonliquefaciens* and *Cutibacterium acnes* are more susceptible to the extract with inhibition zones at 22.4, 21.6 and 20.2 mm, respectively, as compared to the standard antibacterial agents such as penicillin, ampicillin and tobramycin. Similarly, the extracts obtained from the plant leaf showed strong antibacterial effects on *Staphylococcus aureus* than those of the standard antibacterial antibiotics as tobramycin. In addition, the extracts against the other bacterial cultures are far below than those of the standard antibacterial antibiotics used in this study.

Table 1. Antibacterial activity of Stachys sylvatica against some human eye pathogens

Pathogens	**Diameter of zone of inhibition (mm)***			
	EtOH extract of plant (50 mg/mL)	**Standard Antibiotics**		
		P (10 µg/disc)	**AMP (20 µg/disc)**	**TOB (10 µg/disc)**
Bacillus cereus	12.8	16.2	14.2	17.2
Pseudomonas aeruginosa	13.2	NT	NT	15.4
Klebsiella pneumoniae	14.2	NT	NT	16.4
Staphylococcus capitis	22.4	16.2	20.4	18.2
Staphylococcus aureus	14.8	22.4	16.2	14.2
Staphylococcus epidermidis	14.8	15.8	18.4	15.4
Moraxella nonliquefaciens	21.6	16.8	20.2	18.2
Cutibacterium acnes	20.2	14.8	16.2	18.2

* Zone of inhibition, including the diameter of the filter disc (6.00 mm); mean value of three independent experiments;NT: Not Tried.
P: Penicillin, AMP: Ampicillin, TOB: Tobramycin

Studies of antimicrobial activity of *Stachys* species are abundant. Furthermore, there is only one report on the antimicrobial activity of *Stachys sylvatic*a. A study on composition and antibacterial activity of the essential oil of six *Stachys* species from Serbia was reported. The oils obtained from six *Stachys* species (including *S. sylvatica*) were tested against three Gram-positive bacteria (*S.aureus* ATCC 6538, *Bacillus cereus* (clinical isolates) and *Micrococcus flavus* ATCC 10240) and three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 2783, *Proteus mirabilis* (clinical isolates) and *Escherichia coli* ATCC 35218) by microdilution assay (Grujic-Jovanovic et al., 2004). The essential oil of *S. sylvatica* proved to be moderate active in that study against the tested bacterial cultures.

In previous study the oil composition of inflorescences and leaves of *Stachys sylvatica* L. from Italy was analysed by GC-MS (Trillini et al., 2004). The most abundant compounds in the oil from the inflorescences were germacrene D (55.2 %), (E)-β-farnesene (9.1 %) and n-tetracosane (6.9 %), whilst germacrene D (31.7 %), n-tetracosane (7.8 %) and mint sulphide (6.4 %) were the main compounds in the oil from the leaves.

Besides, in the literature γ-muurolene, phytol and benzaldehyde were mentioned to be main compounds in the leaf and flower oils of *S. sylvatica* from Bulgaria (Dimitrova-Duyulgerova et al., 2015). In another study, limonene, α- cedrene and γ-muurolene was found as the main constituents of *S. sylvatica* grown in Turkey (Renda et al., 2017).

In conclusion, these compounds given in above may be responsible for their antibacterial activity. The ethanolic extract of *S. sylvatica* has a strong antibacterial effect against the tested bacterial cultures, which may explain why this plant is used in folk medicine to treat eye infections.

Author Contributions

All author contributions are equal for the preparation research in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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