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Research Article

ANTIBACTERIAL ACTIVITY OF ENDEMIC *BALLOTA NIGRA* SUBSP. *ANATOLICA* AGAINST SOME HUMAN EYE PATHOGENS FROM TURKEY

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ABSTRACT

Objective: The ethanol extracts obtained from the leaves of *Ballota nigra* subsp. a*natolica* P.H. Davis (Lamiaceae) were evaluated for their antibacterial activity against some human eye pathogens.

Methods: Hospital isolates of *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus capitis*, *S. aureus*, *Staphylococcus epidermidis*, *Moraxella nonliquefaciens*, and *Propionibacterium acnes* were obtained. Antibacterial activity was determined in the extracts using disc diffusion method. Some antibacterial antibiotics were used as a positive reference standard to determine the sensitivity of the isolates.

Results: The extracts showed strong antibacterial activity against *M. nonliquefaciens* and *P. acnes*, with inhibition zones of 18.2 mm and 19.2 mm, respectively. The extracts also exhibited moderate activity against the other isolates.

Conclusion: The results demonstrate that the leaf extract of the plant has significant activity and suggest that it may be useful in the treatment of eye infections.

Keywords: *Ballota nigra* subsp. *anatolica*, Antibacterial activity, Eye pathogens.

INTRODUCTION

The species of *Ballota* L. are known for their therapeutic uses against various diseases and have been in Turkish folk medicine as antiulcer, antispasmodic, diuretic, choleretic, antihemorrhoidal, and sedative agents [1,2].

Ballota nigra L. is used externally in the treatment of wounds and burns. Aerial parts of some subspecies of *B. nigra* are used internally to treat inflammation, to suppress cough, and against gastrointestinal disorders [3,4]. It is reported that *B. nigra* subsp. *anatolica* has antidepressant activity [1].

B. nigra subsp. *anatolica* P.H. Davis (Lamiaceae) is endemic to Turkey [5]. In literature scanning, it is determined that some investigations have been done on antimicrobial activity of *Ballota* species against eye infections. Hence, here the purpose was to determine the antibacterial effects of the ethanolic leaf extract of *B. nigra* subsp. *anatolica* collected from Turkey, which is used by the local people to cure some illness.

METHODS

The plant material was collected from Bolu-Seben Road, Turkey in July and August 2016. Voucher specimens (GD 109-5) 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°C (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°C (yield 14.24% for ethanol). The resulting dried extract was stored in labeled sterile screw-capped bottles at −20°C. The extract (in the form of sticky black substances) amounting to around 2 g was dissolved in 0.1 mL of dimethyl sulfoxide (5 mg/mL) before testing.

Microorganisms

Eye infected pathogens (*Escherichia coli*, *Enterobacter aerogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus capitis*, *S. aureus*, *Staphylococcus epidermidis*, *Moraxella nonliquefaciens*, and *Propionibacterium acnes*) used in this study were kindly provided research hospital of medical faculty of Canakkale Onsekiz Mart University, Turkey, from Trakya University, Edirne, Turkey and Duzce University, Duzce, Turkey.

Disc diffusion method

The paper disc diffusion method was employed [6]. Sterile 6 mm disc filter paper disc (Schleicher and Schul, 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 hrs at 37±0.1°C. 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 1.0×10^{7}-1.0×10^{8} 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 hr before placing the extract impregnated paper disc on the plates. The bacterial plates were incubated at 37±0.1°C for 24 hrs. 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 disc (10 µg/disc), and ampicillin (20 µg/disc) were used as positive controls.

RESULTS

The significant antibacterial activity of *B. nigra* subsp. *anatolica* extract and the standard antibiotics assessed by inhibition zones are given in Table 1.

The ethanolic extracts of the plant were strong antimicrobial effects against the eye pathogens, with inhibition zones at 11.4-19.2 mm.

Table 1: Antibacterial activity of *B. nigra* subsp. *anatolica* against some human eye pathogens

Pathogens	EtOH extract (50 µg/mL)	Diamater of zone of inhibition (mm)*		
		Standards		
		P (10 µg/disc)	AMP (20 µg/disc)	TOB (10 µg/disc)
B. cereus	11.4	15.4	12.8	17.4
P. aeruginosa	12.8	11.2	12.2	13.6
K. pneumoniae	15.8	19.0	16.4	17.2
S. capitis	14.6	17.8	20.2	19.4
S. aureus	14.2	21.4	16.8	12.2
S. epidermidis	16.4	18.6	19.2	14.8
M. nonliquefaciens	18.2	15.2	17.4	16.4
P. acnes	19.2	16.2	14.8	18.8

B. cereus: Bacillus cereus, P. aeruginosa: Pseudomonas aeruginosa, K. pneumoniae: Klebsiella pneumoniae, K. pneumoniae: Klebsiella pneumoniae, S. capitis: Staphylococcus capitis, S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, M. nonliquefaciens: Moraxella nonliquefaciens, P. acnes: Propionibacterium acnes, *Zone of inhibition, including the diameter of the filter disc (6.0 mm); mean value of three independent experiments; P: Penicillin (10 µg/disc), AMP: Ampicillin (20 µg/disc), TOB: Tobramycin discs (10 µg/disc)

Notably, *M. nonliquefaciens* and *P. acnes* are more susceptible to the extract of the plant with inhibition zones at 18.2 and 19.2 mm, respectively, as compared to standard antibacterial antibiotics such as penicillin, ampicillin, and tobramycin. Similarly, the extracts showed higher antibacterial activity on *S. epidermidis* and *Staphylococcus aureus* than those of the standard antibacterial antibiotics as tobramycin. Besides, the extracts have more antibacterial effect than those of penicillin and ampicillin against *P. aeruginosa*. The extracts against *B. cereus*, *K. pneumoniae,* and *S. capitis* are far below than the standard antibacterial antibiotics.

DISCUSSION

Studies of antimicrobial activity on *Ballota* species are limited. Furthermore, there are some reports on the antimicrobial effects of *B. nigra* subsp. *anatolica*. In previous studies, the antimicrobial activity of some endemic *Ballota* species growing of Turkey was reported [7]. In another study, diterpenoids and flavonoids isolated from *Ballota inaequidens* are investigated for their activities against bacterial and the yeast cultures [8]. In this study, the compounds tested have no important inhibitory activity against bacteria but showed good activities against *Candida albicans* and *Candida krusei.* Furthermore, it is reported that three diterponoids from *Ballota saxatilis* subsp. *saxatilis* were investigated for their effects against some bacteria and *C. albicans* [9]. In addition, it is reported that essential oil of *Ballota pseudodictamnus* has been investigated for their antimicrobial activity against, and some bacteria, the yeast cultures using the dilution technique in a previous study [10]. In the above study, the chemical composition of the essential oil of *B. pseudodictamnus* obtained from the aerial parts was analyzed by gas chromatography-mass spectrometry. From the 52 identified constituents of the oil, caryophyllene oxide, phytol, and **γ**-muurolene were the major components. Essential oil of the plant exhibited strong to moderate activity against all tested bacteria (minimum inhibitory concentrations [MIC] values 0.45-10.15 mg/mL), while it appeared inactive against the tested fungi. In another study, it is reported that ethanol extracts obtained from *B. nigra* subsp. *anatolica* were investigated for their antimicrobial effect against many bacterial and yeast cultures by disc diffusion method and microdilution method [11]. The extracts have shown strong antibacterial activity against *E. coli* with inhibition zone of 13.0 mm with MICs and minimum bactericidal concentrations of 250 (500) µg/mL, respectively. The extracts also exhibited moderate activity against the other test microorganisms. Equally, in this study, all the extracts of *B. nigra* subsp. *anatolica* were presented antimicrobial activity against all the isolated eye pathogens, especially *M. nonliquefaciens* and *P. acnes* with inhibition zones of 18.2 and 19.2 mm, respectively. The differences between present results and the others may be due to several factors, for example, the intraspecific variability in the production of secondary metabolites.

Chaudhary *et al.* [12] reported that the extracts obtained from the leaves of *Ballota limbata* which are used to treat ophthalmia by the local people, determined their antimicrobial activity against *Bacillus subtilis* as the eye pathogens. In this study, *B. limbata* is evaluated as an alternative medicine for eye disease. In the present study, the ethanolic extracts of the plant have potential activity against the eye pathogens, especially *M. nonliquefaciens* and *P. acnes*. It is parallel to this reported in the mentioned study.

Flavonoids and also phenylpranoids have been reported to exist in some *Ballota* species such as *Ballota acetabulosa*, *Ballota foetida*, *Ballota hirsuda*, and *B. nigra* [13-15]. Flavonoids may be responsible for their antibacterial activity [16]. The result indicated that *B. nigra* subsp. *anatolica* possessed significant activity against the pathogens. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds.

CONCLUSION

The results clearly show that *Ballota* species are interesting source of biologically active compounds. In addition, *B. nigra* subsp. *anatolica* may be useful for therapy against eye infections. Further analyses are necessary to identify the main active constituents.

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