ISSN 1015 • 3918

ANKARA ÜNİVERSİTESİ
ECZACILIK FAKÜLTESİ DERGİSİ

JOURNAL OF FACULTY OF PHARMACY OF ANKARA UNIVERSITY

Cilt/Vol : 32
Sayı/No : 2
Yıl/Year: 2003

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(Ankara Ecz- Fak. Derg.)

Sahibi: Prof. Dr. Seçkin ÖZDEN
Editör : Prof. Dr. Feyyaz ONUR

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Ankara Üniversitesi Basımevi
2003

JOURNAL OF FACULTY OF PHARMACY OF ANKARA UNIVERSITY

(J'.*Fac.Pharm. Ankara)*

Published by Prof. Dr. Seçkin ÖZDEN
Editor : Prof. Dr. Feyyaz ONUR

Journal of Faculty of Pharmacy of Ankara University is an international medium for the publication of original research reports, reviews and short communications on relevant developments in pharmaceutical sciences. This journal is published quarterly. All the articles appeared in this journal are published on the responsibility of the author(s). The manuscript submitted to the journal should not be published previously as a whole or in part and not be submitted elsewhere. The manuscripts should be prepared in accordance with the requirements specified at the end of the issue.

This journal is indexed in Chemical Abstracts (CA), Excerpta Medica Database (EMB ASE), Medicinal Aromatic Plants Abstracts (MAPA) and Turkish Medical Index

Web address: www.pharmacy.ankara.edu.tr/journal

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Ankara Universitesi Basımevi
2003

İÇİNDEKİLER /CONTENTS

Ankara Ecz. Fak. Derg.
32 (2) 93-97, 2003

J- Fac. Pharm, Ankara
32 (2) 93-97, 2003

ANTIMICROBIAL ACTIVITY OF *BALLOTA* SPECIES GROWING IN TURKEY

TÜRKİYE'DE YETİŞEN *BALLOTA* TÜRLERİNİN ANTİMİKROBİYAL AKTİVİTESİ

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ABSTRACT

The antimicrobial activities of ethanol extracts of all Ballota species growing in Turkey were studied. The ethanolic extracts were tested in vitro against Gram-negative strains (Escherichia coli, Pseudomonas aeruginosa) and the Gram positive strains (Staphylococcus aureus, Bacillus subtilisj and the fungi (Candida albicans, Candida galabrata, Candida krusei) by the agar diffusion method. Among all Ballota species studied, Ballota inaequidens was particular^ active against Gram-positive, Gram-negative bacteria and fungi.

Keywords: Ballota, Lamiaceae, Antibacterial activity, Antifungal activity

ÖZET

Bu çalışmada Türkiye'de yetişen turn Ballota türlerinden hazırlanan etanollu ekstrelerin antimikrobiyal aktivitesi bazı Gram-negatif (Escherichia coli, Pseudomonas aeruginosa), Gram-pozitif bakteriler (Staphylococcus aureus, Bacillus subtilis) ile mantarlara (Candida albicans, Candida galabrata, Candida krusei) karşı agar difiizyon metodu ile ölçülmüştür. Bu türler arasında test edilen mikroorganizmalara karşı en yüksek aktiviteyi gösteren tür Ballota inaequidens 'tir.

*Anahtar **Kelimeler:** Ballota, Lamiaceae, Antibakteriyel aktivite, Antifungal aktivite*

INTRODUCTION

Ballota (L.) is represented by sixteen species in Turkey (1). These are *Ballota acetabulosa* (L.) Benth., *B. pseudodictamnus* (L.) Benth. subsp. *lycia* Hub.-Mor., *B. inaequidens* Hub.-Mor & Patzak, *B. cristata* P.H. Davis, *B. saxatilis* Sieber ex. J & C.Presl subsp. *saxatilis, B.saxatilis* Sieber ex. J & C.Presl subsp. *brachyodonta* (Boiss.) P.H. Davis & Doroszenko, *B. glandulosissima* Hub.-Mor & Patzak, *B. larendana* Boiss. & Heldr., *B. latibracteolata* P.H.Davis & Doroszenko, *B. rotundifolia* C. Koch, *B.macrodonta* Boiss. & Bal., *B. nigra* L. subsp. *nigra, B. nigra* L. subsp *anatolica* P.H. Davis, *B. nigra* L. subsp. *foetida* Hayek, *B. nigra* L. subsp. *uncinata* (Fiori & Beg.) Patzak and *B. nigra* L. subsp. *kurdica* P.H. Davis.

* Correspondence

Ballota species have been used in Turkish folk medicine as antiulcer, antispasmodic and sedative agent (2). *B. nigra* is used externally, in the treatment of wounds and burns. It is used internally to supress coughs and upper respiratory inflammation (3,4).

In our previous studies, three diterpenoids (hispanolone, ballonigrine, dehydrohispanolone) and ten flavonoids (kumatakenin, pakipodol, 5-hydroxy 7,3',4' trimethoxy flavone, velutin, corymbosin, 5-hydroxy 3,7,4'-trimethoxyflavone, retusine, 5-hydroxy 7,4'-dimethoxy flavone, filindulatin, ladanein) were isolated, chemically characterised and analysed by HPLC in different species of *Ballota* (2, 5, 6).

As a continuation of our research on the *Ballota* species (2,5) we investigated their antimicrobial properties.

MATERIAL AND METHODS

Plant material

Sixteen species of *Ballota* were collected from different parts of Turkey. Designation of the individuals and their origin are given in Table 1.

Table 1. The names and origins *of Ballota* species used in this study

B. acetabulosa	Bl izmir:Yenifoca,10m, 18.6.1998,AEF 21602
B. pseudodictamnus subsp. *lycia*	C2 Mugla:Fethiye, 20m, 12.6.1997 AEF 21603
B. cristata	C3 Isparta:Egridir, 910 m, 17.7.1997,AEF19899
B. inaequidens	C3 Antalya: Alanya,200m, 20.7.1997,AEF 19901
B. saxatilis subsp. *saxatilis*	C4 icel: Anamur, 1530m, 20.7.1997,AEF 19904
B. saxatilis subsp. *brachyodonta*	C4 İçel: Silifke, 1400 m, 3.7.1998, AEF 21505
B. glandulosissima	C3 Antalya:Kumluca, 500m, 19.7.1997,AEF 19900
B. larendana	A4 Ankara:Kızılcahamam, 830m, 28.6.1998, AEF 21604
B. latibracteolata	C3 Antalya: Gazipaşa, 425 m, 20.7.1997, AEF 19902
B. rotundifolia	A8 Erzurum: Tortum Lake, 1200 m, 1.9.1998, AEF 21606
B.macrodonta	B5 Kayseri:Yahyalı, 1150 m, 2.8.1997, AEF 19907
B. nigra subsp. *nigra*	A5 Sinop: Boyabat, 370 m, 9.10.1998, AEF 21607
B. nigra subsp. foetida	C2 Muğla: Dogiisbelen, 600m, 12.7.1999, AEF 21608
B. nigra subsp. *uncinata*	Bl İzmir: Gökçealan, 250 m, 19.6.1998, AEF 21607
B. nigra subsp. *anatolica*	B4 Ankara: Gölbaşı, 800 m, 28.6.1998, AEF 21601
B. nigra subsp. *kurdica*	We could't find this species at the mentioned localities

Extraction of plant materials

Sixteen samples of dried aerial parts of the plants (20 g each) were extracted with ethanol (150 ml) for 24 hours by using a soxhlet appratus, respectively (7).

Antimicrobial activity

All the extracts were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Shüll No. 2668, Germany) in the amount of 20 ul. Discs injected with pure ethanol served as negative control. Standard antibiotic discs such as ampicillin, cephazoline, gentamycine and fluconozole were used for positive control. *Escherichia coli* ATCC 23556, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Candida galabrata* (from patient) and *Candida krusei* ATCC 6258 were used in this investigation.

The disc-diffusion method (8) was used as a screening test for antimicrobial activity. The antimicrobial screening was performed using Mueller-Hinton Agar (Oxoid) for bacteria and Sabouraud Dextrose Agar (Oxoid) for yeast.

All the extracts were dissolved in 75% sterile ethanol to obtain 133 mg/ml extract concentration. These solutions were impregnated on sterile paper discs of 6 mm diameter (15 for per disc). Discs injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4°C for 2 hours and then incubated at 35±0.1 °C for 24 hours. At the end of the period, inhibition zones formed on the medium were measured with a transparent ruler in millimeters and compared with the reference drugs. These experiments were carried out in duplicate.

RESULTS AND DISCUSSION

The *in vitro* antimicrobial activities of the extracts of *Ballota* species are given in Table 2.

Table 2. Antimicrobial activities of the ethanolic extracts of *Ballota* species

	Diameter of Inhibition zone (mm)						
	E.coli	*P.aeruginosa*	*B.subtilis*	*S.aureus*	*C.albicans*	*C.galabrata*	*C.krusei*
B.acetabulosa	15	0	5	9	12	13	12
B.pseudodictamnus	0	6	0	9	0	17	16
B.cristata	0	0	7	0	14	17	18
B. inaec/uidens	12	12	17	12	18	20	23
B.saxatilis subsp.*saxatilis*	11	10	0	12	11	16	13
B.saxalilisssp.brachyodonta	0	10	0	11	8	13	11
B. glandulosissima	0	0	6	8	12	9	15
B. larendana	0	4	0	8	0	0	7
B. latibracteolata	8	17	7	10	13	15	17
B. rotundifolia	12	13	5	11	18	15	19
B.macrodonta	10	6	0	9	7	14	10
B.nigra ssp. *nigra*	0	6	6	7	11	12	7
B.nigra ssp. *anatolica*	9	6	0	12	13	14	15
B.nigra ssp. *uncinata*	11	11	0	10	16	6	15
B.nigra ssp.foetida	12	10	0	12	13	17	14
B.sechmenii	13	13	5	7	12	15	13

In addition, the inhibition zones formed by standard antibiotic discs (positive control) and the discs injected with only ethanol (negative control) are also given in Table 3.

Table 3.The inhibition zones formed by standard antibiotic discs and the disc injected with only ethanol

	Diameter of Inhibition zone (mm)						
	E.coli	*P.aeruginosa*	*B.subtilis*	*S.aureus*	*C.albicans*	*C.galabrata*	*C.krusei*
Ampicillin (25 ug)	-	-	-	22	-	-	-
Cephazoline(30 ug)	25	20	20	30	-	-	-
Gentamycin(IO ug)	20	23	30	20	-	-	-
flucouazole(25 ug)	-	-	-	-	20	26	20
control	-	-	-	-	-	-	-

(-) not tested

As can clearly be seen in Table 2, the extract of *B.inaequidens* have the greatest antimicrobial efficacy, followed by *B. saxatilis* subsp. *saxatilis* and *B. rotundifolia*. In our previous studies on *Ballota* species, we observed *B. inaequidens* and *B. saxatilis* subsp. *saxatilis* were rich flavonoid content (5,6).

We also previously reported three diterpenoids (hispanolone, ballonigrine, dehydrohispanolone) obtained from the aerial parts of *B.saxatilis* subsp. *saxatilis* and their effects against gram- positive (5. *aureus, S. faecalis)* and gram-negative *(P. aeruginosa, E. coli, K. pneumoniae)* microorganisms and *C. albicans.* All compounds were found effective against *C. albicans* in low concentrations and active bacteria (2).

In addition, previously we isolated some flavonoids and diterpenoids (5-hydroxy 3,7,4'-trimethoxyflavone, retusin, 5- hydroxy-7,4'-dimethoxyflavone, pachypodol, flindulatin, 5-hydroxy-7,3\4'-trimethoxyflavone, hispanolone, ballonigrine) from *B. inaequidens* and tested against *B. subtilis, S. aureus, E. coli, P. aeruginosa, C. albicans* and *C. krusei.* All the compounds tested had inhibitory activity against bacteria and showed good activities against C. *albicans* and *C. krusei* (9).

It is noteworthy to mentioned that the antifungal efficacy of the all extracts more than their antibacterial efficacy.

One of the undisputed functions of flavonoids is to play a role in protecting plants against microbial invasion. Thus, the antimicrobial activity was established to be mainly due to flavonoids (10, 11).

The results obtained herein is likely to shed light on the antimicrobial activity of all *Ballota* species grown in Turkey.

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Received: 10.04.2003

Accepted: 02.06.2003