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Antifungal Activity of *Ballota acetabulosa* Against Yeast *Candida* and *Cryptococcus* Species

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The ethanol extracts prepared from the leaves, rootstock and the combined formulation of *Ballota acetabulosa* (L.) Benth. have been investigated for their antifungal activities against medical yeast *Candida* species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803 and *C. guilliermondii* ATCC 6260) and *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* ATCC 34142) by the visual broth macrodilution method. All extracts exhibited a strong antifungal effect against the yeast cultures. The MIC values ranged from 3.12 to 25 mg/mL. Besides, extracts exhibited greater antifungal effect against *Candida* species than *Cryptococcus* species. It is therefore suggested that extracts could be used traditionally in the treatment of fungal infections especially against candidiasis.

Key Words: *Ballota acetabulosa*, Antifungal activity, Medicinal plants.

INTRODUCTION

Plants have long been in use as medicine all over the world. More recently, plant extracts have been developed and proposed for use as antimicrobials.

The genus *Ballota* L. (Lamiaceae) consists of about 33 species growing mainly in the Mediterranean region. In Turkey, the genus *Ballota* is represented by 11 species, 6 subspecies, 10 of which are endemic[1]. Plants of this genus have been used traditionally for nausea, vomiting, nervous dyspepsia, specifically for vomiting of central origin and also are used for antiemetic, sedative, antibacterial and mild astringent properties[2,3].

Ballota acetabulosa (L.) Benth. is a herbaceous plant growing in rocks and rough ground in dry hills up to 900 m in Greece and Western Anatolia[4]. During our field excursions, it was determined that these plants have been used externally in the treatment of wounds and burns. Aerial parts of the plant are used internally to treat inflammation, to suppress cough and against gastrointestinal disorders. So, the aim of this works is to evaluate the antimicrobial activity of the plant as wild-growing in Turkey.

EXPERIMENTAL

The plant material was collected from Gokceada, Canakkale, Turkey in September, 2009. Voucher specimens of the plant were deposited in the Biology Department of Canakkale Onsekiz Mart University, Canakkale, Turkey.

Preparation of extract: The plant parts were air-dried. Each dry powdered plant material (20 g) was soaked in the ethanol (50 % concentration) until complete saturation of the plant material. The extract was filtered using Whatman No. 1 and the filtrates were evaporated under reduced pressure and dried using a rotary evaporator at 55 ºC. Dried extracts were stored in labeled sterile screw-capped bottles at -20 ºC. The extract (in the form of sticky black substances) amounting *ca.* 2 g was dissolved in DMSO before testing. The combination of plant extracts (1:1 ratio) was used in this test[5]. *Candida* species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803 and *C. guilliermondii* ATCC 6260) and *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* ATCC 34142) as the test fungi were obtained from Microbiology Research Laboratory in Canakkale Onsekiz Mart University, Department of Biology, Turkey and pure cultures were maintained on Sabouraud Dextrose Agar (SDA) plates and Sabouraud Dextrose Broth (SDB) in tubes.

Minumum inhibitory concentration (MIC) determination: MICs were performed by the visual broth macrodilution method[6]. Fungal suspensions were diluted into RPMI-1640 medium without bicarbonate (pH 7.0 with 0.165 morpholine propane sulfonic acid) broth supplemented with glutamine, to a concentration of approximately 0.5×10^5 cfu/mL, verified by colony count in SDA. A two fold serial dilution of 0.2 mL each of extract was added to 1.8 mL of the RPMI-1640 medium. The concentration were 0.390-200 mg/mL. Controls with medium without antifungal samples were used in the test.

To compare the results with standard, ketoconazole was used. Tubes were defined as the lowest concentration which did not yield visual growth. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The MICs values concerning *in vitro* antifungal activities of the extracts are presented in Table-1. The MIC results for the ethanol extracts of leaf, rootstock and the combinations ranged from 3.12-25.00, 6.25-25.00 and 1.56-12.50 mg/mL, respectively and showed that susceptibility of the extracts varied from one fungal strain to another. Comparing the results with those of the antifungal agent ketoconazole used as a reference, it was noted that the combination of the plant extracts (both leaves and rootstock) was stronger antifungal activity than the others. *Candida tropicalis* with the MIC value 1.56 mg/mL is more susceptible than other fungi, followed by *Candida albicans*, *C. guilliermondii* and *Cyrptococcus neoformans* with the same MIC value 3.12 mg/mL. *Candida glabrata* and *Cyryptococcus laurentii* have susceptible to the extract at MIC of 6.25 mg/mL. The highest MICs of the extract were 12.5 mg/mL against *Candida krusei* and *Candida parapsilosis*. Notably, as compared to standard antifungal antibiotic ketoconazole, the combine extracts have a strong antifungal effect against *Candida tropicalis* and *C. guilliermondii*.

TABLE-1
MINUMUM INHIBITORY CONCENTRATION OF THE ETHANOL LEAF AND ROOTSTOCK EXTRACTS

Microorganisms	Minimum inhibitory concentration (MIC)			
	Leaf (mg/mL)	Rootstock (mg/mL)	Leaf and rootstock (mg/mL)	Ketoconazole (µg/mL)
Candida albicans	6.25	12.50	3.12	0.25
Candida tropicalis	3.12	6.25	1.56	4.00
Candida guilliermondii	6.25	6.25	3.12	5.00
Candida krusei	25.00	25.00	12.50	4.00
Candida glabrata	12.50	25.00	6.25	2.00
Candida parapsilosis	25.00	25.00	12.50	2.00
Cryptococcus neoformans	6.25	12.50	3.12	0.25
Cryptococcus laurentii	12.50	25.00	6.25	4.00

In a previous study, ethanol was observed as the best solvent for extracting antimicrobial substances[7]. The result in this study with ethanol is similar to those reported in the mentioned study. It is important to keep in mind that the concentration of extract used in the test may be correlated with a high activity of its chemical components.

Fungi used in this study were chosen primarily on the basis of their importance as opportunistic pathogens of humans. According to findings from the National Infection Surveillance System (NNIS), 61 % of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cyrptococcus* spp.[8].

Ballota species have been used in Turkish folk medicine as antiulcer, antispasmodic, diuretic choleretic, antihaemorrhoidal and sedative agent[9,10]. *Ballota nigra* subsp. *anatolica* and *Ballota larendana* have antidepressant activity[9]. Another study reported that *Ballota acetabulosa* is used for the treatment of hemorrhoids as infusion in folk medicine[11]. The methanol extracts of *Ballota pseudodictamnus* and *Ballota acetabulosa* have been reported to have antioxidant activities[12]. The antimicrobial activities of ethanol extracts of 16 *Ballota* species growing in Turkey were studied. The ethanolic extracts were tested *in vitro* against gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*) and gram-positive strains (*Staphylococcus aureus*, *Bacillus subtilis*) and the yeast cultures (*Candida albicans*, *Candida glabrata*, *Candida krusei*) by the agar diffusion method. Among *Ballota* species studied, *Ballota acetobulosa* has a strong antibacterial activity against bacterial strains. In addition, the extracts have antifungal activity against *C. albicans*, *C. glabrata* and *C. krusei*, with inhibition zones varied from 12, 13 and 12 mm, respectively[13]. Besides, ethanol extracts of some *Ballota* species were tested against four different *Listeria* isolates (*Listeria monocytogenes*, *L. ivanovii*, *L. innocua* and *L. murrayi*) by the agar diffusion method. Among *Ballota* species studied, *Ballota acetabulosa* have a strong antilisterial effects against all *Listeria* species except for *L. innocua*[14].

Flavonoids and phenylpranoids have been reported to exist in some *Ballota* species such as *Ballota acetabulosa*, *B. foetida*, *B. hirsuta* and *B. nigra*[11,15-19]. Flavonoids may be responsible for their antibacterial activity[20]. The result indicated that *Ballota acetabulosa* possessed significant activity against both bacteria and yeast cultures. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds. So, this plant extracts should be analyzed further, as it might provide a new compound effective against pathogens.

Fungi used in this study were chosen primary on the basis of their importance as opportunistic pathogens of humans. Accordance of the findings from National Nosocomial Infection Surveillance System (NNIS), 61 % of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp.[21]. *Candida albicans*, while naturally occurring in the intestinal flora, can cause oral thrush and systematic infections. *Cryptococcus neoformans* causes cryptococcosis, an opportunistic infection of the lungs especially in AIDS patients.

This study provides data on the antifungal properties of the extract obtained from *B. acetabulosa* against some clinically relevant fungi such as *Candida* and *Cryptococcus* species that could be able to studies for therapeutically useful. These extracts may be applied clinically for fungal infections, especially against Cryptococcosis and Candidiasis.

REFERENCES

1. P.H. Davis, Flora of Turkey and the East Aegean islands, Vol. 7, Edinburgh University Press, Edinburgh, pp. 156-160 (1982).
2. G. Citoglu, M. Tanker, B. Sever, J. Englert, R. Anton and N. Altanlar, *Planta Med.*, **64**, 484 (1998).
3. C.A. Newall, L.A. Anderson and J.D. Philipson, Herbal Medicines, a Guide for Health-Care Professionals, The Pharmaceutical Press, London, p. 164 (1996).
4. S. Sahpaz, A.L. Skaltsounis and F. Bailleul, *Biochem. Syst. Ecol.*, **30**, 601 (2002).

5. H. Singh and I.B. Gulati, *Wear*, **139**, 425 (1990).
6. J.P. Dzoyem, J.G. Tangmouo, D. Lontsi, F.X. Etoa and P.J. Lohoue, *Phytother. Res*., **21**, 671 (2007).
7. S.G. Jonathan and I.O. Fasidi, *Afr. J. Biomed. Res*., **6**, 85 (2003).
8. J.J. Walsh, Invasive Fungal Infections: Problems and Challenges for Developing New Antifungal Compounds, In eds: J.A. Sutcliffe, N.H. Georgapapadakov, Merging Targets in Antibacterial and Antifungal Chemotherapy, New York, Chapman and Hall, pp. 349-373 (1992).
9. K. Vural, N. Ezer, K. Erol and F.P. Sahin, *J. Fac. Pharm. Gazi*, **13**, 29 (1996).
10. T. Baytop, Therapy with Plants in Turkey (Past and Present), Istanbul, Nobel Medical Books, edn. 2, p. 371 (1999).
11. A.H. Mericli, F. Mericli and E. Tuzlaci, *Acta Pharm. Turc*., **30**, 143 (1988).
12. M. Couladis, I.B. Chinou, O. Tzakou and A. Loukis, *Phytother. Res*., **16**, 723 (2002).
13. G.S. Citoglu, B.S. Yilmaz and N. Altanlar, *J. Fac. Pharm*., **32**, 93 (2003).
14. B.S. Yilmaz, N. Altanlar and G.S. Citoglu, *J. Fac. Pharm. Ankara*, **34**, 155 (2005).
15. N. Darbour, F. Baltassa and J. Raynaud, *Pharmazie*, **41**, 605 (1986).
16. F. Ferreres, F.A. Tomas-Barberana and F. Tomas-Lorente, *J. Nat. Prod*., **49**, 554 (1986).
17. V. Seidel, F. Bailleul and F. Tillequin, *Planta Med*., **62**, 186 (1996).
18. N. Didry, V. Seidel, L. Dubreuil, F. Tillequin and F. Bailleul, *J. Ethnopharmacol*., **67**, 197 (1999).
19. M.C. Bertrand, F. Tillequin and F. Bailleul, *Biochem. Syst. Ecol*., **28**, 1031 (2000).
20. M. Saeedi, K. Morteza-Semnani, M.R. Mahdavi and F. Rahimi, *Indian J. Pharm. Sci*., **70**, 403 (2008).
21. J.W. Warren, Nosocomial Urinary Tract Infections, In eds.: G.L. Mandell, J.E. Bennett, R. Dolin, Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, Philadelphia: Churchill Livingstone, edn. 6, pp. 3370-3381 (2005).