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ESSENTIAL OILS OF THREE SPECIES OF *Heracleum.* ANTICANDIDAL ACTIVITY

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Fruits of Heracleum crenatifolium *Boiss.*, Heracleum sphondylium *L. subsp. ternatum (Velen.) Brummitt, and* Heracleum platytaenium *Boiss. (Umbelliferae) were hydrodistilled to obtain essential oils that were then analyzed by GC and GC/MS. The major component was identified as octyl acetate (93.7, 87.6 and 31.6% respectively). Octyl butyrate was also characterized as the main component in* H. platytaenium *oil. Furthermore, anticandidal activity of the oils was evaluated using the microdilution broth method. All the oils showed good inhibitory effects against* C. glabrata.

Key words: *Heracleum*, anticandidal, essential oil.

A rapid increase is observed in the number of *Candida* infections on people with compromised immune systems such as cancer and AIDS patients. Infections are usually difficult to eradicate completely in patients receiving antifungal agents continuously for long periods of time [1, 2]. Anticandidal drugs such as fluconazole have been reported to cause resistance [3].

Previously, antifungal resistance used to be rare on *Candida* species; however, at present resistance is a very serious problem. Furthermore, *Candida* species other than *C. albicans* have, in recent years, shown to be responsible for fungemia. *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* infections are more common now than before [4].

Since the antimycotic activity of plants remains largely unexplored, interest has grown in the search for anticandidal agents from plants [5]. Our group has previously reported on the occurrence of over 5.000 natural compounds with anticandidal activity [6].

Here we report on anticandidal effects of the fruit essential oils of *Heracleum crenatifolium* Boiss., *Heracleum sphondylium* L. subsp. *ternatum* (Velen.) Brummitt, and *Heracleum platytaenium* Boiss. *Heracleum crenatifolium* is an endemic plant and this is the first report on its essential oil composition and anticandidal activity. The essential oils were analyzed using both gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS) techniques.

The composition and relative percentages of individual components of the oil were determined by chromatographic and spectroscopic means. Major components were identified as octyl acetate (93.7, 87.6 and 31.6% respectively) for each essential oil. In the oil of *H. platytaenium*, octyl butyrate (37.8%) was the main component. Complete GC/MS analysis of the oils will be reported elsewhere [9]. Main components in the oils are shown in Table 2. Results of anticandidal assays are given in Table 3.

The essential oils did not show outstanding inhibitory effect. They showed moderate activity (MIC values 0.25 to 1.0 mg/mL) against most *Candida* species. However, their effect was close to that of the standard antifungal agent ketoconazole on *Candida dubliniensis* and *Candida glabrata*. The anticandidal effects were better expressed on *C. glabrata*.

Our group has previously reported on the antimicrobial activity of *H. sphondylium* subsp. *ternatum* (collected from another region) essential oil that has 1-octanol (50.3%) as the main component [10]. The oil was shown to inhibit *Candida albicans* (clinical isolate) with an MIC value of 0.5 mg/mL better than the present one with MIC 2 mg/mL. Poorer activity of the oils is possibly due to the presence of octanol esters rather than octanol itself. Octanol was also reported to inhibit *C. albicans* with an MIC value of 500 μg/mL consistent with our previous report [11].

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TABLE 1. The Source of Plant Material and the Dry Weight Basis Oil Yield

Plants	Collection Region	Date	Oil Yield, %	Voucher*
Heracleum crenatifolium	Hadim-Kizilkaya-Karaman road, Konya	June, 2003	3.66	6224
H. platytaenium	Resadiye, Kizilcaoren Village, Tokat	July, 2003	5.33	6321
H. sphondylium subsp. ternatum	Ankara-Kirikkale road, Ankara	July, 2003	2.0	6319

*The Herbarium of Assoc. Prof. Dr. Ahmet Duran (ADO), Kirikkale University, Faculty of Sciences and Letters, Department of Biology, 71451, Kirikkale, Turkey.

TABLE 2. Main Components of the Essential Oils, %

Compounds	*H. crenatifolium*	*H. platytaenium*	*H. sphondylium*
	Esters		
Octyl acetate	93.7	87.6	31.6
Octyl butyrate	Tr.	Tr.	37.7
(Z)-4-Octenyl acetate	Tr.	2.1	3.3
(Z)-4-Octenyl butyrate	-	Tr.	2.6
Octyl 3-methyl butyrate (=Octyl isovalerate)	1.0	Tr.	Tr.
Octyl hexanoate	Tr.	3.0	0.9
Octyl octanoate	-	0.8	Tr.
Hexyl 2-methylbutyrate	0.8	Tr.	Tr.
Hexyl 3-methylbutyrate (=Hexyl isovalerate)	0.7	Tr.	Tr.
Decyl acetate	0.7	Tr.	0.5
	Alcohols		
Octanol	1.1	1.0	3.7
Linalool	-	0.6	-
	Aldehydes		
Octanal	Tr.	0.5	0.7
Decanal	0.1	0.8	Tr.
	Others		
Apiole	-	-	9.3

Tr.: Trace (<0.5%).

TABLE 3. Anticandidal Activites of *Heracleum* Essential Oils, mg/mL

Microorganisms	*H. crenatifolium*	*H. platytaenium*	*H. sphondylium*	Ketoconazole
C. albicans (NRRL Y-12983)	0.5	0.5	1.0	0.0039
C. albicans (Clinical Isolate)	0.5	2	2	0.0625
C. parapsilosis (NRRL Y-12969)	1.0	1.0	1.0	0.0039
C. dubliniensis (NRRL Y-17841)	1.0	1.0	1.0	0.25
C. glabrata (NRRL Y-65)	0.25	0.25	0.25	0.125
C. tropicalis (NRRL Y-12968)	0.5	0.5	0.5	0.0039
C. zeylanoides (NRRL Y-1774)	0.5	0.5	0.5	0.0039

The incidence of *Candida* infections has increased in recent decades due to the use of broad-spectrum antibiotics, steroidal and anticarcinogenic drugs, the increase in the number of immune suppressive patients, transplantations, and implantations of prosthesis apparatus [12]. Therefore, there is an urgent need to screen new effective anticandidal agents from natural compounds. Although the present results may not look promising, they at least give an idea on the activity scale such oils may have.

EXPERIMENTAL

Plant Material and Isolation of Essential Oils. The sources of plant material are shown in Table 1.

The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus for 3 h, from air-dried and crushed fruits. The essential oil and its constituent was analyzed both by GC and GC/MS at the conditions.

Gas Chromatography (GC). GC analysis was carried out using a Hewlett Packard 6890 GC system. HP-Innowax FSC column (60 m × 0.25 mm inner diameter, 0.25 mm film thickness) was used with nitrogen (1.2 mL/min ramp flow) as carrier gas. The oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and then kept constant 220°C for 10 min and programmed to 240°C at a rate of 1°C/min. Split flow was adjusted at 12 mL/min. The injector and FID detector temperatures were at 250°C. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

GC/MS Analysis. A Hewlett Packard G 1800A GCD system with HP-Innowax column (60 m × 0.25 mm, 0.25 mm film thickness) was used with helium (0.7 mL/min) as carrier gas. GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and then kept constant 220°C for 10 min and programmed to 240°C at a rate of 1°C/min. Split flow was adjusted to 50 mL/min, and the injector temperature was at 250°C. Mass spectra were recorded at 70 eV and the mass range was between *m/z* 35 to 425. Library search was carried out using Wiley GC/MS Library, Mass Finder Library, Adams Library and BASER Library of Essential Oil Constituents.

Microorganisms. *Candida albicans* (NRRL Y-12983), *C. albicans* (Clinical isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey), *C. zeylanoides* (NRRL Y-1774), *C. parapsilosis* (NRRL Y-12969), *C. dubliniensis* (NRRL Y-17841), *C. glabrata* (NRRL Y-65), and *C. tropicalis* (NRRL Y-12968) were used as test fungi in the anticandidal assays.

Inoculum Preparation. All the microorganisms were stored at –85°C in 15% glycerol. The yeasts were refreshed in Mueller Hinton Broth (Merck) at 35–37°C except for *C. zeylanoides*. It was incubated at room temperature for 24–48 h. Then the microorganisms were inoculated on Mueller Hinton Agar (Mast Diagnostics) plates for checking purity. Overnight grown *Candida* suspensions in double strength Mueller Hinton Broth were standardized to approximately 10^6 CFU/mL using McFarland No: 0.5 standard solutions.

Anticandidal Assay. Microdilution broth susceptibility assay [7, 8] was used for the anticandidal assay. Stock solution of essential oils and the standard antifungal agent ketoconazole were prepared in DMSO (Carlo-Erba). Dilution series were prepared from 4 mg/mL to 0.003 mg/mL in sterile distilled water in micro-test tubes (Eppendorf) from where they were transferred to 96-well microtiter plates. 100 µl of previously prepared Candidal suspensions were then added to each well. The last row containing only the serial dilutions of antifungal agent without microorganism was used as a negative control. Sterile distilled water and medium served as a positive control in the last column on each plate. After incubation at 37°C and room temperature (just for *C. zeylanoides*) for 24–48 h, the first well without turbidity was determined as the minimal inhibitory concentration.

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