Chemistry of Natural Compounds, Vol. 42, No. 2, 2006

COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *Prangos platychlaena* AND *P. uechtritzii*

A. Uzel,[1] T. Dirmenci,[2] A. Celik,[3] and T. Arabaci[4]

UDC 547.913;576.8

The chemical compositions and antimicrobial activities of the essential oils from two Turkish endemic species, Prangos platychlaena *and* P. uechtritzii, *were investigated. Hydrodistillation was used to isolate the essential oils, and the chemical analyses were performed by GC and GC-MS. The antimicrobial activity was tested by the microdilution technique against* Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Candida albicans, C. krusei, *and* C. tropicalis. δ*-3-Carene (3.39%) and* p*-cymene (3.38%) were identified as major components in* P. platychlaena, *and* α*-pinene (40.82%), nonene (17.03%),* β*-phellandrene (11.14%),* δ*-3-carene (7.39%), and* p*-cymene (4.90%) in* P. uechtritzii.

Key words: *Prangos platychlaena*, *Prangos uechtritzii*, essential oil, antimicrobial activity, microdilution.

The Genus *Prangos* Lindley (*Umbelliferae*) is represented by 13 species in Turkey, including five endemic species [1, 2]. *P. platychlaena* Boiss. and *P. uechtritzii* Boiss. & Hausskn are both endemic species with limited distribution and included in the lower risk and least concern category in the red data book of Turkey [3]. The former is found in the East and North East Anatolian Regions, whereas the latter are distributed in the Central, South, and East Anatolian Regions of Turkey. Here we report on the antimicrobial activity of the essential oils from *P. platychlaena* and *P. uechtritzii* because very little information is available on these endemic species.

The yields of *P. platychlaena* and *P. uechtritzii* on a dry weight basis were 0.4% (v/w) and 2.1% (v/w) respectively. Table 1 shows the percentages of the main components present in the essential oils isolated from the fruits of *P. platychlaena* and *P. uechtritzii* collected in June and July from different regions of Turkey. Fifteen components in *P. platychlaena* (98.82%) and 18 components in *P. uechtritzii* (97.42%) were identified. The components are listed in order of their elution time on the HP 1 MS column. Among the compounds, α-pinene (69.75%), β-phellandrene (10.58%), δ-3-carene (3.39%), and *p*-cymene (3.38%) were identified as major components in *P. platychlaena*, and α-pinene (40.82%), nonene (17.03%), β-phellandrene (11.14%), δ-3-carene (7.39%), and *p*-cymene (4.90%) in *P. uechtritzii*. The essential oils isolated from the fruits of *P. platychlaena* and *P. uechtritzii* were characterized by their richness in α-pinene, which was 69.75% for *P. platychlaena* and 40.82% for *P. uechtritzii*.

The antimicrobial activity of the essential oils measured by the microdilution method is given in Table 2. The essential oils isolated from *P. platychlaena* and *P. uechtritzii* fruits showed antimicrobial activity but differences in microbial susceptibility were observed. In general the essential oil of *P. uechtritzii* is more active against the test microorganisms than *P. platychlaena*. *P. uechtritzii* essential oil had the highest activity against *E. coli* (9 mg/mL), and *P. platychlaena* against *B. subtilis* (36 mg/mL). *E. faecalis* was the most resistant bacterium. *P. platychlaena* and *P. uechtritzii* oils had MIC values 144 mg/mL and 72 mg/mL respectively. No significant differences were seen between *P. platychlaena* and *P. uechtritzii* essential oils in terms of their antifungal activity against *Candida* species. However, *C. tropicalis* seems to be more resistant to *P. platychlaena* essential oil with a 144mg/mL MIC value.

1) Department of Biology, Faculty of Science, Ege University, Izmir, Turkey, fax: (+90) 0 2323881036, e-mail: atac.uzel@ege.edu.tr; 2) Department of Biology, Necatibey Education Faculty, Balikesir University, Balikesir, Turkey; 3) Department of Biology, Science & Arts Faculty, Pamukkale University, Denizli, Turkey; 4) Department of Biology, Science & Arts Faculty, Inonu University, Malatya, Turkey. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 139-140, March-April, 2006. Original article submitted June 20, 2005.

0009-3130/06/4202-0169

TABLE 1. Percentage Composition of the Essential Oils Isolated from *P. platychlaena* and *P. uechtritzii* Fruits (% of Total Ion Current)

Component[a]	Rt[b]	*P. platychlaena*	*P. uechtritzii*	Component[a]	Rt[b]	*P. platychlaena*	*P. uechtritzii*
Nonene	5.23	-	17.03	Phenylacetaldehyde	8.36	-	0.46
α-Pinene	5.57	69.75	40.82	Carvacrol	9.46	-	1.22
Camphene	5.70	0.43	1.14	Thymol	9.52	-	1.05
Sabinene	5.82	0.32	-	β-Bourbonene	11.36	0.65	-
Myrcene	5.86	0.86	2.07	Caryophyllene	11.94	-	0.53
β-Pinene	5.91	2.33	2.07	β-Bisabolene	12.47	1.34	2.15
α-Phellandrene	6.09	2.09	2.57	Germacrene D	12.86	1.18	-
δ-3-Carene	6.17	3.39	7.39	Bicyclogermacrene	13.12	0.74	-
p-Cymene	6.23	3.38	4.90	Valencene	13.42	-	0.32
β-Phellandrene	6.33	10.58	11.14	Spathulenol	14.38	1.42	0.65
Terpinolene	6.92	0.36	0.93	Total		**98.82**	**97.42**
Menthyl acetate	8.04	-	0.98				

[a]Compounds listed in order of elution from on HP 1 MS column.
[b]Retention time (as minutes).

TABLE 2. Antimicrobial Activity of the Essential Oils of *P. platychlaena* and *P. uechtritzii* Fruits Using Microdilution Method

Microorganisms	MIC[a]		MIC[b]
	P. platychlaena	*P. uechtritzii*	antibiotics
Staphylococcus aureus	72	18	4.5*
Enterococcus faecalis	144	72	72*
Bacillus subtilis	36	18	2.25*
Escherichia coli	72	9	2.25*
Pseudomonas aeruginosa	72	18	9*
Salmonella typhimurium	72	18	2.25*
Candida albicans	72	72	144**
Candida krusei	72	72	72**
Candida tropicalis	144	72	144**

[a]Values given as mg/mL (for the essential oils).
[b]Values given as μg/mL (for antibiotics). *Gentamycin; **fluconazole.

Our findings indicate that essential oils isolated from *P. platychlaena* and *P. uechtritzii* have antimicrobial activity and could be used to control microorganisms since these have been used for folk medicine for decades. It will be worthwhile to investigate the individual components in antibacterial and antifungal assays.

EXPERIMENTAL

Plant Material. The fruits of *Prangos platychlaena* were collected from the city of Yuksekova-Semdinli (Hakkari) at an altitute of 1850 m and those of *Prangos uechtritzii* Boiss.& Hausskn. from Akcadag-Dedeyazi village (Malatya) at an altitude of 1900–2100 m in the East Anatolian region of Turkey on 09th June 2002 and 17th July 2002 respectively. The voucher specimens are deposited at the herbarium of Balikesir University, Faculty of Science & Art, Biology Department (herbarium no. T.D. 1807, 1998).

Essential Oil Analysis. The air-dried fruits of *P. platychlaena* and *P. uechtritzii* were hydrodistilled separately for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia [4]. The essential oil was dried over anhydrous sodium sulfate and stored at 4°C before analysis. The essential oil was analyzed by gas chromatography-mass spectroscopy (GC-MS). The analysis of the essential oil was also performed using a Hewlett-Packard (HP) 6890 gas chromatograph, coupled with an HP 5973 mass selective detector and HP-5 capillary column (60m × 0.25 mm i.d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed from 50°C to 300°C at 10°C/min. Injector temperature was 150°C and detector temperature was 250°C. Diluted samples (1/100 in ethyl acetate, v/v) of 1.0 μL were injected by an auto-sampler in the splitless mode.

Identification of essential oil compounds was based on comparison of their relative retention time and mass spectra with those of commercial standards (for the main components) and retention indices (RI) relative to a C_8–C_{32} *n*-alkane mixture. The results were also confirmed by computer matching of mass spectra with the Wiley 275 L mass spectra data library [5]. The relative percentage of the essential oil constituents was calculated from the GC peak areas.

Microbial Strains. The *in vitro* antimicrobial activity of *P. platychlaena* and *P. uechtritzii* essential oils were measured using a panel that included Gram-positive, and Gram-negative bacteria and yeast-like fungi. The Gram-positive bacteria were *Staphylococcus aureus* (ATCC 6538P), *Enterococcus faecalis* (ATCC 29212), and *Bacillus subtilis* (ATCC 6633). The Gram-negative bacteria were *Escherichia coli* (ATCC 29998), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella typhimurium* (CCM 5445). The yeast fungi used were *Candida albicans* (ATCC 10239), *C. krusei* (ATCC 6258), and *C. tropicalis* (RSSK665).

Evaluation of Antimicrobial Activity. The minimum inhibitory concentration (MIC) was determined by means of the broth microdilution method described by the National Committee for Clinical Laboratory Standards [6]. All tests were performed in Mueller-Hinton Broth (MHB, Oxoid) supplemented in Tween 80 (final concentration of 0.5% (v/v)), with the exception of the yeasts (RPMI-1640 medium with glutamine and phenol red, without bicarbonate, Sigma). After aerobic incubation for 18–24 h at 37°C, the bacterial and fungal cultures were diluted to a turbidity of 0.5 McFarland (1.5×10^8 CFU/mL) and further diluted in saline solution to obtain an inoculum of 5.0×10^5 CFU/ well in a final volume of 100 mL and these were confirmed by viable counts. Geometric dilutions ranging from 288.0 mg/mL to 0.070 mg/mL of the essential oils were prepared in a 96 – well microtitere U –shaped plate in triplicate. One positive control (MHB or RPMI-1640 + Tween 80) and one negative control (MHB or RPMI-1640 + Tween 80 + essential oils) were also prepared for each experiment. The plates were incubated aerobically at 37°C for about 18 h. The MIC is defined as the lowest concentration at which there was no visible growth after incubation at 37°C for 18 h.

REFERENCES

1. P. H. Davis, *Flora of Turkey and East Aegean Island*, Edinburgh University Press, Edinburgh, **4**, 1972, p. 382.
2. K. H. C. Baser, B. Demirci, F. Demirci, E. Bedir, P. Weyerstahl, H. Marschall, H. Duman, Z. Aytac, and M. T. Hamann, *Plant. Med.*, **66**, 674 (2000).
3. T. Ekim, M. Koyuncu, M. Vural, H. Duman, Z. Aytac, and N. Adiguzel, *Red Data Book of Turkish Plants*, Bariscan offset, Ankara, 2000, p. 246.
4. *European Pharmacopoeia*, *Maissonneuve Sainte-Ruffine*, 1975, p. 68.
5. R. P. Adams, *Euro. Pharm.*, **3**, 68 (2001).
6. NCCLS (National Committee for Clinical Laboratory Standards), *Performance Standards for Antimicrobial Susceptibility Test. 9th International Supplement*, M100-S9, Wayne, PA, 1999.