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Short Communication

Volatile constituents and antimicrobial activities of *Pterocephalus canus*

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The volatile constituents from the aerial parts of *Pterocephalus canus* growing wild in Iran was obtained by hydrodistillation and analyzed by GC and GC-MS. Twenty three components representing 95.3% of the oil were identified. The main constituents of the oil were naphthalene (42.4%), tridecanoic acid (7.9%), hexadecanoic acid (7.9%), tetradecamethylcycloheptasiloxane (3.4%), 2,3-butanediol (3.1%) and dodecanoic acid (2.1%). Antimicrobial activity of the oil against 6 Gram-positive and negative bacteria was determined by measuring the growth inhibition zone. The oil showed significant antimicrobial activity against *Staphylococcus saprophyticus* and *Escherichia coli*. The bacteria *Staphylococcus aureus*, *Sataphylococcus epidermidis*, *Salmonella typhi* and *Shigella flexnery* were insensitive to the oil.

Key words: *Pterocephalus canus*, Dipsacacae, essential oil composition, antimicrobial activity.

INTRODUCTION

The genus *Pterocephalus*, one of the most important genus of Dipsacacae family is widely used in flavouring and folk medicine all around the world (Ghahreman, 1995). Its main habitat being in sunny, dry, rock crevices mostly found in Western Asia (Iran, Turkey). Its leaves belong to elliptic, entire, tomentose. The floral heads are 25 mm in diameter and with height of 5 to 10 cm which blossoms at summer time. *Pterocephalus* species was known to exhibit various biological activities such as anti-oxidant activities. Antibacterial, spasmolytic, hemostatic, astringent and many other activities of some *Pterocephalus* species have been reported (Zargari, 1992; Newall and Anderson, 1996; Bisset, 1994; Perry et al., 2003; Graikou et al., 2002; Hung et al., 2006; Li, 1998). Recently more attention has been directed to the water soluble biologically active components of this genus (Tian et al., 1993; Zheng et al., 2004). A literature survey revealed that no chemical and biological studies had been performed on the essential oil of *Pterocephalus Canus*. The aim of our study was to evaluate the chemical composition of *P. canus* essential oil and its antimicrobial activities.

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EXPERIMENTAL

Plant material

Aerial parts of *P. Canus* were collected at the flowering stage from Tehran, Iran in June 2007 and identified at the Research Institute of Forest and Rangelands, Tehran, Iran. A voucher specimen has been deposited in the Herbarium of Research Institute for Forests and Rangelands (voucher specimen number is 25418).

Isolation of the essential oil

Aerial parts of *P. Canus* were air-dried for 5 days before isolation of essential oil. The plant material (100 g) was cut into small pieces and the essential oil obtained by hydrodistillation with a Clevenger-type apparatus until there was no significant increase in the volume of the oil collected (5 h). The yield of the yellow oil was 0.02% (w/w) based on the dry weight of the plant.

GC and GC-MS analysis

GC analysis of the oil from the aerial parts of the plant was performed using a Shimadzu 14A gas chromatograph equipped with flame ionization detector (FID) and a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was programmed 50 to 250°C at a rate of 5°C/min; the injector

Table 1. Percentage composition of the essential oils isolated from aerial parts of *Pterocephalus Cannus*.

S/N	Compounds	RI[a]	Yield (%)
1	3-Hydroxy-2-butanone	707	1.4
2	2,3-Butanediol	780	3.1
3	1,1-Diethoxy ethane	806	1.4
4	4-Hydroxy-4-methyl-2-pentanone	933	0.5
5	Ethyl formate	855	0.2
6	Linalool	1103	1.1
7	Naphthalene	1179	42.4
8	α - Terpineol	1191	0.3
9	Geraniol	1285	0.2
10	Dodecamethyl cyclohexasiloxane	1321	1.0
11	*E*-β -Damascenone	1385	0.2
12	Tetradecamethylcycloheptasiloxane	1498	3.4
13	Dodecanoic acid	1573	2.1
14	Caryophyllen oxide	1585	1.1
15	Nonanoic acid	1770	2.1
16	Tetradecanoic acid	1771	2.0
17	Isopropyl myristate	1828	0.2
18	1,2-Benzendicarboxylic acid	1870	0.9
19	Tridecanoic acid	1971	7.9
20	Hexadecanoic acid	1972	7.9
21	Ethyl linoleate	2145	1.8
22	Tetracosane	2302	1.8
23	Hexatriacontane	3600	0.8

[a]Retention indices on HP-5 MS.

and detector temperatures were 260°C; the carrier gas was helium with a flow rate of 1 ml/min. The sample was injected using the split sampling technique of 1:50. The percentage composition of the oil was calculated automatically from peak areas without any correction. GCMS analysis was carried out on a Hewlett-Packard 5890 GC with an HP5970 MSD system using an HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.32 µm).

The oven temperature was as earlier mentioned, the transfer line temperature was 260°C; ionization energy in mass was 70 eV; mass range was 40 to 300 amu and scan time was 1 s. Retention indices (RI) of compounds were determined by comparing to the retention times of a series of *n*-alkanes with linear interpolation. Identification of the oil components was obtained by comparison of their mass spectra with the Wiley GC-MS library as well as by comparing with those reported in the literature. The identification of each component was confirmed by comparison of its retention index either with those of authentic compounds or with data in the literature (Jennings and Shibamoto, 1980; Adams, 1995; Davies, 1990).

Antimicrobial assay

The antibacterial activities of the essential oil of *P. Canus* were determined by measuring the growth inhibitory zones (well method) (Baver et al., 1986) against 6 Gram-positive and negative bacteria. The Gram-positive bacteria included *S. aureus* PTCC1113, *S. epidermidis* PTCC1349, and *S. saprphyticus* PTCC1379, and Gram-negative bacteria included *Salmonella typhi* PTCC1185, *Shigella flexnery* PTCC1234 and *E. coli* PTCC1330. The microorganisms were obtained from the Research Center of Science and Industry, Tehran, Iran.

Microorganisms (obtained from enrichment culture of the microorganisms in 1 ml Mueller–Hinton broth incubated at 37°C for 12 h) were cultured on Mueller–Hinton agar medium. The inhibitory activity was compared with that of standard antibiotics such as tetracycline (30 µg) and gentamicine (10 µg) which were obtained from the Iran Daru Company, Tehran, Iran. The essential oil of *P. Canus* was dissolved at 10% in *n*-hexane, and 50 µl of solution were poured into each well. After 24 h of incubation at 37°C, the diameter of the inhibition zone was measured to the nearest millimeter. Each test was carried out in triplicate and the average was calculated for inhibition zone diameters. A blank containing only *n*-hexane showed no inhibition in a preliminary test.

RESULTS AND DISCUSSION

From the aerial parts of *P. canus* at flowering stage, a yellowish oil was obtained at a yield of 0.02% (w/w). 23 components were identified accounting for 95.3% of the total oil. The oil of *P. Canus* was characterized by naphthalene (42.4%), tridecanoic acid (7.9%), hexadecanoic acid (7.9%) as the major components followed by tetradecamethylcycloheptasiloxane (3.4%), 2,3-butanediol (3.1%), dodecanoic acid (2.1%), nonanoic acid (2.1%), linalool (1.1%) and caryophyllene oxide

Table 2. Antimicrobial activities of *Pterocephalus Canus* oil.

Microorganisms	Growth inhibitory zone (mm)[a]	Standard antibiotics
Staphylococcus aureus PTCC 1113 (GP)	-	16[b]
Staphylococcus epidermidis PTCC 1349 (GP)	-	19[b]
Staphylococcus saprophyticus PTCC 1379 (GP)	12	23[b]
Salmonella typhi PTCC 1185 (GN)	-	26[b]
Shigella flexnery PTCC 1234 (GN)	-	24[c]
Escherichia coli PTCC 1330 (GN)	10	25[c]

GP: Gram- positive; GN: Gram-negative; [a]values are the mean diameter of inhibitory zones (mm); [b]gentamicine; tetracycline, (-) resistant.

(1.1%). The percentage composition of the various oil components are listed in Table 1.

Antimicrobial activities

The antimicrobial activities of *P. Canus* oil was assayed against 6 Gram (±) bacteria and results presented in Table 2 were compared with standard antibiotics. The present study revealed that the plant showed only anti-bacterial activity on *Staphylococcus saprophyticus* and *Escherichia coli*. This study confirms the importance of the correlation between the chemical content of the oils and antibacterial activities.

Conclusions

The aerial parts of *P. Canus* wildly grown in Iran was hydrodistilled and analyzed by GC and GC-MS. It was found that the main constituents of the oil were naphthalene (42.4%), tridecanoic acid (7.9%), hexadecanoic acid (7.9%), tetradecamethylcycloheptasiloxane (3.4%) and 2,3-butanediol (3.1%). The results of the antimicrobial activity of the oil against 6 Gram-positive and negative bacteria showed significant antimicrobial activity against *S. saprophyticus* and *E. coli*. A comparsion of antimicrobial activities between *P. canus* and other species of this family showed antimicrobial activity on *E. coli*, *Salmonella typhimurium* and *S. aureus* for *Pterocephalus pulverulentus* (Talib and Mahasneh, 2010) and on *S. aureus*, *S. epidermidis*, *E. coli*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* for *Pterocephalus perennis* (Graikou et al., 2002). This study confirms the importance of the correlation between the chemical content of the oils and antimicrobial activities.

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