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PHYTOCHEMICAL SCREENING AND ESSENTIAL OIL ANALYSIS OF ONE OF THE PERSIAN SEDGES; *CYPERUS ROTUNDUS* L.

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ABSTRACT

Phytochemical investigations of tuber extracts and evaluation of the hydro-distilled essential oil, obtained from *Cyperus rotundus* L. (Cyperaceae Family) growing wild in Isfahan Province (Iran) were studied. Phytochemical surveys revealed the presence of flavonoids, tannins, alkaloids and essential oils. Chemical composition of dried tubers essential oil was also analyzed by GC/MS. Sixty natural compounds consisting 95.8% of the total components were identified from the essential oil obtained with a yield of 0.2% (w/w). Sesquiterpene compounds have been found to occur in largest amount in the oil. Among the oil constituents, cyperene (16.9%), caryophyllene oxide (8.9%), α-longipinane (8.4%) and β-selinene (6.6%) were the major components.

INTRODUCTION: The family Cyperaceae from Poales order is one of the numerous families within Iran flora. It comprises about 14 genera in Iran which one of the most important of them is *Cyperus*. The genus *Cyperus* L. is distributed throughout the world and has six subgenera and about 45 species in Flora Iranica area [1-3]. It is also called sedges, flat sedges and cypress or nut grass. *Cyperus* species are used for edible, medicinal and ornamental purposes [2-5].

Cyperus rotundus L. that is widely distributed in Iran as an invasive known weed has been chosen for phytochemical screening and essential oil analysis. The popular names of the plant in Iran are Ouyarsalam and So'ad. This is a perennial herb with a height of up to 40 cm [2-4]. It has been used in popular and traditional medicines for many centuries in Iran. The plant is mentioned in several Iranian traditional medicine texts like Rhazes's "al-Hawi". It has been introduced in Iranian traditional and folk medicine to treat several disorders like some dermatological, gastrointestinal, gynaecological and psychological diseases [4, 6].

Biological and pharmacological studies on this genus revealed antibacterial, antimalarial, antihelmintic, insecticidal, antioxidant, antipyretic, anti-inflammatory, hepatoprotective and antidiabetic activities [4, 7-9].

There are some reports on the phytochemical analysis of species belonging to *Cyperus* found in the literature. These scientific studies on the species of this genus showed the presence of constituents belonging mainly to the groups of sesquiterpenes, flavonoids, tannins,

sterols, alkaloids, benzoquinones and essential oils [4, 7-10]. The flavor composition in *Cyperus* has been studied by various authors [7, 8, 10-16].

The present paper deals with the phytochemical investigations and detailed analysis of the essential oil of dried tubers of *C. rotundus* from Isfahan Province, Iran by GC-MS. This *Cyperus* species was not well studied from phytochemical and essential oil analysis point of view in Iran.

MATERIALS AND METHODS:

Plant materials: Tubers of *C. rotundus* were collected on October 2010 from plants growing wild in Baharan homesteads in Kelishad area in Isfahan Province, Iran at an altitude of ca. 1650 m. The plant specimen was identified by Dr. Lili Ghaemmaghami in Department of Biology, Isfahan University, Isfahan, Iran. A voucher specimen of the plant with number 2262 is deposited in the herbarium of School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. The dried tubers of the plant (100 g) were chopped in distilled water and its hydro-distilled fraction was isolated by hydrodistillation for 3 h according to the method recommended in British Pharmacopoeia. Essential oil sample was homogenized and dried over anhydrous sodium sulfate and stored in a freezer [17, 18].

Phytochemical screening of different extracts: The different qualitative micro-chemical tests are to be performed for establishing profile of the extracts for its nature of chemical composition. The preliminary phytochemical tests were carried out to detect the presence of steroids, alkaloids, anthraquinones, tannins and phenolic compounds, cardiac glycosides, essential oils, flavonoids and saponins [17-20].

GC-MS Study: GC/MS analysis was performed on a Hewlett Packard 5972A mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30m × 0.25 mm, film thickness 0.25 µm).

The GC operating conditions were as follows: carrier gas, helium with a flow rate of 2 mL/min; column temperature, 60°-275°C at 4°C/min; injector and detector temperatures, 280°C; volume injected, 0.1 µL of the oil; split ratio, 1:25.

The MS operating parameters were as follows: ionization potential, 70 ev; resolution, 1000; ion source temperature, 200°C.

Identification of components in the oil was based on GC retention indices relative to *n*-alkanes and computer matching with the Wiley 275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [18, 19, 21].

RESULTS AND DISCUSSION: The extract so obtained from *C. rotundus* tubers were subjected to preliminary phytochemical screening for detection of natural compounds present in them. The result of phytochemical screening of extracts revealed that flavonoids, tannins, alkaloids and essential oils were present in them.

The essential oil was a yellow liquid bearing the characteristic pungent and aromatic odor of Cyperaceae plants. The essential oil content of the plant was 0.2% (w/w) yield. Sixty compounds were identified, accounting for 95.8% of the oil. The identities of the components of the oil, the retention indices and the percentages are given in **Table 1.**

The major constituents of the oil were cyperene (16.9%), caryophyllene oxide (8.9%), *α*-longipinane (8.4%), *β*-selinene (6.6%), eugenol (4.7%), aristolone (3.5%), *β*-calacorene (3.3%), α-copaene (3.2%), *trans*-*γ*-bisabolene (3.1%) and α-cyperone (3.0%). Other components were present in amounts less than 2.5%. *C. rotundus* produces an essential oil which is relatively different from those known *Cyperus* oils. The oil was rich in sesquiterpene hydrocarbones.

Contrary to the earlier reports in Nigeria [12, 15] that *α*-pinene, *α*-thujene and humulene were present as major compounds in the oil of different species of *Cyperus*, in the present study these compounds could not be found or just found in trace amounts. In accord with the results of some other studies [10, 16, 22], the most prominent component of our oil, has been found in high amounts in *C. scariosus*, *C. distans* and Tunisian *C. rotundus* essential oils.

TABLE 1: RETENTION TIME (RT), COMPOSITION (%) AND RETENTION INDICES (RI) OF *CYPERUS ROTUDUS* ESSENTIAL OIL

S. No.	RT	Compound	%[a]	RI[b]
1	2.11	α-pinene	1.3	938
2	2.25	verbenene	0.6	972
3	2.44	β-pinene	0.5	977
4	2.83	o-cymene	0.2	1024
5	2.90	limonene	0.2	1030
6	2.93	1,8-cineole	2.4	1031
7	3.55	p-cymene	0.1	1096
8	3.98	α-fenchol	0.1	1119
9	4.41	trans-pinocarveol	0.2	1145
10	4.47	cis-verbenol	0.3	1147
11	4.80	pinocarvone	0.5	1171
12	5.10	isopinocamphon	0.2	1181
13	5.17	para-cymen-8-ol	0.2	1189
14	5.28	α-terpineol	0.4	1196
15	5.44	myrtenol	1.3	1204
16	5.69	verbenone	0.3	1215
17	5.81	trans-carveol	0.1	1227
18	6.00	trans-myrtenyl acetate	0.2	1244
19	6.20	cuminic aldehyde	0.3	1251
20	6.34	carvone	0.1	1256
21	6.92	cinnamaldehyde	0.1	1275
22	7.07	trans-anethole	0.1	1290
23	7.38	thymol	0.5	1299
24	7.45	2,4-decadienal	0.1	1302
25	7.83	carvacrol	1.0	1309
26	8.98	eugenol	4.7	1362
27	9.40	α-ylangene	2.3	1379
28	9.56	α-copaene	3.2	1381
29	9.91	β-elemene	0.2	1397
30	10.14	cyperene	16.9	1403
31	10.65	β-caryophyllene	2.3	1425
32	10.80	β-gurjunene	0.3	1429
33	10.88	α-guaiene	2.0	1439
34	10.97	aromadendrene	2.1	1443
35	11.03	isoaromadendrene	1.6	1446
36	11.09	α-humulene	1.5	1455
37	11.12	α-caryophyllene	0.7	1457
38	11.19	rotundene	0.9	1461
39	11.31	γ-gurjunene	0.3	1474
40	11.47	γ-muurolene	0.6	1477
41	11.59	n-dodecanol	0. 8	1483
42	12.53	β-selinene	6.6	1497
43	12.63	α-selinene	0.8	1499
44	12.72	α-longipinane	8.4	1502
45	12.97	α-farnesene	1.9	1508
46	13.17	cis-γ-bisabolene	2.1	1520
47	13.38	trans-calamenene	0.6	1535
48	13.43	trans- γ-bisabolene	3.1	1540
49	13.59	α-calacorene	0.5	1551
50	13.66	β-calacorene	3.3	1565
51	13.72	γ-elemene	0.4	1568
52	14.34	spathulenol	0.1	1578
53	14.77	caryophyllene oxide	8.9	1601
54	14.89	humulene epoxide II	0.1	1603
55	15.56	γ-gurjunene epoxide	0.2	1658
56	16.12	aristolone	3.5	1757
57	16.40	α-cyperone	3.0	1772
58	24.74	n-hexadecanoic acid	0.3	1942
59	28.79	phytol	0.2	2096
60	28.93	methyl linoleate	0.1	2120

[a]Retention indices on HP-5MS capillary column; [b]%: Calculated from TIC data

Essential oil of Persian *C. rotundus* is a source of a tricyclic sesquiterpene, cyperene. Caryophyllene oxide, which was found as a second major component of the oil, has been reported in several oils of Cyperaceae family and demonstrated antifungal, anti-inflammatory, anti-carcinogenic and skin enhancing activities [23]. These high content natural compounds are the characteristic components of *C. rotundus* and can be used as the index for quality controls of the medicines.

In conclusion, the tuber extracts of plant contain flavonoids, tannins, alkaloids and essential oils and the latter contains cyperene, caryophyllene oxide, *α*-longipinane and *β*-selinene. The pharmacological properties of *C. rotundus* appear to be due to the presence of these active constituents.

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