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In vitro Antimicrobial activity and phytochemical analysis of *Cyprus routduns* volatile oil

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Abstract
Volatile oil of *Cyperus rotundu* L. rhizomes, which were collected from Kosti, White Nile Province, Sudan was obtained by hydro-distillation method. Yield percentage of oil was found to be 0.49 %. Oil was tested for its antimicrobial activity against five standard organism of bacteria and two standard organisms of fungi, it showed moderate activity against all organisms except *E, coli*, which was sensitive to the two concentrations used. GC MS analysis of the oil showed the presence of sixty compounds. The major compounds were cyperene (16.9 %), caryophylene oxide (8.9), β-selinene (6.6 %), α-longipinane (8.4 %), eugenol (4.7 %), aristolone (3.5 %), β-calacorene (3.3 %), α-copaene (3.2 %), trans- γ-bisabolene (3.1 %) and α-cyperone (3.0 %.).

Keywords: *Cyprus routdunus*, volatile oil, antimicrobial

Introduction
Cyperus rotundu L. synonyms *Cyperus tuberosus* Roxb, Arabic name Seida, common names Purple Nustsedge and Nutgrass belongs to the family Cyperaceae. Its erect, grass – like, dark green perennial sedge, rhizomatous and tuberous, up to 0.6 m high, usually around 0.3 m around or shorter. In the Sudan it distributed in Nile banks, Gezira and Rahad (Braun *et. al.* 1991) [2].

Cyperus rotundus is a multipurpose plant, widely used in traditional medicine around the world to treat stomach aliments, wounds, boils and blisters (Oliver-Bever 1986, Puratuchikody *et al*. 2006, Joshi and Joshi 2000 and El-Kamali and El-Khalifa 1999) [14-15, 9, 7] A number of pharmacological and biological activities including anti-Candida, anti-inflammatory, antidiabetic, antidiarrhoeal, cytoprotective, antimutagenic, antimicrobial, antibacterial, antioxidant, cytotoxic and apoptotic, anti-pyretic and analgesic activities have been reported for this plant (Durate 2005 and Dhillon 1999**)** [4]. Also it has as anti-inflammatory, estrogenic, antipyretic, anti-emetic, diuretic and hypotensive agent (Aslam 2002) [1].

Previous phytochemical studies on C. rotundus revealed the presence of alkaloids, flavonoids, tannins, starch, glycosides and furochromones, and many novel sesquiterpenoids (Raut and Gaikwad 2006 and Thebtaranonth *et. al*. 1995) [16, 19].

Cyprus routduns rhizomes contain volatile oil range between 0.16 % to 0.5 % (Oladipupo *et. al.* 2009 and El-Gohary 2004) [13, 6]. The rhizome oils of this plant from different countries also showed compositional differences, suggesting the existence of phytochemical varieties. Cyperene (19.2-30.9%) and α-cyperone (4.5- 25.2%) were the most abundant constituents of the oils of Nigerian and Tunisian species, but the concentrations of other main components varied (Ekundayo 1991 *et. al.* and Kilani *et. al.* 2005) [5, 11]. The Brazilian species was found to contain α- cyperone (22.8%) and cyperotundone (12.1%) as the main compounds of the oil [34]. The rhizome oils of C. rotundus from India were reported to have α-copaene (11.4-12.1%), cyperene (8.4-11.7%), valerenal (8.7-9.8%), caryophyllene oxide (7.8-9.7%) and trans-pinocarveol (5.2-7.4%), some of which were absent in the species from other countries (Jirovetz 2004) [8]. Sonwa and Koenig (Sonwa and Koenig 2010) [17] investigated the essential oil of C. rotundus from Germany, and found the oil to be dominated by cyprotene, α-copaene, cyperene, α-selinene, rotundene, cadalene and nootkatene, among others.

Fig 1: *C. routduns* aerial Parts.

Fig 2: *C. routduns* rhizomes.

Materials and Methods
Plant Material
Plant material was collected from Kosti, White Nile Province, identified by Dr. Hayder Adbalgader and herbarium sheet was deposit at the herbarium of Medicinal and Aromatic Plants Research Institute.

Distillation of volatile oil

Distillation of volatile oils was carried out using the method described by (Sukhdev *et. al*. 2008) [18]:

250 g of the plant sample was placed in 2000 ml rounded bottom capacity flask. 1000 ml of distilled water was added and the Clevenger receiver (lighter than water) (Duran West Germany) and condenser attached to the top of the flask. System was heated at 100 C for about four hours till the volume of oil above water layer at the receiver constant. Oil was pipetted, dried over sodium sulphate anhydrous (BDH Chemicals Ltd, Poole. England) and stored in a dark container in a refrigerator till used. Distillation was repeated three times to calculate the mean of the yield percents Yield percentages were calculated as followed:

Volume of oil / weight of plant sample * 100

GC / MS analysis of the oil

GC-MS analyses of the oils were performed on a Hewlett Packard HP 6890 Gas Chromatography interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from ° 240 -70C at the rate of 5 °C/min. The ion source was set at 240 °C and electron ionization at 70 eV Helium was used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu1.0. µL of diluted oil in hexane was injected into the GC/MS.

Antimicrobial activity of volatile oil

Preliminary antimicrobial study was carried out using the method adopted by (Kavanagh 1972) [10].

Tested organisms

Fungal micro-organisms

Aspergillus Niger	AT cc 9763
Candida albicans	AT cc 7596

Bacterial micro-organisms

Escherichia coli	ATCC 25922
Klebsiella pneumonia	ATCC 53651
Pseudomonas aeruginosa	ATCC 27853
Staphylococcus	ATCC 25923
Proteus vulgaris	NCTC 8196

Preparation of suspension

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37◦ C for 24 hours. The bacterial growth was harvested, washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about (108 – 109) colony forming units per ml. (Miles and Misra 1938) [12]. The suspension was stored in the refrigerator at 4◦ C till use.

Screening the antibacterial activity of the extracts

3 ml of each of the 4 bacterial stock suspensions were thoroughly mixed with 300 ml of sterile melted nutrient agar which was maintained at 45◦ C. 20 ml of each of the inoculated nutrient agar were distributed into 6 sterile Petri-dishes. The agar was left to set and in each of these plates, which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 40,). The agar discs were removed, alternate cups were filed with 0.1 ml of oil using adjustable volume automatic micro-titre pipette, and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position at 37◦C for 18 hours. After incubation the diameters of the inhibition zones were measured.

Results

Table 1: Yield percentage of oil:

Weight of sample	Volume of oil	Yield %
100 g	0.49 ml	0. 49 %
100 g	0.49 ml	0.49 %
100 g	0.50 ml	0.50 %

Means of oil percent = 0.49 %

Table 2: Inhibition zones (mm) of C. *rotundus* Volatile oil against standard organisms

Organisms Conc.	E. c	P. a	K. p	P. v	S. a	Ca. a	As. n
10 %	19	14	16	15	14	14	14
20 %	20	14	16	16	15	14	15

Table 3: Chemical composition of essential oil from *Cyperus rotundus*

No.	RT	Compound	%a	RIb
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	cuminicaldehyde	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

Discussion

Mean of the oil yield found to be 0.49 % using hydro – distillation technique. Obtained yield percentage found to be on line with different previous studies of many authors around the world. El-Gohary (2004) [6] from Egypt reported that the yield percentage of C. *rotundus* volatile oil ranged between 0.19 to 0.46 % and Oladipupo *et al*. (2009 [13] who, stated that two samples of *C. rotundus* from South Africa resulted 0.16 and 0, 20 % of volatile oil. Antimicrobial activity of volatile oil using cup diffusion agar method and two concentrations (10 % and 20 %) against five standard organisms of bacteria and two fungi showed moderate activity against all used organisms except *E, c* which was sensitive to the two concentrations used. Inhibition zones ranged between 14 to 16 mm for all organisms, while for *E. c* was 19 to 20 mm. Obtained results of antimicrobial activity found to be on line with the findings of El-Gohary (2004) [6], who stated that the volatile oil of *C. rotundus* showed antimicrobial activity ranged between 10 to 23 mm against different standard organisms of bacteria and fungi including the same species of this study.

Gas chromatography coupled with Mass Spectroscopic analysis of the oil showed the presences of sixty different compounds. The mijor compound of the volatile oil were cyperene (16.9 %), caryophylene oxide (8.9), β-selinene (6.6 %), α-longipinane (8.4 %), eugenol (4.7 %), aristolone (3.5 %), β-calacorene (3.3 %), α-copaene (3.2 %), trans- γ-bisabolene (3.1 %) and α-cyperone (3.0 %. These results was found to in agreement with the findings of El-Gohary (2004) [6], who stated that the volatile oil of C. rotundus contains sixty

four different compounds and the major compounds were (+) oxo-α-ylangene (9.35%), (+) α-cyperone (9.07%) transpinocarveol (7.92%) and cyperene (7.83%). Also our result showed some differences and some similarity with the report of Oladipupo *et al.* (2009) [13], who stated that the essential oils from the rhizomes of C. rotundus L. collected from two different locations (Empangeni-A and Kwa Dlangezwa-B; both in the Kwa-Zulu Natal Province of South Africa) representing 89.9% and 92.0%of sample A and sample B, respectively. α-Cyperone (11.0%), myrtenol (7.9%), caryophyllene oxide (5.4%) and β-pinene (5.3%) were major compounds in the oil ofsample A. The main constituents of the oil of sample B were β-pinene (11.3%), α-pinene(10.8%), α- cyperone (7.9%), myrtenol (7.1%) and α-selinene (6.6%). The differences between the number and percentages of compounds may be due the type of soil, season, harvesting date and irrigation.

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