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Volatile Constituents and Antimicrobial Activities of Dried Rhizome of *Cyperus rotundus* Linn.

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ABSTRACT

Keywords

Cyperus rotundus Linn., dried rhizomes, benzene, chloroform and methanolic extracts, volatile oil, sesquiterpenes, α -Gurjunene.

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Cyperus rotundus Linn, a sedge of the family of Cyperaceae, is a perennial herb. Commonly known as Nagarmotha is widely distributed in the Mediterranean basin areas. This plant grows naturally in tropical, subtropical and temperate regions and is found throughout India. Volatile constituents of the dried rhizomes of Nagarmotha by GC-MS resulted in the identification of thirty four components. The oil was characterized by seven monoterpenes (11.03%), twenty-five sesquiterpenes (78.25%) and two unknown compounds (10.72%). The monoterpenes present were four alcohols, two hydrocarbons and one ketone. The sesquiterpenes found were twelve hydrocarbons, eight ketones, three alcohols and one oxide and epoxide each. The monoterpene found in the highest proportion was Pinacarveol (2.64%) and the sesquiterpene found in the highest proportion was α -Gurjunene (21.99%). Volatile oil of dries rhizomes of *Cyperus rotundus* Linn in higher concentrations showed significant antibacterial activity against the strains of *Bacillus subtilis* (23mm) followed by *Bacillus pumilis* (17mm) and significant antifungal activity against *Candida albicans* (23mm). The benzene extract of rhizomes showed more potent anti-microbial activity against *Bacillus subtilis* and *Candida albicans* in comparison to chloroform and methanolic extracts.

Introduction

Cyperus rotundus Linn, a sedge of the family of Cyperaceae, is a perennial herb. Commonly known as Nagarmotha is widely distributed in the Mediterranean basin areas. This Plant, which grows naturally in tropical, subtropical and temperate regions (Boulos and El-Hadidi, 1984). About 60

different species of Cyperaceae occur in India of which *Cyperus rotundus* Linn. is important for its medicinal uses. This plant is found throughout India upto an elevation of 1800m from Kashmir to Simla, Garhwal & Khasia hills, throughout the plains of almost all the states & ascending the mountains of

the Central table-land from Mount Abu & Pune to the Nilgiri hills (Sharma *et al.*, 2002). *Cyperus rotundus* rhizomes & leaves on hydrodistillation yield a volatile oil which possess anti-microbial and anti-inflammatroy properties. 27 compounds from the essential oil including copadiene, epoxyguaione, rotundone, eugenol were indentified cyperene, selinene, cyperenone and cyperone were reported as major constituents of essential oil from rhizomes of *C. rotundus* (Komai *et al.*, 1977). *C.rotundus* contains an essential oil that provides for the characteristic odour and taste of the herb, comprised mostly sesquiterpene hydrocarbons, epoxides, ketones, monoterpenes and aliphatic alcohols. Sesquiterpenes include selinene, isocurcumenol, nootkatone, aristolone, isorotundene, cypera-2,4(15)-diene, and norrotundene, as well as the sesquiterpene alkaloids rotundines A-C. Other constituents include the ketone cyperadione, and the monoterpenes cineole, camphene and limonene. *C.rotundus* has also been shown to contain miscellaneous triterpenes including oleanolic acid and sitosterol, as well as flavonoids, sugars and minerals

The rhizomes of *C. rotundus* have been used in ancient medicine in India for fever, dysentery, pruritis, pain, vomiting and various blood disorders (Kirtikar *et al.*, 1944). It is also a home remedy for indigestion disorders of stomach & irritation of bowel (Joshi SG, 2000). The plant possesses anti-malarial (Thebtaranonth *et al.*, 1995), anti-diabetic (Bowden *et al.*, 1999), & insecticidal properties (Morimato *et al.*, 1999)

Experimental

Plant material

The plant material was collected from the local market from M/s Gattumal and Sons,

Alamgiri Ganj, Bareilly (U.P.). Identification and authentication was done by Dr Alok Kumar Khare, Associate Professor, Department of Botany, Bareilly College, Bareilly (U.P.) reference number HBCB/588 dated 6th Jan 2015. A voucher specimen was kept in the herbarium of the Department of Botany, Bareilly College, Bareilly (U.P.).

Isolation

The dried rhizomes (1 kg) were hydro-distilled for 3 hours according to the method recommend in the British Pharmacopoeia 2003. The yield of volatile oil obtained was 0.6% v/w. The amber coloured volatile oil was collected in the graduated tube. The collected volatile oil was dried over anhydrous sodium sulphate and stored in the dark.

GC Analysis

Analytical GC was carried out on a Varian 3300 GC fitted with a silicone DB-1 capillary column (30m × 0.25mm), film thickness 0.25µm, carrier gas Nitrogen, flow rate 1.5 ml/min., split mode, temperature programmed 80-250 °C at 4 °C/min. Injector temperature and detector temperature were 250 °C and 300 °C respectively. Detector used was FID. Injection volume for all samples was 0.1µ.

GC-MS Analysis

GC-MS Analysis was carried out on a QP-2000 instrument at 70eV and 250°C. GC column Ulbon HR-1 fused silica capillary 0.25mm × 50m with film thickness 0.25µm. The initial temperature was 100 °C for six minutes and then heated at a rate of 10 °C per min. to 250 °C. Carrier gas Helium, flow rate 2ml/min., detector used was FID.

Identification of volatile constituents

The individual compounds were identified by comparing their retention indices (RI) of the peaks on ULBON HR-1 fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries and also with those reported in the literature (Adams, 1995; Libey, 1991; Jennings and Shibamoo, 1980; Swigar and Silverstein, 1981; Anderson and Falcone, 1969; Ali, 2001).

Relative amounts of identical components were based on peak areas obtained without FID response factor correction. Volatile constituents were identified by comparing their Kovats indices and retention times with those of authentic standards available in the author's laboratory and with those of literature are summarized in Table 1. The constituents were arranged in order of GLC and GC-MS elution on silicon DB-1 and ULBON HR-1 fused silica column, respectively.

Antimicrobial activity

Preparation of sample

The volatile oil (0.1% v/v, 0.5 % v/v, 1% v/v) and dried benzene, chloroform and methanolic extracts were dissolved in dimethyl sulfoxide (DMSO) for anti-microbial activity.

Preparation of Standard Drugs Solution

Chloramphenicol and Ketoconazole were used as standard solutions for comparison of anti-bacterial and anti-fungal studies. Both

the standard drugs were taken in DMSO. The concentration of both standard drug solutions was 10 mg / ml.

Anti-microbial Activity

The antimicrobial activities of volatile oil and dried alcoholic extract of dried rhizome of *Cyperus rotundus* were collected and the experiments were performed in Microbiology laboratory, Sri Ram Murti Smarak Institute of Medical Sciences, Bareilly, U. P. The identification of microbial strains was based on morphological, cultural and biochemical tests. The antibacterial activities of various oil concentrations and dried alcoholic extract of the dried rhizome of *Cyperus rotundus* were studied by the cup plate method (Pharmacopoeia of India, 1996; Macki and McCartney, 1980; Singh *et al.*, 2008) against various microorganisms mentioned in the Table 2. Chloramphenicol and Ketoconazole were used as standard and the activity of each concentration was compared with corresponding concentration of standard drugs. The plates were incubated at 37 ± 2 °C for antibacterial activity and 25 ± 2 °C for anti fungal activity, after 48 hrs of incubation. The Petri dishes were taken out from the incubator and the anti-microbial activity of different concentrations of oil and dried alcoholic extract of dried rhizome of *Cyperus rotundus* were compared by measuring the diameter of the zone of inhibition. (Table 2)

Result and Discussion

The volatile components of the *Cyperus rotundus* Linn. are listed in table No.1. Components are arranged in order to GC-elution. The oil was characterized by seven monoterpenes (11.03%), twenty-five sesquiterpenes (78.25%) and two unknown compounds (10.72%). The monoterpene

alcohols (6.54%) present in our sample included Pinacarveol (2.64%), Myrtenol (1.87%), p Menth -1 en-8-ol (1.21%), trans - carveol (0.82%). The monoterpene hydrocarbons (2.69%) found were β - Pinene (1.51%) and α - Thujene (1.18%). The monoterpene ketone found was Verbenone (1.80%). The sesquiterpene hydrocarbons (51.8%) present were α - Gurjunene (21.99%), Delta Guaiene (7.31%), Valencene (5.74%), α - Cubebene (4.67%), β - selinene (4.12%), allo aromadendrene (1.91%), β - Selinene (1.69%), α - humulene (1.37%), α - selinene (1.23%), β - elemene (0.71%), Eudesma – 2,4,11 - triene (0.53%) and Oxo-alpha ylangene (0.53%). The sesquiterpene ketones (16.08%) included Isolongifolen -5-one (6.41%), longiverbenone (4.62%), Nootkatone

(1.62%), Oxo- cyperone (0.89%), α - cyperone (0.84%), Aristolone (0.68%), Oplopenone (0.57%) and Solavetivone (0.45%). The sesquiterpene alcohols (4.53%) found in our sample were Globulol (2.23%), Patchenol (1.39%) and Spathulenol (0.91%). The sesquiterpene oxide and epoxide present were Caryophyllene oxide (5.13%) and Aromadendrene epoxide (0.71%) respectively.

Volatile oil of dried rhizomes of *Cyperus rotundus* Linn in highest concentrations(1%v/v) showed significant antibacterial activity against the strains of *Bacillus subtilis* (23mm) followed by *Bacillus pumilis* (17mm) and significant antifungal activity against *Candida albicans* (23mm).

Table.1 Chemical composition of volatile constituents of *Cyperus rotundus* Linn. rhizomes

S. No.	Component	RI	% age	S. No.	Component	RI	% age
1.	α - Thujene	915	1.18	18.	α - selinene	1490	1.23
2.	β - Pinene	980	1.51	19.	Spathulenol	1552	0.91
3.	Pinacarveol	-	2.64	20.	Oplopenone	1608	0.57
4.	p Menth -1 en-8-ol	-	1.21	21.	Caryophyllene oxide	1615	5.13
5.	Myrtenol	1195	1.87	22.	Globulol	1631	2.23
6.	Verbenone	1205	1.80	23.	Isolongifolen -5-one	-	6.41
7.	trans - carveol	1202	0.82	24.	Patchenol	1628	1.39
8.	α - Cubebene	-	4.67	25.	Aromadendrene epoxide	-	0.71
9.	β - elemene	1383	0.71	26.	α - cyperone	-	0.84
10.	α - Gurjunene	1431	21.99	27.	longiverbenone	-	4.62
11.	β - Selinene	1454	1.69	28.	Oxo- cyperone	--	0.89
12.	Delta Guaiene	-	7.31	29.	C ₁₅ H ₂₂ O	-	9.14
13.	α - humulene	1460	1.37	30.	Oxo-alpha ylangene	1739	0.53
14.	Valencene	-	5.74	31.	C ₁₅ H ₂₂ O	-	1.58
15.	allo aromadendrene	1468	1.91	32.	Aristolone	1752	0.68
16.	Germatrene E	1471	0.53	33.	Solavetivone	1816	0.45
17.	Eudesma – 2,4,11 - triene	1476	4.12	34.	Nootkatone	1820	1.62

RI-Retention index

Monoterpenes (7) = 11.03%; Hydrocarbons (2) = 2.69%; Alcohols (4) = 6.54%; Ketone (1) = 1.8%

Sesquiterpenes (25) = 51.8%; Hydrocarbons (12) = 51.8%; Alcohols (3) = 4.53%; Ketones (8) = 16.08%; Oxide (1) = 5.13%; Epoxide (1) = 0.71%

Unknown compounds (2) = 10.72%

Table.2 Antimicrobial activity of volatile oil and different extracts of *Cyperus rotundus* Linn. of dried rhizome

S.No	Test organism	Zone of Inhibition (in mm) ^a											Erythromycin estolate	Ketoconazole	
		Conc. of Volatile Oil			Dried benzene extract (mg/ml)			Dried chloroform extract (mg/ml)			Dried methanolic extract (mg/ml)			(mcg/ml)	(mcg/ml)
		0.1 %v/v	0.5 %v/v	1.0 %v/v	50	100	150	50	100	150	50	100	150	50	50
1.	<i>Bacillus subtilis</i>	17	19	23	10	14	17	9	12	15	02	05	08	29	-
2.	<i>Bacillus pumilis</i>	12	14	17	5	7	9	5	8	9	01	04	09	24	-
3.	<i>Pseudomonas aeruginosa</i>	9	10	11	5	7	9	3	5	8	02	04	07	15	-
4.	<i>Shigella flexneri</i>	12	13	15	6	7	9	4	5	9	01	03	06	18	-
5.	<i>Candida albicans</i>	18	20	23	11	14	17	8	10	14	03	06	10	-	28
6.	<i>Aspergillus niger</i>	16	17	19	10	12	15	6	9	12	02	05	09	-	23

a - an average of triplicate.

Erythromycin estolate - against bacterial strains only.

Ketoconazole – against fungal strains only.

The benzene, chloroform and methanolic extracts of rhizomes showed anti-microbial activity against *Bacillus subtilis*, *Bacillus pumilis*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Aspergillus niger* & *Candida albicans* in comparison with Erythromycin estolate & Ketoconazole standard antibiotics respectively.

The benzene extract showed antimicrobial activity at concentration 50mg/ml, 100mg/ml & 150mg /ml against *Bacillus subtilis* (10,14,17 mm), *Bacillus pumilis* (5,7,9 mm), *Pseudomonas aeruginosa* (5,7,9 mm), *Shigella flexneri* (6,7,9 mm) *Aspergillus niger* (10,12,15mm) & *Candida albicans* (11,14,17 mm) in comparison with Erythromycin estolate & Ketoconazole standard antibiotics respectively.

The chloroform extract also showed antimicrobial activity at concentration 50mg/ml,100mg/ml &150mg /ml against

Bacillus subtilis (9,12,15mm), *Bacillus pumilis* (5,8,9mm), *Pseudomonas aeruginosa* (3,5,8 mm) , *Shigella flexneri* (4,5,9mm) *Aspergillus niger* (6,9,12 mm) & *Candida albicans* (8,10,14mm) in comparison with Erythromycin estolate & Ketoconazole standard antibiotics respectively.

The methanol extract showed antimicrobial activity at concentration 50mg/ml, 100mg/ml & 150mg /ml against *Bacillus subtilis* (2,5,8 mm) , *Bacillus pumilis* (1,4,9 mm), *Pseudomonas aeruginosa* (2,4,7 mm), *Shigella flexneri* (1,3,6 mm) *Aspergillus niger* (2,5,9 mm) & *Candida albicans* (3,6,10 mm) in comparison with Erythromycin estolate & Ketoconazole standard antibiotics respectively.

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