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Analysis and antimicrobial activity of the essential oil of *Cyperus rotundus* L. rhizomes

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Abstract
The rhizomes of *Cyperus rotundus* L. (Cyperaceae), a native to India, are used to treat amenorrhoea, bronchitis, dyspepsia, stomach disorders, fever, malaria, cough, vomiting, renal and vesical calculi, skin diseases, wounds, dysmenorrhoea, deficient lactation, loss of memory, insect bites, nausea, dysuria, infertility, cervical cancer and menstrual disorders. Hydrodistillation of the rhizomes yielded a pale yellowish essential oils (0.6%). GC-MS analysis of the oil showed the presence of sesquiterpenes β-selinene (23.7%), α-cyperone (8.1 %), caryophyllene (4.1 %) and α-selinene (3.5 %), monoterpenes anethole (16.2 %) and cuminaldehyde (9.2 %), fatty acids viz., arachidic ((9.4 %), stearic (8.7 %) and palmitic (2.2 %) acids and *n*-pentane (5.8%) as the main constituents. The significant antimicrobial activities were observed with the essential oil of the rhizomes against *Bacillus subtilis, B. pumilus, Pseudomonas aeruginosa, Shigella flexneri, Aspergillus niger* and *Candida albicans.* A benzene extract of the rhizomes exhibited potent antibacterial effects against *B. subtilis, P. aeruginosa, S. flexneri and antifungal activity against C. albicans and A. niger.* The chloroform extract of the rhizomes elicited marked antibacterial activity against *B. pumilus* only.

Keywords: *Cyperus rotundus*, rhizomes, essential oil analysis, antimicrobial activity

Introduction
Cyperus rotundus L. (Cyperaceae), syn. *C. maritimus* Bojer; *Pycreus rotundus* (L.) Hayek (Cyperaceae), known as nagarmotha, saad kufi and nut grass, is considered as one of the world's worst weeds. It is indigenous to India, but now found in tropical, subtropical and temperate regions of the world. It is a smooth, erect and perennial herb having wiry, slender, scaly, creeping, dark and persistent rhizomes [1]. The rhizomes are considered as an analgesic, anthelmintic, antiseptic, antispasmodic, antitussive, aphrodisiac, aromatic, astringent, carminative, diaphoretic, diuretic, emmenagogue, litholytic, sedative, stimulant, stomachic, vermifuge and tonic; used to treat amenorrhea, loss of appetite, bleeding, blisters, boils, bronchitis, cervical cancer, colic, constipation, cough, diarrhea, dysentery, dysmenorrhea, dyspepsia, dysuria, fever, flatulence, food toxicity, indigestion, infertility, inflammation, insect bites, intestinal parasites, lacteal disorders, malaria, loss of memory, menstrual disorders, nausea, pyrexia, renal and vesical calculi, rheumatoid arthritis, stomach ailments, excessive thirst, skin rashes, urinary tenesmus, vomiting, worm infestation and wounds [1-3]. A decoction of the rhizomes with stem bits of *Tinospora cordifolia* and dried ginger is given to alleviate malarial fever. The rhizome decoction with the leaves of *Fumaria indica*, *Swertia chirayita*, black pepper and ginger is taken to relieve typhoid fever. The rhizome mixed with ginger and honey is ingested against dysentery and gastric and intestinal troubles. A fresh tuber paste is applied to the breast as a galactagogue. An essential oil of the tuber is used in perfumery and to make soap and insect repellent creams. A decoction of the roots and tubers is an excellent antidote to all poisons. The tubers improve blood circulation and are effective in gynecological diseases caused by blood stagnation [1-3].

The main constituents of the rhizome essential oil were cyperene, cyperotundone, humulene, β-selinene, myrtenol, β-caryophyllene, caryophyllene oxide, α- and β-selinenes, zierone, campholenic aldehyde, α.- and β-pinenes, longiverbenone, isolongifolen-5-one, rotundene, cyperorotundene α-cyperone and β.-vatirenene [4-12].

The rhizome essential oil is effective for decreasing the growth of axillary hair [13, 14], fumigant effect against *Blattella germanica* [15], anti-allergic [16], heme oxygenase-1 induction [17], antioxidant, cytotoxic, and apoptotic [8] and antiplatelet activities [18]. The tuber essential oil showed remarkable ovicidal and larvicidal activities against *Aedes albopictus* mosquitoes [19].

A hexane extract of the tubers was effective for repellency of the mosquito vector *Anopheles culicifacies*, *An. stephensi* and *Culex quinquefasciatus* even at a low dose [20]. The tuber essential oil elicited antimicrobial activity against various bacterial and fungal strains in different concentrations [9]. Sesquiterpenes from the tubers displayed antimalarial activity [21]. The *C. rotundus* oil exhibited remarkable antibacterial activity against Gram-positive bacteria, less antibacterial effect against Gram-negative bacteria and no activity against *P. aeruginosa* and *P. vulgaris* [22]. Sesquiterpenes from the rhizomes inhibited LPS- induced nitric oxide production [23]. The present paper describes the isolation and characterization of essential oil components and antimicrobial activity from the tubers of *C. rotundus* collected from Delhi.

Fig 1: *Cyperus rotundus* plant and rhizomes

Materials and Methods

Plant Material

The rhizomes of *C. rotundus* were procured from the AIMIL Pharmaceutical (I), Ltd, New Delhi and authenticated by Prof. M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is preserved in the Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Preparation of extracts

The coarse powders of the rhizomes (100 g each) were extracted individually with benzene (350 ml), chloroform (350 ml) and methanol (350 ml) in a Soxhlet apparatus exhaustively. Each solvent extract was dried under reduced pressures to get dark brown 790 mg, 950 mg and 11.2 g semi solid masses, respectively.

Isolation of essential oil

The finely chopped rhizomes (500 g) were added to deionized water (1.5 L) and subjected to hydrodistillation in a Clevenger apparatus for 4 h. The essential oil was evaporated together with water vapour and finally collected in a condenser. The essential oil was separated, measured, dried over anhydrous sodium sulphate and stored at 4ºC in the dark. This oil was used for GC and GC-MS analysis and evaluation of antimicrobial activity. The yield of essential oil obtained was 0.6% *v/w*. It was dried over anhydrous sodium sulphate and stored at 4^0C in the dark until analysis.

GC Analysis

Analytical gas chromatographic analysis was carried out on a Varian 3300 Gas Chromatograph equipped with a flame ionization detector (FID) and a silicone DB-1 capillary column (30 m x 0.25 mm i.d.), film thickness 0.25µm, carrier gas nitrogen, flow rate 1.5 ml/min., split mode ratio was 1:25. Injector and detector temperatures were 250ºC and 300ºC, respectively. An aliquot (0.5 µL of the diluted oil) was injected into the GC. Component separation was achieved following a linear temperature programmed from 50 to 230°C at a rate of 3 °C per min and then held at 230 °C for 10 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas. A homologous series of *n*-alkanes was run under the same conditions for determination of retention indices.

GC-MS analysis

The GC-MS analysis of the oils was 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 70 - 240 °C at the rate of 5 °C/min. The ion source was set at 240 °C and electron ionization at 70 eV and mass scan range (*m/z*) was 40-850 amu. Helium was used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. 1.0 µL of diluted oil in hexane was injected into the GC/MS. The percentage composition of the oil was calculated automatically from the FID peak area without any correction.

Identification of components

The individual compounds were identified by comparing their Kovat's indices (KI) of the peaks on Innowax 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 carried out by comparison of fragmentation pattern of the mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K L, WILEY 8 libraries and published literature [24,25]. Relative amounts of identical components were based on peak areas obtained without FID response factor correction.

Antimicrobial activity

All anti-microbial activities were performed at Arbro laboratories, Kirti Nagar, New Delhi.

Microbes used for antimicrobial activities were *Bacillus subtilis* (MTCC 441), *B. pumilus* (ATCC 7061), *Pseudomonas aeruginosa* (MTCC 424), *Shigella flexneri* (SC602), *Aspergillus niger* (MTCC 404) and *Candida albicans* (MTCC 227) and identification of each culture was done by conventional methods. All strains were maintained at 4 °C over nutrient agar slants throughout the experiment and used as stock cultures. Pure Erythromycin estolate and ketoconazole (Ranbaxy Ltd., Gurgaon) 50 mcg/ml were used as standards for comparison of antibacterial activity.

Preparation of media

All media were prepared in distilled water by dissolving agar (15 g), peptone (5 g), beef extract (1.5 g), dextrose (1 g), sodium chloride (3.5 g), dipotassium-hydrogen phosphate (3.68 g) and potassium dihydrogen phosphate (1.32 g), and pH of the media was adjusted between 6.95-7.05. The prepared medium was sterilized at 121^0C temperature and 15 lb pressure for 30 minutes.

Sabouraud dextrose agar media was composed of dextrose (40.0 g), mycological peptone (10.0 g), agar (15.0 g) and distilled water (1.0 L).

Preparation of standard solution

For the preparation of standard solution, pure ketoconazole and erythromycin estolate were dissolved in dimethyl formamide (DMSO), 50 mcg/ml. The further 10 µg/ml of ketoconazole and erythromycin estolate were obtained by diluting 1 ml of stock solution up to 10 ml of (DMSO).

Preparation of test solutions

For antimicrobial activities of dried benzene, chloroform and methanolic extracts in various concentrations (50 mg/ml, 100 mg/ml and 150 mg/ml) were dissolved in methanol.

Preparation of organisms or inoculums

The test organisms were maintained on freshly prepared medium slants. The slants were incubated at 37 °C for 24 h. The organisms from the medium slants were washed using 3 ml of saline solution and incubated for 24 h at 37 ± 2 °C. The developed organisms from the nutrient media were washed using 50 ml of distilled water. A dilution factor was determined which gave 25% light transmission at 530 nm. The amount of suspension to be added to each 100 ml nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

Determination of zone of inhibition

The antibacterial activity of the benzene, chloroform and methanolic extracts were studied against five bacterial strains such as *Bacillus subtilis, B. pumilus, Pseudomonas aeruginosa, Shigella flexneri, Aspergillus niger* and *Candida albicans.* The dried benzene, chloroform and methanolic extracts were dissolved in methanol. Pure erythromycin estolate and ketoconazole, 50 c mg/ml each, were used as standard for comparison of antimicrobial activity. The antimicrobial activities were screened by the agar well diffusion method. Nutrient agar plates were swabbed with the respective broth culture of the organisms and kept for 15 minutes in laminar chamber for absorption to take place. Wells were made in agar plates using a sterile cork borer and 10µl of different concentrations of extracts were added to different wells. The plates were incubated at 37± 2^0C for bacteria and 25 ± 2^0C for fungus for 24 hours. The diameters of the zone of inhibitions were measured in millimeter. The observations are tabulated in Table-2.

In the case of fungi, the test was performed in sterile petri dishes containing sabouraud dextrose agar (SDA). The oil was adsorbed on sterile paper disc and placed on the surface of the medium previously inoculated with a suspension of fungus. Control discs were saturated with erythromycin estolate (10 µg/disc). All petri dishes were sealed with a sterile laboratory film to avoid evaporation of the test samples and incubated at 27 °C for 48 h. The zone of inhibition was determined by measuring the diameter in mm of the clear zone around each disc.

Results and Discussion

Hydrodistillation of the rhizomes of *C. rotundus* yielded 0.6 % pale yellowish essential oils. The composition of the oil is displayed in Table 1. The constituents are listed in order of their elution on the (DB-1) column. A total 16 components were identified. The oil was characterized by larger amounts of sesquiterpenes (45.6 %) than monoterpenes (29.2%). The sesquiterpene composition of the oil was dominated by β-selinene (23.7%), α-cyperone (8.1 %), caryophyllene (4.1 %) and α-selinene (3.5 %). The compounds anethole (16.2 %) and cuminaldehyde (9.2 %) were the major representative of monoterpenoids. The oil was consisted three fatty acids (20.3 %) including arachidic ((9.4 %), stearic (8.7 %) and palmitic (2.2 %) acids. An aliphatic alkane, viz. *n*-pentane (5.8%) was also present in the oil. The composition profile of the oil showed that it was richer in sesquiterpenes and, therefore, was similar to other reported essential oil compositions of *C. rotundus* from different countries.

The rhizome essential oils of this plant from different regions showed variation in chemical composition suggesting the existence of phytochemical varieties. The plant essential oil contained α-cyperone, cyperene, cyperotundone and β-selinene as the major compounds along with other constituents such as, α-copaene, valerenal, caryophyllene oxide, patchoulene acetate and sugeonyl acetate [11]. However, cyperene (37.9 %) and cyperotundone (11.2 %) were the major components in the essential oils of C. rotundus from Iran [11]. 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 [26]. The essential oil of *C. rotundus* from Germany was dominated by cyprotene, α-copaene, cyperene, α-selinene, rotundene, cadalene and nootkatone [4]. The rhizome essential oil from southern India was consisted mainly of cyperene (9.7%), humulene (7.9%), β- selinene (7.8%), zierone (4.6%), campholenic aldehyde (3.8%) and α-pinene (3.5%) [12]. The essential oils from South African species contained α-cyperone (11.0%), myrtenol (7.9%), caryophyllene oxide (5.4%) and β-pinene (5.3%) in one sample and β-pinene (11.3%); α-pinene (10.8%), α-cyperone (7.9%), myrtenol (7.1%) and α-selinene (6.6%) in another sample [8]. An *n*-hexane soluble fraction of the rhizomes was composed of hentriacontane (7.15%), triacontane (6.12%), nonacosane (5%), octacosane (4.38%), octadecane (2.35%) and hexadecane (2.32%) [27]. Humulene, β-caryophyllene and their isomeric epoxides accounted for more than 70 % of the essential oil from a Nigerian species [28]. The Japanese species was found to contain α-cyperone (36.6%), β-selinene (18.5%), cyperol (7.4%) and caryophyllene (6.2%) [5,7]. The *C. rotundus* from China, Hong Kong, Taiwan and Vietnam had α-cyperone (30.7%), cyperotundone (19.4%), β-selinene (17.8%), cyperene (7.2%), cyperol (5.6%) and β-elemene (5.2%) [8]. The Hawaiian *C. rotundus* had cyperotundone (25.0%), cyperene (20.7%), patchoulene acetate (8.0%) and sugeonyl acetate (6.9%) as the major compounds [29, 30]. 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 [5, 31]. The Brazilian species was found to contain α-cyperone (22.8%) and cyperotundone (12.1%) as the main compounds of the oil [32].

Table 1: Chemical composition of essential oil from the rhizomes of *C. rotundus*

S. No.	Component	Kovat's index	% Area
1	n-Pentane	500	4.8
2	α-Pinene	936	1.2
3	β-Pinene	979	1.7
4	Myrtenol	1201	0.9
5	Cuminaldehyde	1223	9.2
6	Anethole	1255	16.2
7	Cyperene	1390	2.7
8	β-Selinene	1485	23.7
9	α-Selinene	1494	3.5
10	Caryophyllene oxide	1584	1.2
11	Caryophyllene	1614	4.1
12	Cyperotundone	1680	2.3
13	α-Cyperone	1772	8.1
14	Palmitic acid	1950	2.2
15	Stearic acid	2124	8.7
16	Arachidic acid	2218	9.4

The significant antimicrobial activities were observed with the essential oil of the plant rhizomes against *Bacillus subtilis, B. pumilus, Pseudomonas aeruginosa, Shigella flexneri, Aspergillus niger* and *Candida albicans.* The zone of inhibitions of the microorganisms were compared with standard samples erythromycin estolate and ketoconazole. The zones of inhibition were in the range of 11.0 to 14.5 mm at 0.01 mg/ml, 14.0 to 16.5 at 0.05 mg/ml and 16.0 to 20.0 at 0.1 mg/ml for the essential oil (Table 2). The dried benzene extract exhibited potent antibacterial effects against *B. subtilis, P. aeruginosa, S. flexneri,* and antifungal activity against *C. albicans* and *A. niger.* The dried chloroform extract showed potent antibacterial activity against *B. pumilus* only. These results showed that the benzene extract of the rhizomes was highly potent against microorganisms in comparison of chloroform and alcoholic extracts and with erythromycin estolate and ketoconazole as standard antibiotics. It was reported that the antibacterial activity of oil from tubers of *C. rotundus* showed more important activity against Gram-positive bacteria specially *Staphylococcus aureus* than Gram-negative bacteria. The variation of the antimicrobial activities of the rhizome essential oils was due to difference of the chemical compositions of the oils of different regions.

Table 2: Antimicrobial activity of essential oil and benzene, chloroform and methanol extracts of rhizomes of *Cyperus rotundus* L.

S. No.	Test organism	Zone of Inhibition (in mm)[a]											
		Essential oil mg/ml			Dried benzene extract (mg/ml)			Dried chloroform extract (mg/ml)			Dried methanolic extract (mg/ml)		
		0.01	0.05	0.1	50	100	150	50	100	150	50	100	150
1.	*Bacillus subtilis*	14.5	16.5	20.0	12.0	15.0	18.0	9.0	12.0	15.0	2.0	5.0	8.0
2.	*Bacillus pumilus*	13.5	16.0	17.5	6.5	7.0	9.5	5.0	8.0	10.0	1.0	4.5	9.5
3.	*Pseudomonas aeruginosa*	11.5	15.5	17.0	5.0	7.5	9.0	3.0	5.0	8.5	2.0	4.0	7.5
4.	*Shigella flexneri*	11.0	14.0	16.0	6.5	7.5	10.0	4.5	5.0	9.0	1.0	3.0	6.0
5.	*Candida albicans*	14.5	16.5	19.0	11.0	14.0	18.0	8.5	10.0	14.0	3.0	6.5	10.0
6.	*Aspergillus niger*	13.0	15.5	18.0	10.0	12.0	15.0	6.0	9.5	12.0	2.0	5.0	9.5

An average of triplicate was taken. There was no growth with the control compound.

Erythromycin estolate (25.0 -18.5 mcg/ml) was used against bacterial strains only. Ketoconazole was used as a standard against fungal strains only.

Conclusion

The rhizome essential oil of *Cyperus rotundus* was composed mainly of β-selinene (23.7%), anethole (16.2 %), cuminaldehyde (9.2 %), arachidic acid ((9.4 %), stearic acid (8.7 %) and α-cyperone (8.1 %). It exhibited antimicrobial activities *Bacillus subtilis, B. pumilus, Pseudomonas aeruginosa, Shigella flexneri, Aspergillus niger* and *Candida albicans.* A benzene extract of the rhizomes was active against *B. subtilis, P. aeruginosa, S. flexneri, C. albicans* and *A. niger.*

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