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

Vijender Singh, Mohammed Ali, Archana Negi and Shahnaz Sultana

Abstract

The rhizomes of *Cyperus rotundus* L. (Cyperaceae), a native to India, are used to treat amenorrhea, bronchitis, dyspepsia, stomach disorders, fever, malaria, cough, vomiting, renal and vesical calculi, skin diseases, wounds, dysmenorrhea, deficient lactation, loss of memory, insect bites, nausea, dysuria, infertility, cervical cancer and menstrual disorders. Hydrodistillation of the rhizomes yielded a pale yellowish essential oil (0.6%). GC-MS analysis of the oil showed the presence of sesquerpenes 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 world’s worst weeds. It is a smooth, erect and perennial plant, tuberous, with a terminal tuft of leaves. The rhizome is fleshy and yellowish. The rhizome is used in various traditional medicines throughout the world. The rhizome is used in Ayurvedic medicine for the treatment of various ailments such as bronchitis, dyspepsia, stomach disorders, fever, malaria, cough, vomiting, renal and vesical calculi, skin diseases, wounds, dysmenorrhea, deficient lactation, loss of memory, insect bites, nausea, dysuria, infertility, cervical cancer and menstrual disorders. Hydrodistillation of the rhizomes yielded a pale yellowish essential oil (0.6%). GC-MS analysis of the oil showed the presence of sesquerpenes 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.
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 Cleveger 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 4°C 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°C 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. Detector and detector temperatures were 250°C and 300°C, 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°C 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, P. 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°C for bacteria and 25 ± 2°C for fungus for 24 hours. The diameters of the zone of inhibitions were measured in millimeter. The observations are tabulated in Table 1. 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 cumbinaldehyde (9.2 %) were the major representative of monoterprenoids. The oil was consisted mainly of cyprotene, α-copaene, cypere, α-selene, rotundene, cadalene and nootkatone [24]. The rhizome essential oil from southern India was dominated by cyprotnene, α-copaene, cyperene, α-selene, rotundene, cadalene and nootkatone [24]. The rhizome essential oil from southern India was consisted mainly of cyprotene (9.7 %), humulene (7.9 %), β- selinene (7.8 %), zierone (4.6 %), campholenic aldehyde (3.8 %) and α-pinene (3.5 %) [21]. 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-selene (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 %) [25]. 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-selene (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 %), parchouline 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 Tusinian species, but the concentrations of other main compounds 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].

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, patchouline 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 cyprotenne, α-copaene, cypere, α-selene, rotundene, cadalene and nootkatone [24]. The rhizome essential oil from southern India was consisted mainly of cyprotene (9.7 %), humulene (7.9 %), β- selinene (7.8 %), zierone (4.6 %), campholenic aldehyde (3.8 %) and α-pinene (3.5 %) [21]. 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-selene (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 %) [25]. 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-selene (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 %), parchouline 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 Tusinian 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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Component</th>
<th>Kovat's index</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Pentane</td>
<td>500</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>α-Pinene</td>
<td>936</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>β-Pinene</td>
<td>979</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>Myrtenol</td>
<td>1201</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>Cumaraldehyde</td>
<td>1223</td>
<td>9.2</td>
</tr>
<tr>
<td>6</td>
<td>Anethole</td>
<td>1255</td>
<td>16.2</td>
</tr>
<tr>
<td>7</td>
<td>Cypere</td>
<td>1390</td>
<td>2.7</td>
</tr>
<tr>
<td>8</td>
<td>β-Selene</td>
<td>1485</td>
<td>23.7</td>
</tr>
<tr>
<td>9</td>
<td>α-Selene</td>
<td>1494</td>
<td>3.5</td>
</tr>
<tr>
<td>10</td>
<td>Caryophyllene oxide</td>
<td>1384</td>
<td>1.2</td>
</tr>
<tr>
<td>11</td>
<td>Caryophyllene</td>
<td>1614</td>
<td>4.1</td>
</tr>
<tr>
<td>12</td>
<td>Cyperotundone</td>
<td>1680</td>
<td>2.3</td>
</tr>
<tr>
<td>13</td>
<td>α-Cyperone</td>
<td>1772</td>
<td>8.1</td>
</tr>
<tr>
<td>14</td>
<td>Palmitic acid</td>
<td>1950</td>
<td>2.2</td>
</tr>
<tr>
<td>15</td>
<td>Stearic acid</td>
<td>2124</td>
<td>8.7</td>
</tr>
<tr>
<td>16</td>
<td>Arachidic acid</td>
<td>2218</td>
<td>9.4</td>
</tr>
</tbody>
</table>
The significant antimicrobial activities were observed with the essential oil of the plant rhizomes against Bacillus subtilis, P. pavilus, 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.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test organism</th>
<th>Essential oil mg/ml</th>
<th>Dried benzene extract (mg/ml)</th>
<th>Dried chloroform extract (mg/ml)</th>
<th>Dried methanolic extract (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacillus subtilis</td>
<td>14.5</td>
<td>15.5</td>
<td>16.0</td>
<td>15.0</td>
</tr>
<tr>
<td>2.</td>
<td>Pseudomonas aeruginosa</td>
<td>13.5</td>
<td>16.0</td>
<td>17.5</td>
<td>16.5</td>
</tr>
<tr>
<td>3.</td>
<td>Shigella flexneri</td>
<td>11.5</td>
<td>15.5</td>
<td>17.0</td>
<td>16.0</td>
</tr>
<tr>
<td>4.</td>
<td>Candida albicans</td>
<td>14.5</td>
<td>16.5</td>
<td>19.0</td>
<td>18.0</td>
</tr>
<tr>
<td>5.</td>
<td>Aspergillus niger</td>
<td>13.0</td>
<td>15.5</td>
<td>18.0</td>
<td>17.0</td>
</tr>
</tbody>
</table>

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, P. pavilus, 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.

Acknowledgement

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