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Extraction of *Cyperus Rotundus* Rhizomes Oil, Identification of Chemical Constituents and Evaluation of Antimicrobial Activity of the Oil in North Kordofan State

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Abstract: *Cyperus rotundus plant belongs to (Cyperaceae) family has a common local name siada. It has many different medical uses and these were based on the different parts of the plant. The aim of this work to extract Cyperus rotundus rhizomes oil, identify chemical components, test the antioxidant and antimicrobial effect of the oil. Three samples from cyperus rotudus rhizomes were collected from different locations in North kordofan State (Elrahad(A), Elobeid(B) and Bano(C)). The extraction process was carried out by hydrodistillation. The percentage yield of the hydrodistilled essential oil prepared from the three samples (A, B and C) gave 2.9%, 0.6% and 1.8% respectively. Analysis of the oils by GC/MS resulted in the identification of 75, 83 and 79 compounds for samples A, B and C respectively. The composition of the essential oils differed qualitatively and quantitatively according to the growing places. The antimicrobial activity was tested by cup-plate agar diffusion method using four different concentrations of the three extracted oil samples (100, 50, 25 and 12.5mg/ml) against four types of bacteria (Staphylococcus aurous, Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli) and two fungi strains (Candid albican and Aspergillusnige). Candid albican was the most susceptible to extract (A) at higher concentration (100mg/ml) among the tested microorganisms the inhibition zone diameter was found to be 20mm. Extract B have been shown to possess the strongest antimicrobial activity against Aspergillusniger at higher concentration 100mg/ml with an inhibition zone diameter 20mm. Extract C at concentration 100mg/ml exhibited moderate antimicrobial effect in the range 14-17mm towards investigated microorganisms. Other concentrations for the three samples A, B and C have been shown to possess an inhibition zone diameter in the range of 13- 17 mm. DPPH and iron chelating ability assays were used to test the antioxidative properties of the extracted oils. The radical scavenging assay percentage (%RSA ±SD) was found to be 01±0.04, 0.0, 06±0.11 and 88±0.04 for samples A, B, C and the standard respectively. The iron chelating ability percentage was found to be 01±0.02, 55±0.03, 29±0.2 and 98±0.01 for the samples A, B, C and standard respectively. The study recommended further study on other part of the plant.*

Keywords: *Cyperus rotundus, essential oil, chemical constituents, antimicrobial activity.*

1. INTRODUCTION

Cyperus rotundus, (family Cyperaceae), also known as purple nutsedge or nutgrass, is a common perennial weed with sender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long. The tubers are externally blackish in colour and reddish white inside, with a characteristic odour. The stems grow to about 25 cm tall and the leaves are linear, dark green and grooved on the upper surface. Inflorescences are small, with 2-4 bracts, consisting of tiny flowers with a red-brown husk. The nut is three angled, oblong-ovate, yellow in colour and black when ripe. (Auld and Medd 1987, Gunasekera and Fernando 1994, Parsons andCuthbertson 2001).

Essential oils are complex mixtures, constituted of terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristic aroma. The various applications of essential oils may be found in the cosmetic industry, as ingredients of fragrances, decorative cosmetic, fine fragrances and flavouring, in the food industry, as aromas and flavours, in the pharmaceutical industry, as active components of medicines and as antibacterial/antimicrobials, and in aromatherapy. It has been used in the production of lubricants, soap and personal care products, as well as in the tropical treatment of various conditions such as hair dandruff, muscle spasms, varicose veins and wounds (zimba et al.,

2005,Chivandi et al.,2008). Due to toxic effects of synthetic oils, there is a growing trend to replace them and revert to the natural oils in cosmetic and pharmaceutical industries (Shackleton et al., 2006).

The most common methods used for the industrial extraction of these oils are steam distillation, extraction with solvents and pressurized liquid extraction. Their selection will depend on the characteristics of the material from which the oil will be extracted, since they can be present in different parts of the plant, like the roots, the stem, the leaves, the fruits and/or the seeds (Harbone, 1973).

The rhizomes essential oil of the plant in this study have medicinal properties widely used in traditional medicine around the world to treat stomach aliments, wounds, boils and blisters. 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 (Majid et al., 2008) and (Sivapalan 2013). *Cyperus rotundus* rhizomes are considered astringent, diaphoretic, diuretic, analgesic, antispasmodic, aromatic, carminative, antitussive, emmenagogue, litholytic, sedative, stimulant, stomachic, vermifuge, tonic and antibacterial. It may be a good remedy for indigestion in the light of constituents present in it, for example, there are many enzymes for carbohydrates and minerals which act as catalyst for various biochemical reactions and helps indigestion. It is also useful for dietary management of psychotic diseases and metabolic disorders. It is used in treatment of Nausea and vomiting, dyspepsia, colic, flatulence, diarrhoea, dysentery, intestinal parasites, fever, malaria, cough, bronchitis, renal and vesical calculi, urinary tenesmus, skin diseases, wounds, amenorrhoea, dysmenorrhoea, deficient lactation, loss of memory, insect bites, food poisoning, indigestion, nausea, dysuria, bronchitis, infertility, cervical (Sivapalan 2013).

Cyperus rotundus is a weed in tropical and warm-temperate countries including India, China, Taiwan, Korea, Philippines, Thailand, Vietnam, Malaysia, Indonesia, the Pacific Islands, Africa, South America, the Middle East, North America, Mexico, New Zealand and Australia (Gunasekera and Fernando, 1994, Parsons and Cuthbertson 2001).

The antioxidant activity of essential oils is another biological property of great interest because they may preserve foods from the toxic effects of oxidants. Moreover, essential oils being also able of scavenging free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals (Miguel, 2010).

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. (Prakash2000).

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Miller et.al. 2000). Free radicals are highly reactive species having single unpaired electron on the outer orbit. Most of the free radicals are the reactive oxygen species (ROS) like superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) etc. They are produced by ultraviolet ray (uv), environmental pollution, hydrolysis, radiation etc. The substances, with which they react, are also converted to free radicals and thus they can setup an autocatalytic chain of reaction. They can

react with most of the biomolecules like proteins, fats,carbohydrates. Thus they can set up a large number of disorders including aging. They can damage the cell wall by causing peroxidation of the lipid layer of the cell wal1. These different types of toxic effects of the free radicals can be blocked by the antioxidants which either scavenge the free radicals or block their synthesis. Various synthetic antioxidants are now available but they are costly and many of them are associated with various side effects such as anorexia, nausea, diarrhea (Sies, 1997). Various research works have shown that, food rich in antioxidant play essential role to prevent different modern health hazards, i.e. thalassaemia, neuro – degenerative diseases, as well as inflammation, aging (Ames, 1993). Free iron plays an important role to form the free radicals. Moreover, excessive iron deposition in different vital organs can lead to the loss of function of those organs like liver, kidney. So, chelation of this free iron can prevent the formation of free-radicals as well as can prevent the impairment of vital organ function. Various synthetic iron chelators are available but they are costly & some are associated with side effects. Research works have demonstrated that some natural food supplement can chelate iron (Gerber, 2002).

1.1. The Objective of the Study

- Extraction of *Cyperus rotundus* rhizomes oil.
- Chemical constituents of the extracted oil.
- Antimicrobicial activities of the extracted oil against four types of bacteria and two fungi.

2. MATERIALS AND METHODS

2.1. Plant Materials

Three samples (A, B and C) of Cyprus retendus rhizomes were collected from Elrahad, Elobeid, and Bano areas- North Kordofan State (July 2013). The rhizomes of C. retendus were dried at room temperature for two weeks and grounded into powder.

2.2. Chemicals

Distilled water, DMS, ethanol and nutrient agar.

2.3. Microorganisms

Microorganisms were obtained from central of medicinal and aromatic plant researches.

The test organisms used in this study were *Bacillus subtilis* NCTC 8236 (Gram + ve bacteria), *Escherichia coli* ATCC 25922(Gram -ve bacteria**)**, *Pseudomonas aeruginosa* ATCC 27853 (Gram -ve bacteria), *Staphylococcus aurous* ATCC 25923(Gram +ve Bacteria), *Aspergillusniger* ATCC9763 and *Candida albicans* ATCC7596.

National Collection of Type Culture (NCTC), Colindale, England.American Type Culture Collection (ATCC) Rockville, Maryland, USA.

3. METHODS

3.1. Extraction of oil

From the powdered samples (A, B and C) 150-250-200g respectively were placed in 1000 ml conical flasks and subjected separately to hydrodistillation for 6hrs. The oil was separated from water by a syringe, injected into dark sterilized container and kept at 4 ^{o}C until further use.

3.2. Preparation of Bacterial Suspensions

One ml aliquots of a 24 hrs broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hrs. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for

two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hrs. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

3.3. Preparation of Fungal Suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

3.4. Testing of Plant Extracts for Antibacterial Activity

The cup-plate agar diffusion method **(**Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8 –10^9 C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45 ºC. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars were left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each of the oils dilutions in methanol using automatic microlitre pipette, and allowed to diffuse at room temperature for two hrs. The plates were then incubated in the upright position at 37 ºC for 18 hrs. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

3.5. Testing for Antifungal Activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 ºC for two days for the *Candida albicans* and three days for Aspergillusniger.

3.6. Gas Chromatography/ Mass Spectrometry (GC/MS) Analysis

GC/MS analysis was carried out with a Fisons Instrument GC2010, equipped with mass selective detector and quad rupole analyzer (FTD).The injection mode is split, and column oven temperature 50^{o}C.The flow control mode by pressure 90.0KPa, column flow 1.53ml/min. The electron ionization energy was 70 eV, ion-source temperature 200°C and the interface temperature 240°C. A split-splitless injection (split ratio-1.0) at 270°C injector temperature was employed.

A fused silica column 5% phenyl-poly-dimethyl-siloxane (DB-5MS 30m x 0.32 mmi.d. and 0.25µm film thickness) was used. The oven temperature was programmed as follows: from 50°C (5min hold 8d) raised at 2°C/min to 280°C (31min hold). Helium was used as the carrier gas at a linear velocity 44.8cm/sec. Injector and transfer line temperature were 200^{o}C and 280^{o}C respectively. 0.5ml of volatile oil was diluted by 1ml ethanol; 1µl was taken from diluted oil and injected on injection point at 200^{o}C. The evaporation of sample entered automatically into the column at 280^{o}C, after 31min from a starting time the compound were detected. Data acquisition was performed with Mass Lab Software for the mass ranges 30 - 600 u with a scan speed of 833scan/s. The identification of compounds was performed by comparing their mass spectra with data mass spectra library spectra. The identification of compounds was also based on the Kovats retention indices. The Kovats retention indices were calculated using n-alkanes C8-C20 and the experimental values were compared with those reported.

4. RESULTS AND DISCUSSION

4.1. Antimicrobial results

Essential oils obtained from cyperus rotundus were screened for their antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aurous, and

antifungi activity againt Candida albican and Aspergillusniger. The results of these screening were shown in tables (1), (2) and (3).

According to the results of antimicrobial screening shown in tables 1-3, the strongest antimicrobial activities against tested microorganisms were obtained for extracts A and B. The extract A has been shown to possess the strongest antimicrobial effect against *Candida albican*. The plant extract exhibited intermediate antimicrobial effect against others investigated microorganisms. The extract B has been shown to possess the highest antimicrobial effect against Aspergillusniger and intermediate effect against others tested microorganisms. The extract C has an intermediate antimicrobial activity against the investigated microorganisms. The highest concentration (100 mg/ml) for extract A has been shown to possess the strongest antimicrobial effect except for *Bacillus subtilis* where lower concentration (12.5 mg/ml) showed the strongest effect. The highest concentration (100 mg/ml) for extract B exhibit intermediate effect against *Escherichia coli* and higher antimicrobial effect for other microorganisms. Extract C showed irregular antimicrobial effect with the different concentrations against *Candid albican* and *Aspergillusniger*. These results indicated the similarities of the antimicrobial effect of the three samples in spite of their different growing places.

Table1. *Antimicrobial properties of plant extract A at four different concentrations (inhibition zone diameter in mm)*

Concentration mg/ml	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aurous*	Candid albican	*Aspergillusniger*
100	14	16	16	16	17	20
50	16	17	15	17	16	17
25	15	15	14	15	15	16
12.5	14	14	13	14	14	15

Table2. *Antimicrobial properties of plant extract B at four different concentrations (inhibition zone diameter in mm).*

Concentration mg/ml	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aurous*	Candidaalbican
100	16	16	16	17	20
50	15	15	15	16	17
25	13	14	14	15	16
12.5	14	16	17	14	14

Table3. *Antimicrobial properties of plant extract C at four different concentrations (inhibition zone diameter in mm).*

Concentration mg/ml	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aurous*	Candid albican	*Aspergillusniger*
100	16	17	15	17	15	14
50	15	16	14	16	14	17
25	14	15	13	15	16	16
12,5	13	14	12	14	15	15

4.2. The Percentage of the Oil

The percentage of the oil in the three samples (A, B and C) was found to be (2.9, 0.6, and 1.8) respectively as shown in table (4). These results indicated the dependence of percentage yield on the sample location.

Table4. *Percent extraction for oil of the three samples A, B and C (150, 250 and 200g from powder samples respectively)*

Sample	**Extracted oil (g)**	**Extraction percent(w/w)%**
A	4.385	2.9
B	1.589	0.6
C	3.596	1.8

4.3. GC/MS Analysis

GC/MS analysis are shown in Tables (6), (7) and (8) for the three samples (A, B and C) with the following compounds were detected in the three samples: alpha,beta Pinene, D-Limonene, Camphene, Thymol, Copaene, Caryophyllene Isoledene, and cyclo hydrocarbon like Bicyclo[3.1.0]hex-2-ene,4-methylene-1-(1-methylethyl), Bicyclo [2.2.1]heptan-2-ol,1,3,3 trimethyl, Cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl), Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene,and aromatic compound like (Benzene, 1-methyl-4-(1-methylethyl), Naphthalene, 1,6-dimethyl-4-(1-methylethyl)), and alcohol Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl), p-menth-1-en-8-ol, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl), Methanol, (1,4-dihydrophenyl).and ester like -Tetradecynoic acid, methyl ester.and aldhed like Cyclohexanebutanal, 2-methyl-3-oxo .

GC/MS study for sample (A) revealed the presence of seventy five compounds in *Cyprus rotundus* rhizomes oil these are shown in table 6. 1,5,5,8-tetramethyl 12-oxabicyclo[9.1.0] dodeca-3,7-diene (19.24%), 2,4,6-trimethyl 3-cyclohexene-1-carboxaldehyde (11.16%) and methyl (z)-5,11,14,17-eicosatetraenoate (8.86%) were the main components in sample (A). other components detected in lower amounts.

Table6. *Identified compounds of Cyprus rotundus rhizomes for sample (A) determined by GC/MS*

Peak	Retention time	Area %	Name
1	3.183	0.06	Methanol, (1,4-dihydrophenyl)-
2	3.717	2.02	.alpha.-Pinene
3	4.133	0.07	Camphene
4	4.458	0.17	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-
5	4.692	1.62	.beta.-Pinene
6	5.233	0.02	Cyclohexane, 1,3-butadienylidene-
7	5.475	0.02	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-
8	5.633	0.39	D-Limonene
9	6.075	0.39	Benzene, 1-methyl-4-(1-methylethyl)-
10	7.000	0.02	Cyclohexene, 1-methyl-4-(1-methylethylidene)-
11	7.233	0.05	3-Methyl-4-cyclohexene-1,2-dicarboxylic anhydride
12	7.567	0.03	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, [1S-(1.alpha.,3.beta.,5.alpha.)]-
13	7.800	0.06	Camphenol, 6-
14	8.058	0.17	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-
15	8.425	0.07	2-Bicyclo[3.3.1]non-6-en-3-ylpropan-2-ol
16	8.700	0.10	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-
17	8.883	2.11	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-
18	9.275	0.16	p-Mentha-1,5-dien-8-ol
19	9.400	0.50	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-
20	9.700	0.53	Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-
21	9.867	1.55	p-menth-1-en-8-ol
22	9.942	0.49	Bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene-
23	10.042	0.06	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-
24	10.133	1.03	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-
25	10.400	0.06	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-
26	10.467	0.13	Thymol
27	10.717	1.33	(1R)-(-)-Myrtenal
28	10.958	0.04	Carveol 2
29	11.292	2.01	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-
30	11.517	0.08	cis,cis,cis-7,10,13-Hexadecatrienal
31	11.733	1.62	Copaene
32	12.258	0.15	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-
33	12.417	0.57	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-

			tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-
34	12.825	0.28	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-
35	12.917	0.08	Caryophyllene
36	13.008	0.03	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,
37	13.083	0.05	Limonene-1,2-epoxide(fr.1)
38	13.683	0.50	.alpha.-Caryophyllene
39	13.908	2.32	1,10-Decanediol
40	14.058	0.58	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-
41	14.200	0.13	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-
42	14.517	0.21	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-
43	14.992	0.37	Isoledene
44	15.150	0.14	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-
45	15.383	0.12	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-
46	15.467	0.19	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-
47	15.817	0.11	1H-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-
48	15.908	0.07	(-)-Spathulenol
49	16.042	0.24	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-
50	16.133	0.68	Caryophyllene oxide
51	16.525	0.42	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl
52	16.817	8.86	Methyl (Z)-5,11,14,17-eicosatetraenoate
53	16.958	1.32	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)
54	17.150	0.61	.alpha.-Caryophyllene
55	17.250	0.57	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)
56	17.367	19.24	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl
57	17.617	2.07	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-
58	17.767	11.16	3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl-
59	17.817	4.59	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-
60	18.117	6.61	1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-
61	18.308	3.66	Aromadendrene oxide-(2)
62	18.508	0.88	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)
63	18.792	0.29	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-
64	18.858	0.16	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol
65	18.992	6.19	Longiverbenone
66	19.183	0.48	Megastigmatrienone
67	19.375	0.74	1,2,3,4,6,7,8,8a-Octahydronaphthalene-6,7-diol, 5,8a-dimethyl-3-isopropenyl-, cyclic carbonate, trans-
68	19.550	1.80	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-
69	19.850	0.15	Isolongifolen-5-one
70	20.067	1.51	Cyclohexanebutanal, 2-methyl-3-oxo-, cis-
71	20.375	0.34	2-Butyl-5-methyl-3-(2-methylprop-2-enyl)cyclohexanone
72	20.517	0.20	13-Tetradecynoic acid, methyl ester
73	20.783	2.49	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-
74	21.108	1.51	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-
75	21.533	0.32	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-

Table7. *Identified compounds of* ***Cyprus*** *rotundus rhizomes for sample B determined by GC-MS*

Peak	Retention time	Area %	Name
1	3.717	0.18	.alpha.-Pinene
2	4.458	0.02	Tricyclo[3.2.1.0(2,4)]octane, 8-methylene-, (1.alpha.,2.alpha.,4.alpha.,5.alpha.)-
3	4.692	0.13	.beta.-Pinene
4	5.633	0.04	D-Limonene
5	6.067	0.04	Trifluoroacetyl-.alpha.-terpineol
6	8.058	0.02	Fenchol, exo-
7	8.883	0.33	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-
8	8.992	0.06	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-
9	9.275	0.02	p-Mentha-1,5-dien-8-ol
10	9.400	0.07	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-
11	9.708	0.08	Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-
12	9.867	0.22	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-
13	9.942	0.08	2(10)-Pinen-3-one, (.+/-.)-
14	10.133	0.18	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-
15	10.408	0.04	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-
16	10.483	0.01	Tricyclo[3.3.1.1(3,7)]decane, 2-bromo-
17	10.717	0.20	(1R)-(-)-Myrtenal
18	10.883	0.57	Tricyclo[6.3.0.0(1,5)]undecan-4-one, 5,9-dimethyl-
19	11.142	0.02	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-
20	11.292	0.30	.alpha.-Cubebene
21	11.500	0.03	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-
22	11.550	0.07	(+)-Cycloisosativene
23	11.658	0.47	Ylangene
24	11.733	0.40	Copaene
25	11.867	0.31	Ionone
26	11.933	0.50	Cycloisolongifolene, 8,9-dehydro-
27	12.125	0.34	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-
28	12.258	0.27	1,5-Cycloundecadiene, 8,8-dimethyl-9-methylene-
29	12.442	10.11	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-
30	12.775	0.20	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-
31	12.825	0.20	10-Methoxytricyclo[4.2.1.1(2,5)]deca-3,7-dien-9-ol
32	13.075	0.05	Carane, 4,5-epoxy-, trans
33	13.308	0.33	1H-Naphthalen-2-one, 3,4,5,6,7,8-hexahydro-4a,8a-dimethyl-
34	13.408	0.17	(-)-Isosativene
35	13.708	3.57	1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol
36	14.067	0.14	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-
37	14.350	0.15	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-en-8-one
38	14.533	0.23	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-
39	14.725	0.61	Eudesma-4(14),11-diene
40	15.000	0.49	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-
41	15.150	0.43	3-Ethyl-4,4-dimethyl-2-(2-methylpropenyl)cyclohex-2-enone
42	15.458	0.50	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-
43	15.567	0.10	Santalol
44	15.642	0.13	Cyclohexanone, 2,6-bis(2-methylpropylidene)-
45	15.733	0.14	p-Menth-1-en-3-one, semicarbazone
46	15.817	2.78	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-
47	16.117	0.29	Cadala-1(10),3,8-triene
48	16.392	0.57	Tricyclo[3.2.1.02,7]oct-3-ene, 2,3,4,5-tetramethyl-

49	16.500	0.23	Bergamotol, Z-.alpha.-trans-
50	16.592	0.20	3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
51	16.658	0.41	2(1H)-Naphthalenone, 7-ethynyl-4a,5,6,7,8,8a-hexahydro-1,4a-dimethyl-, (1.alpha.,4a.beta.,7.beta.,8a.alpha.)-
52	16.808	1.70	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-
53	16.958	0.28	Carotol
54	17.142	0.34	3-Trifluoromethylbenzoic acid, dodec-9-ynyl ester
55	17.250	1.25	Cubenol
56	17.342	5.70	3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde
57	17.475	1.89	.alpha.-Cedrene oxide
58	17.642	0.75	4,8,12,16-Octadecatetraen-1-ol, 4,9,13,17-tetramethyl-
59	17.817	1.28	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-
60	18.083	6.81	Isolongifolen-5-one
61	18.325	2.06	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol
62	18.492	0.54	Acetate, (2,4a,5,8a-tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl) ester
63	18.775	2.14	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene
64	18.900	1.88	4,7-Methanoazulene, decahydro-1,4,9,9-tetramethyl-
65	18.975	8.33	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-
66	19.142	3.35	Longiverbenone
67	19.442	1.02	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-
68	19.617	23.74	5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-
69	19.792	2.69	Bicyclo[4.3.0]nonane, 1-isopropenyl-4,5-dimethyl-5-phenylsulfonylmethyl-
70	20.275	0.93	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene
71	20.442	1.49	9H-Cycloisolongifolene, 8-oxo-
72	20.558	0.30	11-Oxatricyclo[5.3.0.1(2,6)]undecan-4-one, 3-endo-5-endo-dimethyl-9-isopropylidene-
73	20.783	0.51	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-
74	21.083	1.20	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol
75	21.683	1.04	1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene
76	22.617	0.52	11-Oxatricyclo[5.3.0.1(2,6)]undecan-4-one, 3-endo-5-endo-dimethyl-9-isopropylidene-
77	23.375	0.19	Naphtho[2,1-d][1,3]dioxepin, dodecahydro-5a,8,8,11a-tetramethyl-, [5aR-(5a.alpha.,7a.beta.,11a.alpha.,11b.beta.)]-
78	24.308	0.38	9-Methoxycalamenene
79	24.650	0.22	3(4H)-Dibenzofuranone, 4a,9b-dihydro-8,9b-dimethyl-
80	24.983	0.17	Ledene alcohol
81	25.092	0.11	5H-Benzo[b]pyran-8-ol, 2,3,5,5,8a-pentamethyl-6,7,8,8a-tetrahydro-
82	26.325	0.04	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-
83	27.833	0.12	Benzofuran, 7-cyclohexyl-2,3-dihydro-2-methyl-

Table8. *Identified compounds of Cyprus rotundus rhizomes for sample C determined by GC-MS*

Peak	Retention time	Area %	Name
1	4.025	0.01	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
2	5.042	0.03	.beta.-Pinene
3	6.008	0.01	Limonene
4	6.458	0.03	Benzene, 1-methyl-4-(1-methylethyl)-
5	9.117	0.02	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-
6	9.308	0.38	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-
7	9.408	0.08	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1.alpha.,2.beta.,5.alpha.)]-
8	9.700	0.03	5,8,10-Undecatrien-3-ol

9	9.817	0.06	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-
10	10.117	0.07	Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-
11	10.325	0.04	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-
12	10.375	0.11	Bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene-
13	10.558	0.30	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-
14	10.825	0.06	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-
15	10.892	0.04	Thymol
16	11.150	0.31	(1R)-(-)-Myrtenal
17	11.325	0.45	Tricyclo[6.3.0.0(1,5)]undecan-4-one, 5,9-dimethyl-
18	11.567	0.03	.alpha.-Cubebene
19	11.725	0.20	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-
20	12.092	0.69	Ylangene
21	12.175	0.38	Copaene
22	12.317	0.26	7-Octylidenebicyclo[4.1.0]heptane
23	12.375	0.35	Cycloisolongifolene, 8,9-dehydro-
24	12.575	0.33	Cycloisolongifolene, 8,9-dehydro-
25	12.700	0.45	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-
26	12.908	7.66	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-
27	13.217	0.12	2,9-Heptadecadiene-4,6-diyn-8-ol, (Z,E)-
28	13.267	0.12	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-
29	13.358	0.22	Caryophyllene
30	13.758	0.34	1H-Naphthalen-2-one, 3,4,5,6,7,8-hexahydro-4a,8a-dimethyl-
31	13.867	0.13	(-)-Isosativene
32	14.175	3.13	1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol
33	14.317	0.11	2,10,10-Trimethyltricyclo[7.1.1.0(2,7)]undec-6-en-8-one
34	14.417	1.37	Eugenol
35	14.775	1.70	Eudesma-4(14),11-diene
36	14.892	1.34	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-
37	14.983	0.42	3-Buten-2-ol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
38	15.192	0.55	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-
39	15.442	0.85	Isoledene
40	15.608	0.44	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-
41	15.892	0.52	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-
42	16.283	3.30	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-
43	16.458	0.08	Cyclohexane-1-methanol, 3,3-dimethyl-2-(3-methyl-1,3-butadienyl)-
44	16.583	0.60	9-Methoxycalamenene
45	16.858	0.72	Benzenebutanoic acid, .beta.,3,4-trimethyl-, methyl ester
46	16.900	0.16	4-Isopropyl-7,11-dimethyl-3,7,11-cyclotetradecatrienone
47	16.950	0.67	Aromadendrene oxide-(2)
48	17.050	0.42	Bicyclo[4.1.0]heptane-7-methanol, 1,5,5-trimethyl-2-methylene-, (1.alpha.,6.alpha.,7.alpha.)-
49	17.133	0.47	Aromadendrene, dehydro-
50	17.275	3.54	Methyl (Z)-5,11,14,17-eicosatetraenoate
51	17.458	0.36	Trichothec-9-en-8-one, 12,13-epoxy-3,4,7,15-tetrahydroxy-, (3.alpha.,4.beta.,7.alpha.)-
52	17.617	0.34	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-.alpha.,5,5,8a-tetramethyl-2-methylene.
53	17.700	0.58	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-
54	17.783	0.84	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol
55	17.942	2.30	.alpha.-Cedrene oxide
56	18.117	1.31	Thunbergol
57	18.333	1.70	Androst-5-en-3-one, 4,4-dimethyl-

58	18.558	4.83	Isolongifolen-5-one
59	18.683	0.97	1H-3a,7-Methanoazulen-5-ol, octahydro-3,8,8-trimethyl-6-methylene-
60	18.783	1.76	Aromadendrene, dehydro-
61	19.292	2.46	1,4-Methanoazulen-7(1H)-one, octahydro-4,8,8,9-tetramethyl-, (+)-
62	19.408	1.44	Tetradecahydro-1-methylphenanthrene
63	19.508	5.82	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-
64	19.675	2.78	Longiverbenone
65	19.900	1.07	trans-3(10)-Caren-2-ol
66	20.017	1.23	Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)-
67	20.225	17.93	Isolongifolen-5-one
68	20.900	8.22	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-
69	21.100	2.09	9H-Cycloisolongifolene, 8-oxo-
70	21.650	0.42	Acetate, (2,4a,5,8a-tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl) ester
71	21.758	0.85	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
72	22.417	1.30	1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene
73	23.442	0.62	11-Oxatricyclo[5.3.0.1(2,6)]undecan-4-one, 3-endo-5-endo-dimethyl-9-isopropylidene-
74	24.958	1.57	Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-
75	25.050	0.72	Caryophyllene oxide
76	25.592	0.23	Cycloisolongifolene, 8-hydroxy-, endo-
77	25.867	0.21	Longipinocarvone
78	26.783	0.09	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-
79	28.225	0.20	Pyrene, 1,2,3,3a,4,5,5a,6,7,8,8a,9,10,10a-tetradecahydro-

5. CONCLUSION

This study revealed that the oil percentage, chemical constituents and the antimicrobial effect depends on the location of the growing plant. The observed compositional difference between *C. rotundus* found in Sudan and the rest of the world could be due to soil, rain fall, nutritional status of the plants, environmental, climatic conditions, and other factors, which can influence essential oil composition. The compositional difference between the three samples of *C.rotundus* shows the existence of more chemical diversity within the *C.rotundus* species. Compounds have been detected in all oil samples were alpha.-Pinene, Camphene, D-Limonene, Camphenol, 6-, p-Mentha-1,5-dien-8-ol, Thymol, (1R)-(-)-Myrtenal, Carveol 2, Copaene, Caryophyllene, Naphthalene, 1,6-dimethyl-4-(1-methylethyl). The oil extracted from sample A was effective against Aspergillusnige at higher concentration and the oil extracted from sample B shows good antimicrobial activity against Candid albican at higher concentration.

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