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Research Article

GC-MS characterization and antioxidant potential of rhizome essential oil from *Cyperus rotundus* L. growing in North Central Nigeria

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Abstract

Oxidative stress has been linked to neurological diseases. The menace of stress is clinically curtailed by using synthetic drugs whose usage is associated with several draw backs. Recently, essential oils have shown promising antioxidant potential devoid of any side effect. The potential is a function of the type of phytochemicals whose presence in the oil varies from plant to plant. It is on this basis that this study investigated the antioxidant potential of characterized essential oil from rhizomes of Nigerian grown *Cyperus rotundus*. Rhizomes (500 g) of *C. rotundus* were pulverized and hydrodistilled for three hours. The hydrodistillation afforded 0.125±0.02% (w/w) of essential oil. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) were used to characterize the oil and the characterization revealed abundance of sesquiterpenoids (85.9%). Major compounds in the oil were α-humulene (14.3%), caryophyllene oxide (12.3%), humulene-1,2-epoxide (9.1%), valencene (8.2%), β-selinene (7.9%), β-caryophyllene (6.5%) and γ-gurjunene epoxide (5.4%). The antioxidant potential of the oil was established using radical scavenging assay. The oil showed antioxidant activity by scavenging free radicals of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) with IC_{50} of 32.60 µg/mL. Although the oil was not as active as butylated hydroxytoluene (BHT, IC_{50} of 18.12 µg/mL) that was used as standard, its activity revealed that it could serve as alternative to synthetic drugs to ameliorate the menace of oxidative stress after clinical approval.

1. Introduction

Recently, men have witnessed several neurogenic diseases such as memory loss, Alzheimer, Parkinson, amyotrophic lateral and multiple sclerosis depression. The development of these diseases has been linked to oxidative stress, caused by excess free radicals in the cell. The damage caused by reactive oxygen species is restored by antioxidants which convert the free radicals generated in the body cells into less harmful substances, thereby curtailing the menace of oxidative stress [1-3]. Commonly used antioxidants are butylated hydroxyl-toluene (BHT), butylated hydroxyl-anisole (BHA), tert-butylated hydroxyquinone (TBHQ) among others [4]. These are synthetic drugs and their high dosages are reported to be responsible for the carcinogenesis of human cells as well as DNA and liver damages [5-7]. As a result of this, there is a need for alternative antioxidants from natural sources with little or no side effects.

Plants produce phytochemicals that have been explored as phytomedicines since ancient times. These phytochemicals are secreted in certain organs and are derived from all parts of plants [8, 9]. *Cyperus rotundus* also known as nut-grass or nutsedge is a perennial plant belonging to the family of *Cypereacea*. The plant is commonly known as "eso koriko", "ayaa ayaa", "ayaare", "ahihia ahihia", and "isholo i toho"

by the Yorubas, Hausas, Fulfulde, Igbos and TIVs people of Nigeria respectively [10]. The plant is used in ethnomedicine for the treatment of stomach disorders, cancers, dysmenorrhea, mensural irregularities, blood disorders, leprosy, dysentery, spasms and inflammatory diseases [11,12]. Crude extracts from the plant have been reported to possess antifungal, anti-inflammatory, antidiabetic, antimalarial, antiviral, antibacterial, antidiarrheal, and antioxidant properties that justify its usage in ethnomedicine [12-14]. Phytochemical screening of extracts from various parts of the plant revealed the presence of terpenoids, polyphenols, saponins, alkaloids, flavonoids, and furochromes [15]. The presence of polyphenols and terpenoids were reported to be responsible for the antioxidant property of the extracts [15].

Essential oil extracted from rhizomes of the plant growing in different parts of the world has been characterized and compositional differences were established. Characterization of rhizome essential oils of the plant grown in two locations (Empangeni and KwalDlangezwa) in the KwaZulu Natal province of South Africa revealed the abundance of α-cyperone, myrtenol, and β-pinene [16]. Saperene, α-cyperone, and α-selinene were the most prominent constituents identified from the rhizome oil of the plant native to China [17]. The oil of the Brazilian grown *C. rotundus* contained cyperotundone, and α-cyperene as the principal constituents, while α-cyperene and cyperone predominated the rhizome oils of the plant indigenous to Tunisia and Nigeria [18–20]. Meanwhile, valerenal, cyperene, *trans*-pinocarveol, α-copaene, and caryophyllene oxide were the major compounds that constituted the rhizome oil of the plant native to India [21]. These phytochemical variations might be due to differences in agroclimatic conditions at different locations of the plant.

The antioxidant activity of rhizome essential oil of *C. rotundus* of Tunisia and China origin have been explored [17, 22]. However, to the best of our knowledge, the antioxidant activity of the rhizome oil of the plant grown in north-central Nigerian has not been studied. This study therefore aimed at characterizing and evaluating antioxidant potential of rhizome essential oil of *C. rotundus* growing in north-central Nigeria.

2. Materials and methods

2.1. Sample collection

Fresh rhizomes of *C. rotundus* (2500 g) were harvested at the Park and Garden, University of Ilorin, Ilorin, Nigeria. Identification of the plant was carried out by Mr. Bolu, at the Herbarium of Plant Biology Department, University of Ilorin, where voucher specimens were deposited [UILH/003/0679].

2.2. Essential oil extraction

Rhizomes (500 g) of *C. rotundus* were blended and hydrodistilled for 3 hours in a Clevenger setup, based on British Pharmacopoeia specification [23]. The extraction was carried out in triplicate. The oils were collected, preserved in a sealed sample tube and stored under refrigeration at 4 ^{o}C until the analyses were carried out.

2.3. *Gas Chromatography – Mass Spectrometry (GC/MS) analysis of the oil*

An Agilent 19091S gas chromatograph coupled with a quadruple focusing mass spectrometer 433HP-5 mass detector was used. Helium was used as the carrier gas at a flow rate of 1.5 mL/min; all analyses were performed at constant flow. The GC was fitted with a 30 m by 0.25 mm fused silica capillary column coated with phenyl methyl siloxane at a split ratio of 1:50. The film thickness was 0.25 µm. The oven temperature was initially kept at 100 ^{o}C for 5 min. Afterwards, 150 ^{o}C at a rate of 4 ^{o}C/min. for 8 min. and to 250 ^{o}C at a rate of 20 ^{o}C/min. Mass detector conditions were as follows: Transfer line temperature at 300 ^{o}C, ionization mode electron impact at 70 eV. The percentage composition of the oil was computed in each case from GC peak areas. The identification of the components was done based on comparison of retention indices.

2.4. Identification of constituents in the oil

The identification of the constituents in the oil was based on (i) comparison of their retention indices (RI), calculated using a homologous series of n-alkanes (C_7–C_{30}, Supelco Bellefonte, PA, USA) under identical experimental conditions, co-injection with standards and compared with those data from Wiley 275 and NIST 08 libraries (ii) comparison of fragmentation pattern in the mass spectra of each constituent with those data from Wiley 275 and NIST 08 libraries [24-27]. The relative quantity of each constituent was calculated based on peak area of the GC (FID response) without using a correction factor.

2.5. DPPH antioxidant assay of the oil

The antioxidant potential of the oil was measured in terms of its hydrogen-donating or radical scavenging ability against DPPH, using the method reported by Ilhami, [28]. In the method, 2,2-diphenyl-1-picryl-hydrazyl, DPPH, solution (1.5 mL of 10^{-4} M, in 95% ethanol) was mixed with the oil (1.5 mL) at various concentrations (12.5-200 μg/mL) in ethanol. Each of the mixtures was shaken thoroughly and incubated in the dark for 30 minutes at ambient temperature. The control was prepared using the same procedure without the oil. The absorbance of the solution was measured at 517 nm using UV-spectrophotometer (UV-550; Jasco Inc., Japan). The assay was carried out in triplicate and the results were expressed as mean values ± standard deviation. The concentration of the oil that gave 50 % inhibition (IC_{50}) was calculated from the graph of percentage inhibition against the oil concentration. Butylated hydroxyl-toluene (BHT) was used as standard. The percentage inhibitions were calculated using the equation (1):

$$\text{Inhibition (\%)} = \frac{A_0 - A_T}{A_0} \times 100 \qquad (1)$$

where, A_0 is the absorbance of the control sample (containing all reagents except the test compound) and A_T is the absorbance of the test samples.

2.6. Statistical analysis

Tests were carried out in triplicates. The mean values were calculated from the three values. The data for various biochemical parameters were expressed as mean ± SD (n = 3) and compared using one-way analysis of variance (ANOVA) test, followed by Dunnett multiple comparison tests with equal sample size test. Values were considered statistically significant at $p < 0.05$. The IC_{50} values were calculated by non-linear regression analysis from the mean values. Statistics were done using SPSS for Windows version 10.

3. Results and discussion

Hydrodistillation of the blended rhizome of *C. rotundus* yielded 0.125 ± 0.02%(w/w) of essential oil. The yield was lower when compared to the yields of essential oils from rhizomes of the plant grown in two different locations, Empangeni (0.20%) and KwaDlangezwa (0.16%), in South Africa [16]. Similarly, higher oil yields of 2.9% and 1.8% were obtained from rhizomes of the plant harvested from Elrahad and Bano parts of the north Kordofan state of Elobeid [29]. The lower oil yield from this study might be due to unfavorable environmental conditions in north-central Nigeria as compared to South Africa and Elobeid. Table 1 shows the percentage composition, identities and retention indices of constituents of rhizome essential oil of *C. rotundus*.

The Table showed nineteen compounds which represented 97.6% of the total oil volume. Oxygenated sesquiterpenoids (44.0%) were the major class of compounds in the oil while hydrocarbon monoterpenoids constituted 41.9% of the oil. Humulene-1,2-epoxide (9.1%), β-caryophyllene (6.5%), γ-gurjunene epoxide (5.4%), β-selinene (7.9%), valencene (8.2%), α-humulene (14.3%), and caryophyllene oxide (12.3%) were the principal constituents in the oil. Other major compounds in the oil include α-copaaene (3.0%), cubenol (1.9%), β-eudesmol (2.7%), aristolone (2.8%), nootkatone (2.9%), pentadecanoic acid (3.1%), stearic acid (4.6%), and oleic acid (4.0%). Compounds that were detected in significant amounts were α-cyperene (0.8%), α-bulnesene (0.6%), α-calamenene (0.6%), and *t*-cadinol (0.9 %).

The rhizome essential oils of the plant indigenous to South Africa and China were of myrtenol chemotype [16,17]. Isologifolen-5-one and cyperene were the chemotypes of the oils of the plant native to Kordofan, Elobeid, and New Delhi, India, respectively [29, 30]. The predominant of α-humulene revealed that the oil of this study was of α-humulene chemotype. The chemotypic variation of rhizome essential oil from *C. rotundus* could be linked to differences in agroclimatic conditions of different locations in the world where the plant is grown.

Synthases of the most predominant monoterpenoids and sesquiterpenoids have been established to aid the biosynthesis of terpenic compounds that constitute essential oil [31-33]. The process involves transformation of the terpenoid precursors to various cationic intermediates followed by their deprotonation or hydration to terminate the process. The biosynthesis of all sesquiterpenoids identified in the essential oil from rhizome of this plant, was facilitated by α-humulene synthase. The rhizome essential oils of the plant grown in Empageni and KwaDlangezwa of KwaZulu, Natal Province of South

Table 1. Chemical composition of rhizome essential oil of *Cyperus rotundus*

S/N	Compounds	Composition (%)	RI[a]	RI[b]	Mass Spectra Data
1	α-Copaene	3.0	1221	1221	93,105,**119**,133,147
2	*α*-Cyperene	0.8	1398	1396	69,91,105,**119**,147
3	β-Caryophyllene	6.5	1418	1418	69,**93**,105,133, 204
4	γ-Gurjunene epoxide	5.4	**1474**	**1474**	**67**,81,95,110,122
5	β-Selinene	7.9	1485	1479	81,93,**105**,121,133
6	Valencene	8.2	1491	1484	119,133,147,**161**,175
7	α-Bulnesene	0.6	1505	1505	204,133,**119**, 93, 79
8	α-Calamenene	0.6	1521	1498	133,147,**161**,179,189
9	α-Humulene	**14.3**	1554	1552	204, 121, **93**, 80,67
10	Caryophyllene oxide	12.3	1581	1580	220, 109, **79**, 69, 41
11	Humulene-1,2-epoxide	9.1	1606	1607	43,55,**67**,81,96
12	Cubenol	1.9	1642	1641	**119**,133,147,161,204
13	β-Eudesmol	2.7	1649	1648	204, 149, 93, **59**, 55
14	*t*-Cadinol	0.9	1653	1653	105,119,**161**,189,204
15	Aristolone	2.8	1756	1752	77,119,161,175, **218**
16	Nootkatone	2.9	1800	1789	119,132,**147**,161,175
17	Pentadecanoic acid	3.1	1878	1869	60,**73**,85,98,115
18	Stearic acid	4.6	2141	2139	**43**,60,73,85,98
19	Oleic acid	4.0	2175	2144	55,69,**83**,97,123
	Compound Classes				
	Hydrocarbon Sesquiterpenoids	41.9			
	Oxygenated Sesquiterpenoids	44.0			
	Non-terpenoids	11.7			
	Total	97.6			

Compounds are listed in order of elution from fused silica capillary column coated on CP-Sil 5; RI[a] = Literature Retention Indices, RI[b] = Calculated Retention Indices, Bolded name = Chemotype.

Africa on the other hand contained monoterpenoids (whose biosynthesis was facilitated by myrtenoland β-pinene synthases respectively) that were not identified in this studied [16]. The absence of monoterpenoids in the oil could be attributed to the inactiveness of monoterpene synthases due to unfavourable agroclimatic conditions of north-central, Nigeria. Caryophyllene oxide and *α*-selinene synthases catalyzed the biosynthesis of sesquiterpenoids in the oils of the plant native to Empageni and KwaDlangezwa respectively. The synthases aided the formation of β-selinene, *α*-copaene, β-caryophyllene, *α*-humulene, caryophyllene oxide, humulene epoxide, and nootkatone. The compounds were of lower quantities compared to their quantities in the oil of this study. The higher quantities of the compounds in this oil showed that the agroclimatic conditions of this location favoured the activity of *α*-humulene synthase, thereby producing the above listed compounds in higher amounts.

Qualitatively, γ-gurjunene, valencene, *α*-bulnesene, *α*-calamenene, cubenol, β-eudesmol, and *t*-cadinol that constituted the oil were not identified in the oils of South African origin. Similarly, *α*-cyperone, caryophellenol-11, vulgarol, caryophella-3,8(13)-dien-5-β-ol, patchenol, *α*-selinene, *allo*-aromadendrene, and β-elemene that were constituents of the oils from the plant native to South Africa were not detected in this oil. This qualitative variation is attributable to differences in environmental conditions of the two countries that subsequently influenced the activities of the synthases in the plant.

The rhizome oil showed antioxidant activity by scavenging DPPH radicals. The activity of the oil was concentration dependent and increased steadily with increasing concentration and a 50% inhibitory concentration (IC_{50}) of 32.60 μg/mL was determined [Fig. 1]. The IC_{50} value of the oil revealed that the oil

was a weaker antioxidant as compared to the activity of BHT (IC_{50} = 18.12 µg/mL) that was used as reference drug. Antioxidant activity of caryophyllene oxide, β-caryophyllene, and α-humulene have been reported [34-36]. The antioxidant activity of the oil in this study could be linked to the presence of β-caryophyllene, caryophyllene oxide, and α-humulene in the oil.

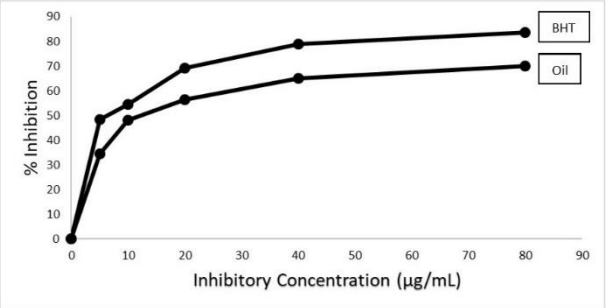

Figure 1. DPPH radical scavenging activity of rhizome essential oil of *Cyperus rotundus* and BHT.

4. Conclusions

The essential oil was isolated from rhizomes of *C. rotundus* and characterized. The characterization revealed abundance of sesquterpenoids most notably α-humullene, humulene-1,2-epoxide, β-caryophyllene, caryophyllene epoxide, valencene and β-selinene. The oil showed moderate antioxidant activity when compared to the activity of butylated hydroxy toluene that was used as standard. The oil could therefore be used to curtail the menace of oxidative stress after clinical trials.

Authors' contributions

Conceptualization, Methodology and Writing of Original Draft: IRO; Methodology, Writing-review, and Editing: ULA.

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Conflicts of interest

The authors declared that there is no conflict of interest in this manuscript.

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