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Chemical constituents and cockroach repellent activity of Sudanese *Cyperus rotundus* rhizomes essential oil

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ABSTRACT: Cockroaches are among the most adaptive creatures to the environment; they dwell on the earth's surface for the past millions of years. The presence of cockroaches is posing serious health issues in many countries of the world. Searching for an effective natural repellent agent has become a highly important attempt to control these insects. Hence, this study aimed to evaluate the chemical compositions of essential oil from Sudanese *Cyperus rotundus* rhizomes and determine their repellent activity against American cockroaches (*Periplaneta americana*). The obtained essential oil was radish yellow in color, the oil content was 0.73% and forty-four constituents were identified. The most major components were: Isolongifolol (7.63%), longiverbenone (5.61%), β-cadinene (5.54%), and longifolenaldehyde (5.16%). The oil was characterized by sesquiterpenes abundance 52.45% (oxygenated sesquiterpenes 30.72% and sesquiterpenes hydrocarbons 21.73%); whereas, monoterpenes comprised 8.43% (oxygenated monoterpenes 6.81%, and monoterpenes hydrocarbons 1.62%). The experiment showed that the essential oil possesses promising results as a repellent against *P. americana*. Therefore, conducting more research on isolation, identification of active compounds and extensive tests on other types of cockroaches are priorities in future studies.

KEYWORDS: *Cyperus rotundus*; steam distillation; essential oil; chemical compositions; repellent activity; cockroaches

1. INTRODUCTION

Cockroaches are among the most adaptive creatures to the environment; they have been in existence for millions of years. Their presence on the earth's surface is generating serious health issues. Cockroaches are widely spread across the different regions of the earth, especially in the subtropics and tropics [1]. They are hardy and common insects that can survive in different environments. A different individual may be allergic to cockroaches after frequent exposure. Sometimes, a cockroach can be a carrier of intestinal diseases such as dysentery, diarrhea, cholera, and typhoid fever [2]. Besides, there is growing interest in the use of repellents in the control of cockroaches. Several synthetic chemicals have been investigated based on their repellent actions against cockroaches, these include N,N-diethyl phenylacetamide (DEPA). Some reports have shown that several essential oils from different parts of plants possess several properties such as insect repellent [3]. Cockroach repellent is being utilized as a preventive agent in the storage and transportation of merchandise; protection of food industry facilities, sensitive communication equipment, and electronic equipment; easy access to hiding places of cockroaches including cabinet voids, plumbing ducts, electrical ducts, and crevices; and protection of sensitive places like hospitals, children's nurseries and kitchens. Although several cockroach repellents are being sold on the market; however, these synthetic-based products (including deet) are being associated with an unpleasant odor and can cause some challenges to health such as potential encephalopathic toxicity, urticaria and depression of the central nervous system [4]. Thus, investigating an eco-friendly repellent, especially from plant sources is inevitable.

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Cyperus rotundus is a plant belonging to the Cyperaceae family, it is locally called "Alsada" in Sudan. It was used as food, fodder, medicine, and insect repellent. The rhizomes of *C. rotundus* have been used in folk medicine in different regions of the world for many purposes such as anti-arthritic, anti-inflammatory, anticonvulsant and analgesic activities, itching, fever, pain, dysentery, various blood disorders, vomiting, and natural repellents [5-9]. The published literature has shown that there was a great variation in the composition of the *C. rotundus* rhizomes' essential oils around the world. The variation could be due to several factors, including the environments, climatic conditions, soil composition, geographical area, plant organ, plants' nutritional level, chemotypes, and many other parameters that can impact or affect the components of the oils. It is worth noting that, the final application of any essential oil depends entirely on its components, both quantitatively and qualitatively. Even though there are very few previous studies regarding Sudanese *C. rotundus* oils; nevertheless, none has evaluated the potency of these oils as cockroach repellent. Therefore, this study aimed to determine the chemical compositions of essential oil from Sudanese *C. rotundus* rhizome and to evaluate the repellent activity against American cockroaches.

2. RESULTS AND DISCUSSION

2.1. Yield of *C. rotundus* essential oil

The *C. rotundus* rhizomes essential oil was obtained by steam distillation. The oil content was expressed on a dry rhizome weight basis (%v/w). The oil content was 0.73% with a radish yellow color. Using a hydrodistillation extraction method, a light-yellow essential oil was achieved from dried *C. rotundus* rhizomes with a yield of 0.83% from the previous studies [10,11]. Lawal and Oyedeji (2009) used hydrodistillation to extract fresh *C. rotundus* rhizomes procured from two diverse areas gave 0.20 and 0.16% yields with pale yellowish oils [12]. Eltayeb et al. (2016) reported about three samples of *C. rotundus* rhizomes essential oils collected from different states in Sudan yielded 1.2% (Dongola) and 0.5% (Khartoum and AlGazera) [13]. In another study by Eltayeib and Um-Ismaeel (2014), it was reported that the yield of three samples of oils from Bano, Elobeid, and Elrahad Areas-North Kordofan State of Sudan were 2.9, 0.6, and 1.8 (w/w), respectively [14]. Moreover, Bajpay et al. (2018) and Al-Snafi (2016) from India and Iraq both found that *C. rotundus* rhizomes have 0.19% essential oils [15,16]. El-Gohary (2004) reported that two samples of *C. rotundus* rhizomes from Egypt have oils of 0.46 and 0.19% [17]. The variations in the recovery yields from *C. rotundus* reflect that geographical locations, soil composition, plant organ, and nutritional states of the plants can greatly influence the oil yields.

2.2. Chemical composition of *C. rotundus* rhizomes essential oil

Table 1 shows the chemical compositions of *C. rotundus* essential oil achieved by steam distillation, and Figure 1 shows the GC-MS chromatogram of the oil. Forty-four components were identified. The major oxygenated sesquiterpenes were (-)-isolongifolol (7.63%), longiverbenone (5.61%) and longifolenaldehyde (5.16%). β-cadinene (5.54%), α-copaene (3.72%), γ-muurolene (1.92%), and rotundene (1.91%) are the main sesquiterpene hydrocarbons. Compounds (IR)-(-)-myrtenal (2.01%) and E-pinocarveol (1.73%) are the important oxygenated monoterpenes; whereas, pseudopinen (0.56%) is the highest monoterpene hydrocarbon in the obtained oil. Janaki et al. (2018) reported that elemenone (13.59%), α-cyperone (13.41%) and caryophllene oxide (13.03%) were the most important sesquiterpenes of *C. rotundus* essential oil from Iran [18]. In addition, Hu et al. (2017) reported a total of 30 identified components from Chinese *C. rotundus* oil [10]. The α -cyperone (38.46%), cyperene (12.84%) and α -selinene (11.66%) were the major components in the essential oil of *C. rotundus* rhizomes from China, followed by β -caryophyllene oxide (4.33%), (d)-limonene (3.62%), α -calacorene (3.14%), and γ -muurolene (3.13%) [11].

Table 1. GC-MS analysis of chemical analysis of chemical constituents of *C. rotundus* rhizomes essential oil.

Compound	Formula	Systematic name	Area (%)
Monoterpene hydrocarbons			
alpha-Pinene	$C_{10}H_{16}$	2,6,6-Trimethylbicyclo[3.1.1]hepta-2-ene	0.44
Thujadiene	$C_{10}H_{14}$	Bicyclo[3.1.0]hex-2-ene,4-methylene-1-(1-methylethyl)	0.05
Pseudopinen	$C_{10}H_{16}$	Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene	0.56
o-Cymene	$C_{10}H_{14}$	Benzene,1-methyl-2-(1-methylethyl)	0.19
Limonene	$C_{10}H_{16}$	Cyclohexene,1-methyl-4-(methylethenyl)	0.32
p-Cymenene	$C_{10}H_{12}$	Benzene,1-methyl-4-(1-methylethenyl)	0.06
Total of monoterpene hydrocarbons			1.62
Oxygenated monoterpenes			
Cineole	$C_{10}H_{18}O$	2-oxabicyclo[2.2.2]octane,1,3,3-trimethyl	0.11
Fenchol	$C_{10}H_{18}O$	Bicyclo[2.2.1]heptan-2-ol,1,3,3-trimethyl	0.19
Camphenol	$C_{10}H_{16}O$	5,5-dimethyl-6-methyenebicyclo[2.2.1]heptan-2-ol	0.09
E-Pinocarveol	$C_{10}H_{16}O$	Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene,[IS-(1.alpha.,3.alpha.,5.alpha.)]	1.73
Cis-Verbenol	$C_{10}H_{16}O$	Bicyclo[3.1.1]hept-3-en-2-ol,4,6,6-trimethyl,(1.alpha.,2.beta.,5.alpha.)	0.17
Pinocarvone	$C_{10}H_{14}O$	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptan-3-one	0.29
Isoborneol	$C_{10}H_{18}O$	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethl,exo	0.21
Terpinenol-4	$C_{10}H_{18}O$	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	0.32
Benzenemethanol	$C_{10}H_{14}O$	1-methyl-4-(.alpha-hydoxyisopropyl)benzene	0.11
Terpineol schlethin	$C_{10}H_{18}O$	3-Cyclohexene-1-methanol,alpha.,alpha.4-trimethyl	0.71
(IR)-(-)-Myrtenal	$C_{10}H_{14}O$	6,6-dimethyllbiclo[3.1.1]hepta-2-ene-2-carbaldehyde	2.01
Vebenone	$C_{10}H_{14}O$	Bicyclo[3.1.1]hepta-3-en-2-one,4,6,6-trimethyl	0.33
Cis-Carveol	$C_{10}H_{16}O$	2-Cyclohexane-1-ol,2-methyl-5-(1-methylethenyl)	0.15
(-)-Carvone	$C_{10}H_{14}O$	2-cyclohexane-1-one,2-methyl-5-(1-methylethenyl)	0.12
Royaltac	$C_{10}H_{22}O$	n-decan-1-ol	0.27
Total of oxygenated monoterpenes			6.81
Sesquiterpene hydrocarbons			
Beta-Vatirenene	$C_{15}H_{22}$	8,8a-dimethyl-2-(1-methylethylidene)-1,23,7,8,8a-hexa hydronaphthalene	0.35
Alpha-Ylangene	$C_{15}H_{24}$	8-Isopropyl-1,3-dimethyltricyclo[4.4.0.0$^{2,7.}$]dec-3-ene	1.65
Alpha-Copaene	$C_{15}H_{24}$	8-Isopropyl-1,3-dimethyltricyclo[4.4.0.0$^{2,7.}$]dec-3-ene	3.72
Beta-Elemene	$C_{15}H_{24}$	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylenyl)-,[1S-(1.alpha.,2.bata.,4.beta.)]	0.53
	$C_{15}H_{24}$	Naphthalen,1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1.alpha.,4a.beta.,8a.alpha.)-(+/-)	5.54
	$C_{15}H_{24}$	1,4,7-Cycloundecatriene,1,5,9,9-tetramethyl-Z,Z,Z	0.87
Rotundene	$C_{15}H_{24}$	1,5,9-Trimethyltricyclo[6.2.2.0$^{2,6.}$]dedec-9-ene	1.91
Gamma-muurolene	$C_{15}H_{24}$	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.alpha.,8a.alpha.)	1.92

Table 1 continued

Compound	Formula	Systematic name	Area (%)
Sesquiterpene hydrocarbons			
Alpha-Muurolene	$C_{15}H_{24}$	[1.alpha.,4a.alpha.,8a.alpha.]-,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-[1-methylethyl]naphthalene	0.55
Trans-calamenene	$C_{15}H_{22}$	4-Isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	1.59
AlphaCalacorene	$C_{15}H_{20}$	1-Isopropyl-4,7-dimethyl-1,2-dihydronaphthalene,(S)	0.60
	$C_{15}H_{24}$	1HCyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-.[3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*	0.65
	$C_{15}H_{24}$	Bicyclo[5.2.0]nonane,4-methylene-2,8,8-trimethyl-2-vinyl	0.62
Longifolene-(V4)	$C_{15}H_{24}$	1,5,5,8a-tetramethyl-1,2,3,3a,4,5,6,8a-octahydro-1,4-methanoazulene	1.23

Total of sesquiterpene hydrocarbons			21.73
Oxygenated sesquiterpenes			
Caryophyllene oxide	$C_{15}H_{24}O$	5-Oxatricyclo[8.2.0.0(4,6)-]dodecane,4,12,12-trimethyl-9-methylene[1R-(1R*,4R*,6R*,10R*)]	0.61
Spathulenol	$C_{15}H_{24}O$	1H-Cycloprop[e]azulen-7-ol,dechydro-1,1,7-tri methyl-4-methylene-[1ar-(1a.alpha.,4a.alpha., 7.beta.,7b.alpha.)]	0.86
(-)-Isolongifolol	$C_{15}H_{26}O$	1,4-Methanoazulene-9-methanol,dehydro-4,8,8-tri methyl-[1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9R*)]	7.63
Isologifolen-5-one	$C_{15}H_{22}O$	Isologifolen-5-one	1.32
Longifolenaldehyde	$C_{15}H_{24}O$	Longifolenaldehyde	5.16
	$C_{15}H_{24}O$	Tetracyclo[6.3.2(2,5).0(1,8)tridecan-9-ol,4,4-dimethyl	3.33
Longiverbenone	$C_{15}H_{22}O$	2,6,6,11-Tetramethyltrcyclo[5.4.0.02,8]undec-10-en-9-one	5.61
Aristol-9-en-8-one	$C_{15}H_{22}O$	2H-Cyclopropa[a]naphthalene-2-one,1,1a,4,5,6,7,7a, 7b-octahydro-1,1,7,7a-tetramethyl-,(1a.alpha.,7.alpha., 7a.alpha.,7b.alpha.)	4.26
Ledol	$C_{15}H_{26}O$	1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetra methyl-,[1ar-(1a.alpha.,4.alpha.,4a.beta.,7.alpha., 7a.beta.,7b.alpha.)]	1.94
Caryophyllene oxide	$C_{15}H_{24}O$	5-Oxatricyclo[8.2.0.0(4,6)-]dodecane,4,12,12-trimethyl-9-methylene[1R-(1R*,4R*,6R*,10R*)]	0.61
Spathulenol	$C_{15}H_{24}O$	1H-Cycloprop[e]azulen-7-ol,dechydro-1,1,7-tri methyl-4-methylene-[1ar-(1a.alpha.,4a.alpha., 7.beta.,7b.alpha.)]	0.86
(-)-Isolongifolol	$C_{15}H_{26}O$	1,4-Methanoazulene-9-methanol,dehydro-4,8,8-tri methyl-[1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9R*)]	7.63
Isologifolen-5-one	$C_{15}H_{22}O$	Isologifolen-5-one	1.32
Longifolenaldehyde	$C_{15}H_{24}O$	Longifolenaldehyde	5.16
	$C_{15}H_{24}O$	Tetracyclo[6.3.2(2,5).0(1,8)tridecan-9-ol,4,4-dimethyl	3.33
Total of oxygenated sesquiterpenes			30.72

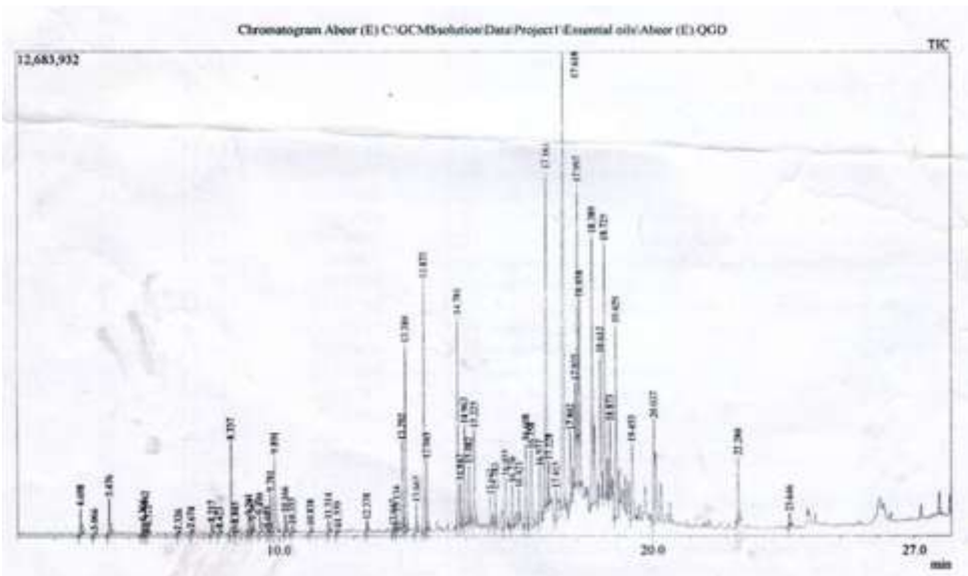

Figure 1. GC-MS chromatogram of the *C. rotundus* rhizomes essential oil.

El-Gohary (2004) mentioned that 52 components were identified in the essential oil of Egyptian *C. rotundus*; α-ylangene (9.35%), α-cyperone (9.07%) transpinocarveol (7.92%), and cyperene (7.83%) were the major constituents in the essential oil [17]. Aghassi et al. (2013) reported that the cyperene (37.90%) was the dominant constituent of essential oil of Iranian *C. rotundus*. Other components present at appreciable content were cyperotundone (11.2%), isorotundene (9.50%), cyperol (6.40%), α-cyperone (4.30%), and β-pinene (3.90%) [19]. Moreover, Morimoto and Komai (2005) claimed that two sesquiterpene ketones namely; cyperotundone (0.26%) and α-cyperone (0.1%) were isolated from dried tubers of Japanese *C. rotundus* as major constituents [20]. In addition, Xiao-shan et al. (2006) and Sofia et al. (2014) reported that the main components of *C. rotundus* essential oil are α-cyperone(26.15%), β-selinene (17.99%), cyperene (15.73%) [21,22].

In this study, the *C. rotundus* rhizomes essential oil was characterized by sesquiterpenes abundance, the percentages are oxygenated sesquiterpenes (30.72%), sesquiterpene hydrocarbons (21.73%), oxygenated monoterpene (6.81%), and monoterpene hydrocarbons (1.62%). The terpenes percentages (%) (mono- and sesquiterpenes) of *C. rotundus* essential oil in this study are shown in Figure 2. Aghassi et al. (2013) reported that the Iranian *C. rotundus* oil has terpenes as sesquiterpene hydrocarbons (54.90%), oxygenated sesquiterpenes (35.60%) and monoterpenes (6.40%) [19]. Janaki et al. (2018) also mentioned that around 74.53% of the total compositions of *C. rotundus* oil from Iran were sesquiterpenes [18]. Moreover, Das et al. (2015) in their review on *C. rotundus* essential oils mentioned that the chemical compositions were quantitatively distinguished through a higher percentage of sesquiterpenes (70%), a lower percentage of oxygenated monoterpenes (10%) and monoterpene hydrocarbons compound (5%) [23]. On the other hand, Lawal and Oyedeji (2009) in their study on two types of essential oils from *C. rotundus*; collected from two separate areas in South Africa namely KwaDlangezwa and Empangeni. It was found that the oil from Empangeni's *C. rotundus* had the highest sesquiterpenes (59.80%) compared to monoterpenes (29.10%). However, the oil from that of KwaDlangezwa had relatively closer contents of sesquiterpenes (45.90%) and monoterpenes (46.10%) [12].

The results from this study compared with the previously published articles clearly showed that there are variations in terms of components of the *C. rotundus* oils; this confirms the existence of more chemical diversities within the *C. rotundus* species. This diversity and variation can be justified for several reasons such as extraction methods, environments, soil composition, geographical area, plant organ, and nutritional status of the plants.

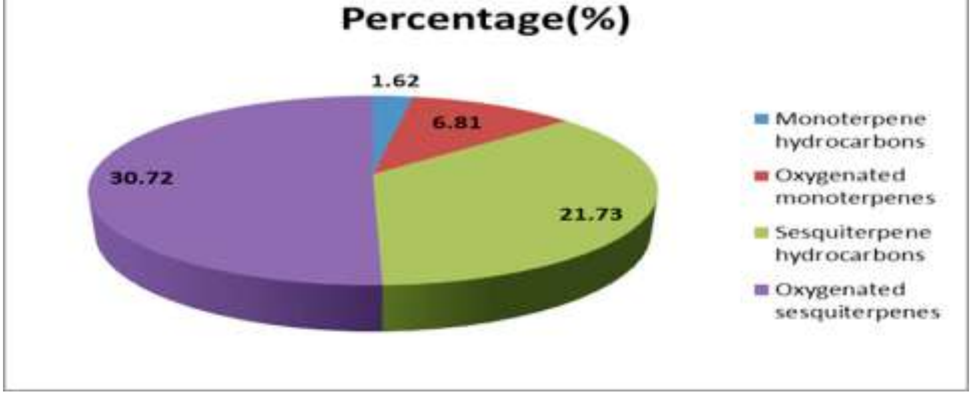

Figure 2. Distribution of mono and sesquiterpenes in *C. rotundus* rhizomes essential oil.

2.3. Repellent activity of *C. rotundus* rhizomes essential oil

The oil showed good repellent activity against American cockroaches. For 100% concentration of the oil, the repellence reached 100% up to 3 h of exposure, while the standard (naphthalene) reached 100% for more than 5 h. However, the repellence reaches 90% for more than 9 h of exposure, and more than 80% after 24 h. Figure 3 shows the repellent activity of *C. rotundus* rhizomes essential oil against American cockroaches after 72 h of treatment. The IC_{50} and IC_{90} values of *C. rotundus* rhizomes essential oil against American cockroaches were 57 and 88%, respectively. Figure 4 shows the IC_{50} and IC_{90} values of *C. rotundus* rhizomes essential oil against American cockroaches after 24 h of treatment. The repellence activity of the oil could be attributed to the presence of compounds such as myrtenal, caryophyllene oxide, pinene, limonene, terpinen-4-ol, o-cymene, carvone, 1,8-cineole, and cymenene. These compounds have previously proven their repellent activity toward certain insects including cockroaches.

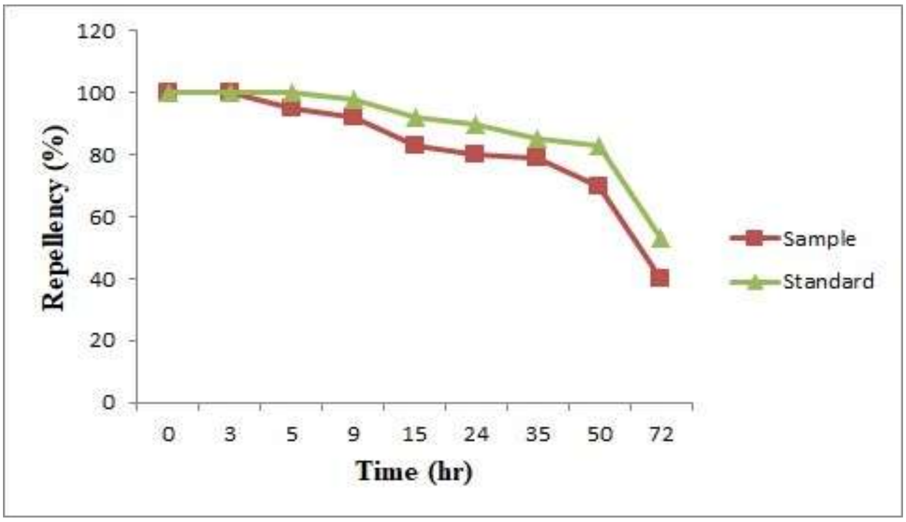

Figure 3. Repellence activity of *C. rotundus* rhizomes essential oil (100%) against American cockroaches after 72 h of treatment.

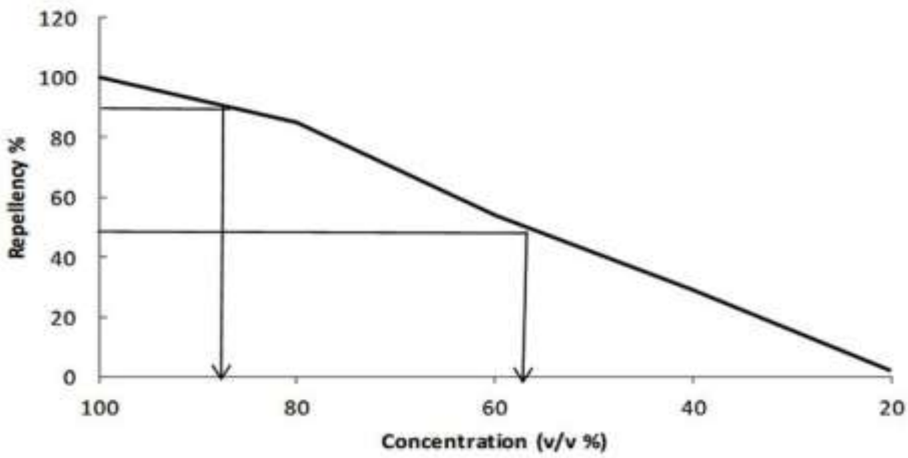

Figure 4. The IC_{50} and IC_{90} values of *C. rotundus* rhizomes essential oil against American cockroaches after 24 h of treatment.

It is noted that about 12.60% of cockroaches died in this repellence test. Then, it can be assumed that the oil is toxic to the cockroaches; this observation agreed with some previous studies. Phillips (2009) reported that the chemical component, α-pinene which has been proved to repel cockroaches was slightly toxic to the adult male of American cockroaches [24]. Moreover, researchers investigated the fumigant toxicity of 12 components of essential oils including 1,8-cineole, carvacrol, trans-cinnamaldehyde, eugenol, citronellic acid, geraniol, S-(-)-limonene, (-)-linalool, (+)-alpha-pinene, (-)-menthone, thymol, and (-)-beta-pinene against German cockroach. It was found that 1,8-cineole was the most toxic component of the essential oils to adult males, females, and nymphs [25]. Therefore, the presence of such compounds (e.g, α-pinene, limonene, alpha-pinene, and 1,8-cineole) in *C. rotundus* rhizomes essential oil can justify the mortality of the cockroaches.

Yoon et al. (2009) investigated the repellent efficacies of certain essential oils components, the results varied with different doses and cockroach species. It was reported that the major components responsible for the repellent activity were γ-terpinene, β-pinene and limonene [26]. El-Seedi et al. (2012) claimed that the oil with main compounds of 1,8-cineole, 4-terpineol and carvone was the most repellent oil among many oils tested against cockroaches [27]. Moreover, Liu et al. (2011) reported that the essential oil of *C. rotundus* contains α-pinene (28.74%), 1,8-cineole (27.18%), spathulenol (6.63%), globulol (6.53%), and ρ-menth-1-en-8-ol showed strong repellence against nymphs of German cockroaches [8]. Also, essential oils containing α-pinene is used as insecticides, solvents, and perfume bases [28]. Paranagama and Ekanag (2004) investigated the repellent properties of fresh rhizomes of *Alpinia calcarata* against *P. Americana,* it was claimed that the oil showed higher repellent properties than the control because the oil has components such as α-pinene, camphene, 1,8-cineole, and camphor [29].

It is worth noting, many single isolated compounds from plants are used and approved to have repellent activity against insects; these include cineole [30, 31], camphene against German cockroaches [32], limonene [33-36], terpinene 4-ol and β-elemene [37-39], caryophyllene oxide [40, 41], o-cymene [1], p-cymene [42], α-copaene, -α-pinene, carvone, γ-muurolene, α-ylangene longifolene, myrtenal, pinocavreol, α-muurolene, and α-calacorene [43].

3. CONCLUSION

The chemical constituents of oil extracted from Sudanese *C. rotundus* rhizome had been studied alongside the evaluation of the extracted oil against American cockroaches. The obtained results established the presence of forty-four chemical components. Out of these chemical components, (-)-isolongifolol, longiverbenone, β-cadinene, and longifolenaldehyde were more prominent. Moreover, the repellence activity against American cockroaches was significant. The repellence efficacy observed in this oil might be because of the existence of compounds such as myrtenal, caryophyllene oxide, pinene, limonene, terpinen-4-ol, o-cymene, 1,8-cineole, carvone, and cymenene. These have potentially positioned *C. rotundus* oil as a useful natural repellent against *P. americana*. Therefore, conducting more studies on the isolation, identification of active compounds and extensive tests on other types of cockroaches are priorities in future findings.

4. MATERIALS AND METHODS

4.1. Plant material

The *C. rotundus* rhizomes were procured on 13 October 2017 from Aldaba (River Nile State), Sudan. The taxonomic identification of *C. rotundus* rhizomes was performed by a botanist from the National Centre for Research, Department of Biology, Khartoum, Sudan. The samples were sealed and kept in a desiccator to avoid any fungal activities.

4.2. Extraction of essential oil

The dry *C. rotundus* rhizomes were used to obtain the essential oil through Steam Distillation (SD). In brief, 100 g of dried and crushed rhizomes were steam distilled for 4 h. The distilled essential oil was dried over anhydrous sodium sulphate, filtered and kept in hermetically closed dark bottles at -4 °C prior to further analysis. The percentage (v/w%) of the essential oil was estimated using Eq. (1).

$$\text{Essential oil } (\%) = \frac{\text{Volum of oil (v)}}{\text{Weight of sample (g)}} \times 100 \qquad (1)$$

4.3. GC-MS analysis

The essential oil extracted from *C. rotundus* was analyzed using an Agilent 7890A Gas Chromatography-Mass spectrometry of Model 3171A. Table 2 shows the chromatographic settings for the analysis.

Table 2. Chromatographic settings for the analysis of *C. rotundus* rhizomes essential oil.

Parameters	Settings
Chromatograph	Agilent Technologies 7890A GC Systems coupled with MS detector
Auto-sampler	GC auto sampler
Column	Nonpolar capillary DB-1 of 100% dimethyl-polysiloxane (30 m, 0.25 mm i.d, film thickness 0.25 µm)
Carrier gas	Helium
Gas flow rate	1 mL/min
Injector mode	Splitless mode
Injector temp.	250 °C
Injection volume	1µL/L
Temp. program	60 °C for 3 min, 240 °C at the rate of 3 °C/min and held for 10 min
Runtime	93 min
Lab data system	NIST Library Chem Station software v.1.7.

4.4. Cockroaches' collection and repellent test

Approximate 600 adults (both male and female) of American cockroaches (*P. americana*) were collected from the University of Khartoum, Sudan. The cockroaches were reared continuously for several generations and kept under 25-30 °C, 70-80% relative humidity, and photoperiod of 13:11 h (light/dark) from September to December 2017. They were kept in boxes and reared by feeding them with biscuits and water in the laboratory. The considered cockroaches for repellent tests were the healthy adults and nymphs (male and female). The temperature was maintained at 28 ± 5 °C. Furthermore, the Ebeling choice box test which was described by Ebeling et al. (1966) with some modifications was used in this experiment [44]. Briefly, one square box (choice box) was divided into two equal-size compartments by partition. In this experiment, three number of choice-boxes were joined together to form a set of the test box. The medium size transparent microwave tableware was used as the choice box for the repellent test. Eight sets of test boxes were set up. The right-side choice box is considered as the untreated zone where 25 g of milled food (Jacob's biscuit) were filled into the centre of the box and about 1% of DMSO was carefully dropped around the food. The same treatments were applied to the other test box. The DMSO was the negative control of the assay which does not affect the repellence activity against cockroaches. The left-side choice box is considered as the treated zone where the test sample (*C. rotundus* rhizomes essential oil) was carefully dropped around the food at the centre of the box. Naphthalene was used as the positive control standard. One piece (1 g) of naphthalene was ground into coarse powder form and placed together as the treatment in the treated zone. The *C. rotundus* rhizomes essential oil was prepared in various concentrations (5, 25, 50, 75, and 100 v/v%) by dissolving in 1% of DMSO. Ten adult and nymph cockroaches (male and female) were then released into the central choice box (untreated zone). Thereafter, the selected boxes (untreated and treated zone) were borne to a photoperiod of 72 h at 27 ºC for 3 days. The cockroaches situated at the untreated and treated zones were carefully monitored and counted for 0, 3, 6, 9, 12, 24, 48, and 72 h of treatments. Each treatment with different concentrations was carried out in three replicates. The percentage of repellence was estimated using Eq. (2).

$$\text{Repellence }\% = 100 - \left(\frac{T}{N} \times 100\ \%\right)$$

Where T is the number of cockroaches situated at the treated zone and N indicates the total number (ten heads) of cockroaches utilized in the repellence test. Then, the mean percentage of repellence was estimated from the values obtained in three replicates.

4.5. Statistical analysis

The statistical analysis of the results was done using MS Excel (2007)- version 12.0.4518.1014. The results were performed in three repetitions and expressed as mean ± standard deviation.

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