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Anticandidal activity of the extract and compounds isolated from *Cyperus conglomertus* Rottb

Ghaferah H. Al-Hazmi [a], Amani S. Awaad [b,*], Monerah R. Alothman [c], Saleh I. Alqasoumi [d]

[a] Department of Chemistry, College of Science, Princess Nourah bint Abdulrahman University, PO Box 84428, Riyadh, Saudi Arabia
[b] Pharmacognosy Department, College of Pharmacy, Prince Sattam bin Abdul-Aziz University, Al-Kharj, Saudi Arabia
[c] Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia
[d] Department of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia

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ABSTRACT

The phytochemical screening of *Cyperus conglomeratus* showed that carbohydrates and/or glycosides, flavonoids, tannins, sterols and/or triterpenes, and proteins and/or amino acids are present. The fatty acid profile comprised major; palmitic, oleic, heptadecanoic, linoleic and minor; arachidonic, lignoceric, stearic, and myristic acid. Two compounds; namely, α-amyrin and β-sitosterol were isolated by the fractionation of unsaponifiable matter.

The acute toxicity study showed that the reported after oral administration of the alcohol extract (**TAE**) showed that the plant was highly safe as the LD_{50} was more than 4000 mg/kg. These results were well supported by the sub-chronic toxicity, as the **TAE** administrated to rats for 15 consecutive days at dose 1000 mg/kg showed no alteration in the liver and kidney functions. Moreover, the extract of the plant exhibited anti-candidal activity against different *Candida* species. The most potent activity, (23.1 ± 2.1, 0.98 μg/ml) and (22.3 ± 0.53, 0.98 μg/ml), was obtained by the chloroform and total extract, respectively against *Candida albicans*.

1. Introduction

The Cyperaceae are one of the largest monocotyledonous family (Filip, 2014). The genus *Cyperus* is comprises approximately 600 species and its widely distributed in different countries in tropical, sub-tropical and temperate regions (Follak et al., 2016). A member of the genus *Cyperus*, *Cyperus rotundus*, have been frequently used as multi-purpose medicinal plant in folk medicine throughout all world for treatment of stomach, wounds, blisters, and boils (Puratuchikody et al., 2006; Joshi and Joshi, 2000). Also *C. rotundus* exhibited different biological and pharmacological activities such as antidiabetic, anti-inflammatory, antidiarrhoeal, antimutagenic, cytoprotective, cytotoxic and apoptotic, antipyretic, analgesic, antioxidant, anticandidal, antimalarial, and antibacterial activities (Hema et al., 2013; Kilani et al., 2008, 2007; Sundaram et al., 2008; Raut and Gaikwad, 2006; Uddin et al., 2006; Durate et al., 2005).

The phytochemical studies of *Cyperus rotundus* showed the presence of flavonoids, alkaloids, glycosides, tannins, polyphenol, furochromones, starch, quinines, and saponins (Seabra et al., 1997). Moreover, α-pinene, β-pinene, α-copaene, cyperene, cyperotundone, α-cyperone, caryophyllene oxide, myrtenol, and α-selinene were isolated from essential oil of *C. rotundus* (Hema et al., 2013; Zoghbi et al., 2008; Kilani et al., 2005; Jirovetz et al., 2004).

Another species of the genus *Cyperus*, *C. conglomeratus*, has been found to overcome the extreme condition (Ghahreman, 2000). *Cyperus conglomeratus* is used in traditional medicine as antimicrobial, emollient, diuretic, stimulant, anthelmintic and analgesic treatment (Feizbakhsh and Naeemy, 2011). The present study aimed to evaluate the anticandidal activity of *Cyperus conglomeratus* and isolate the bioactive compounds from it.

2. Material and methods

2.1. Plant materials

The aerial parts of *Cyperus conglomeratus* Rottb (Cypraceae) were collected during flowering stage in 2014, from Jizan territory

* Corresponding author.
E-mail address: a.awaad@psau.edu.sa (A.S. Awaad).

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of Saudi Arabia. The plant samples were collected in specific containers and identified by Dr. Jacob T. Pandalayil, Assistant Professor of Plant Taxonomy, Botany and Microbiology Department, Faculty of Science, King Saud University and, for conformation, compared with the published plant description in Flora of Saudi Arabia (Migahid, 2002). A voucher specimen has been deposited in the herbarium of Faculty of Sciences, King Saud University. The plant materials were dried in well ventilated shaded place, reduced to fine powder, packed in tightly dark closed containers and stored for the studies.

2.2. Phytochemical screening, extraction and isolation

2.2.1. Phytochemical screening

The plant powder of *Cyperus conglomeratus* Rottb was phytochemicaly screened for its constituents of different phytochemical groups using the standard published methods by Khan et al. (2011).

2.2.2. Extraction and isolation

The dried powder of *Cyperus conglomeratus* Rottb (500 g) were extracted by percolation in 95% aqueous ethanol (1 L) at the room temperature till exhaustion for two days and filtered off. The mark lifted was re-extracted again by the same way, this process was repeated four times till complete exhaustion. The ethanol was removed from the combined extracts using rotatory evaporator under reduced pressure at a temperature not exceeding 25 °C. The obtained total dry extract (120 g) was suspended in water (500 ml) and extracted with chloroform (several times) till complete exhaustion. Chloroform extracts were collected together and dried from any excess of water residue through its filtration over anhydrous sodium sulphate. The extract was freed from chloroform using rotatory evaporator as before to yield 35.8 g.

For isolation of the active compounds, residue obtained from chloroform extract (20 g) was subjected to saponified using the published method described by Mathew et al. (2007). From saponification two portion were obtained (Saponifiable and unsaponifiable matters) with total weight of 10 and 20 g respectively.

Saponifiable matter was investigated using gas-liquid chromatography after methylation as described in published references (Ichihara and Fukubayashi, 2010). On the other hand, unsaponifiable matter was subjected to isolation of its contents using column chromatography. 15 g of un-saponifiable matter was applied on the top of glass column (130 × 3 cm) packed with neutral alumina (200 g), eluted using benzene-ethyl acetate (86:14 v/v). Handed fractions were collected (50 ml each) and reduced to three sub- fraction (according to number, colure and Rf of the spots), each fraction was concentrated and symbolized as A, B & C with total weight of 3.5, 2.8 and 6.5 g. respectively. Each subfraction was subjected to multiple columns, eluted with different proportion of benzene gradually increased by different proportion of chloroform, from which two compounds C1 and C2 were isolated and identified using different instrumental analysis.

2.3. Pharmacological study

2.3.1. Animals

Mice and rats of both sex (Swiss albino) were used their weights were ranged between 25 and 30 g for mice and 180–200 g for rats. All animals were purchased from the animal house of King Saud University, KSA. All animal were maintained under standard conditions (temperature 23 ± 1.0 °C, humidity 55 ± 10%, 12 h light/12 h dark cycle). They allowed to adapt to the laboratory environment for one week before experimentation and fed with a standard pellet diet with water *ad libitum*.

2.3.2. Preparation of the extracts for biological studies

Hundred gram of the dried plant under investigation (*Cyperus conglomeratus* Rottb) were extracted by percolation in 95% ethanol as previously mentioned (Section 2.2.2). The dried plant extract was freshly suspended in distilled water just before administration by the aid of Tween 80.

2.3.3. Acute toxicity (LD_{50}) test

for testing the acute toxicity. Known weight of the extract was suspended in saline by the aid of few drops of tween 80 to be in a concentration of 2 g/ml and dilutions. Five groups of mice each of six were used in the present experiment. Doses of the extract varying from 1 to 16 g/kg body weight were orally administered to mice. Each group was watched for 24 h in order to record any abnormal signs, symptoms or mortality.

2.3.4. Effect on liver and kidney functions

Rats were divided into 3 equal groups each of 10 rats. The 1st group was left as a control, while the 2nd & 3rd groups were orally given the total alcohol and chloroform extracts respectively in a dose of 500 mg/kg for 15 days. Blood samples were collected from the orbital plexus of rats, 6 h after the last dose of each extract. Samples were left to clot at room temperature for 20 min. The obtained sera were collected and used to determine the activity of (AST) aspirate aminotransferase and (ALT) alanine aminotransferase. Levels of urea, creatinine were also estimated according to published (Erbayraktar et al., 2017).

2.4. Anticandidal activity

2.4.1. Anticandidal assay

Pure cultures of *Candida albicans*, *Candida dubliniensis*, *Candida famata*, *Candida glabrata*, *Candida inconspicua* were used as test organisms. The anticandidal activity of *Cyperus conglomeratus* extract was determined by using two methods Disc-diffusion method and well-diffusion method (Murray et al., 1995). Petri dishes containing 20 ml of malt extract agar medium were seeded with 48-h cultures of *Candida* species. Wells, 6 mm, were cut off and 50 μL of *C. conglomeratus* extract was added and incubated at 37 °C for 3 days. The diameter of the inhibition zone formed around the well indicated the presence of anticandidal activity.

2.4.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by well-diffusion method (Akinyemi et al., 2005). The MIC was determined using twofold dilutions for concentrations from 0.0 to 10 mg/ml. A 100 μL of the plant extracts was added into the wells and incubated at 37 °C for 3 days. The MIC was determined as the lowest concentration of the plant extract that produced no visible microbial growth (clear zone) around the well.

3. Results and discussion

3.1. Phytochemical screening

The primary phytochemical screening of *Cyperus conglomeratus* indicated the presence of carbohydrates and/or glycosides, flavonoids, tannins, sterols and/or triterpenes, and proteins and/or amino acids. While different chemical group such as alkaloids and/or nitrogenous bases, cardinolides, saponins, anthraquinones, volatile oil and oxidase enzyme were absent.

The gas liquid chromatography (GLC) of the saponifiable matter showed the presence of 14 fatty acids (Table 1). The major, highest concentration, fatty acids were stearic acid (33.05%), myristic acid (23.60%), palmitolic acid (14.36%) and behenoic acid (10.89%). The

Table 1
The GLC analysis of fatty acid methyl esters of *Cyperus conglomeratus* saponifiable matter.

Peak number	Rt (min)	RRt	No. C	Authentic methyl ester of	Percentage
1	6.40	0.33	10:0	Capric acid	0.04
2	11.58	0.60	12:0	Lauric acid	0.34
3	12.27	0.64	13:0	Tridecanoic acid	0.69
4	13.35	0.69	14:0	Tetradecanoic acid	2.76
5	14.02	0.73	14:0	Myristic acid	23.60
6	15.02	0.78	14:1	Myristiolic acid	5.87
7	15.88	0.83	16:0	Palmitic acid	2.87
8	18.12	0.94	16:1	Palmitolic acid	14.36
9	19.23	1.00	18:0	Steric acid	33.05
10	20.23	1.05	18:1	Oleic acid	0.37
11	21.13	1.09	18:2	Linoleic acid	3.91
12	26.80	1.39	20:4	Arachidonic acid	0.20
13	29.37	1.53	22:6	Henatriacontanoic acid	0.68
14	37.05	1.93	22:0	Behenoic acid	10.89

Rt: retention time, RR_t: relative retention time, No. C: number of carbon.

minor, lowest concentration, fatty acids were caproic acid (0.04%), arachidonic acid (0.20%), lauric acid (0.34%), oleic acid (0.37%).

The fractionation of un-saponifiable matter using column chromatography lead to isolation of two compounds (C1 and C2), which were analyzed and identified using m.p., spectroscopic analysis; IR, and ^{1}H NMR & ^{13}C NMR.

The first compound (C1): obtained as whitish crystal residue (36.8 mg) with R_f = 0.69 using system (Benzene: ethyl acetate 86/14), m.p. (185–186 °C). ^{1}H NMR Spectrum of compound in chloroform-D3 showed the following: Triplet at δ 5.60 ppm (1H t, J = 5.8, H-12), singlet at δ 3.45 ppm (1H, s, —OH), triplet at δ 2.07 ppm (1H, J = 4.8H-3) nearest from —OH, multiplet at δ 1.81 ppm (4H, m, H-1 & H-2), multiplet at δ 1.59 ppm (6H, m, H-23 & H-24), multiplet at 1.48 ppm (6H, m, H-29 & H-30) (Table 2).

^{13}C NMR Spectrum in $CDCl_3$ recorded 30 carbons, and DEPT-135 revealed the presence of seven quaternary carbons. Spectra showed C=C at δ142.07 (C-13), δ122.13 (C-12), also one carbon at δ 76.48 (C-3) next to —OH group, at δ 60.14 (C-18), δ 51.84 (C-9) hithermost of double bound, at δ 50.33 (C-5), at δ 44.36 (C-16), at δ 42.96 (C-4), at δ 40.96 (C-8), at δ39.42 (C-14), at δ38.72 (C-10), at δ 35.52 (C-11), at δ34.93 (C-17), at δ 34.25 (C-2), at δ 29.19 (C-20), CH_3 group at δ 30.92 (C-26), four CH_2 group at δ 29.20 (C-1), δ 29.09 (C- 6), at δ 28.44 (C-7) and 27.89 (C-19), three CH_3 group position see in δ 25.61 (C-25), at δ 23.06 (C- 23) and 22.08 (C-24), at δ 24.16 (C-15), at δ 20.03 (C-22), at δ 18.18 (C-21), also CH_3 group position at δ 17.99 (C-27), δ 16.20 (C-28), δ 15.87 (C-29), and δ 15.12 (C-30) (Table 3). Accordingly, this compound identified as α-amyrin (Fig. 1).

The second compound (C2): obtained as whitish crystal residue (100.8 mg) with R_f 0.56 using system (Benzine :ethyl acetate 86/14), m.p (137–138 °C). ^{1}H NMR Spectrum of compound C2 in chloroform-D3 showed the following: Triplet at δ 5.34 ppm (1H d, J = 2.2 Hz, H-6), singlet at δ 3.51 ppm (1H, s, —OH), at δ 2.26 ppm (2H q, J = 4.5 Hz, H-3) nearest from —OH, triplet at δ 1.98 ppm (2H,t, J = 3.1 Hz, H-5 & H-8), at δ 1.83 ppm (3H, t, H-28), sextet at 1.63 ppm (1H,s, H-18), singlet at 1.57 ppm (8H,s,H-1,H-2,H-15 & H-16), at 1.33 ppm (5H, m, H-9, H-11, & H-12), multiplet at 1.14 ppm (6H, m, H-4,14,21,17, & 22), at 1.12 ppm (6H, d, H-29 & H-30), at 0.91 ppm (4H d, J = 6.4 Hz, H-19 & H-20), singlet at 0.81 ppm (9H, s, H-24, H-25 & H-26), singlet at 0.66 ppm (3H,s, H-23) (Table 4). ^{13}C NMR in $CDCl_3$ showed 30 carbons, and DEPT-135 revealed the presence of three quaternary carbon C-6 at δ 140.87 ppm, δ42.43 (C-10), at δ 36.62 (C-13), rest of the results were listed in Table 5. From previous data this compound was identified as β-sitosterol (Fig. 1).

Table 2
^{1}H NMR chemical shift assignments of C1.

Proton no.	Chemical shift ppm	Proton no.	Chemical shift ppm
H-12	5.60(1H,t,J = 5.8 Hz)	H-25	1.12(3H,s)
—OH	3.45(1H,s)	H-6&H-7	1.03(4H,q,J = 4.9 Hz)
H-3	2.07(1H,J = 4.8 Hz)	H-15&H-16	0.99(4H,t,J = 8.6 Hz)
H-1&H-2	1.81(4H,m)	H-26	0.90(3H,s)
H-23&H-24	1.59(6H,m)	H-19&H-21	0.88(4H,s)
H-29&H-30	1.48(6H,m)	H-22	0.86(2H,s)
H-5	1.3(1H,s)	H-27	0.81(3H,d,J = 6.5 Hz)
H-9	1.23(1H,S, J = 6.2 Hz)	H-28	0.76(3H,s)
H-11	1.17(2H,m)	–	–

3.2. Pharmacological study

The tested extract was characterized by a low degree of toxicity. The obtained results indicated that different doses of the alcohol of extract (500, 1000, 2000 and 5000 mg/kg) did not produce any symptom of acute toxicity and none of the mice died during 24 h of observation. It was suggested that oral LD_{50} of were higher than 5000 mg/kg. Since substances possessing LD_{50} higher than 50 mg/kg are non-toxic (Erbayraktar et al., 2017). The tested extracts were considered safe.

Oral administration of the alcohol extract of *Cyperus conglomeratus* Rottb at the tested dose didn't disturb liver and kidney functions (Table 4). Interestingly, the obtained results revealed that these extracts are safer with negative side effects on liver and kidney function (Erbayraktar et al., 2017).

3.3. Anticandidal activity

The anticandidal activity of *Cyperus conglomeratus* against different *Candida* species were determined (Table 5). The best

Table 3
^{13}C NMR chemical shift assignments of C2.

Carbon no.	Chemical shift ppm	Carbon no.	Chemical shift ppm
7	121.86	9	29.23
3	71.93	16	28.38
17	56.87	5	24.43
14	56.14	11	22.08
21	50.23	15	23.16
22	45.93	27	21.20
19	42.42	23	19.96
4	39.88	24	19.53
12	37.36	25	19.13
2	36.62	29&30	18.90
18	36.27	26	12.10
20	34.04	28	11.98
1	32.03		
8	32.01		

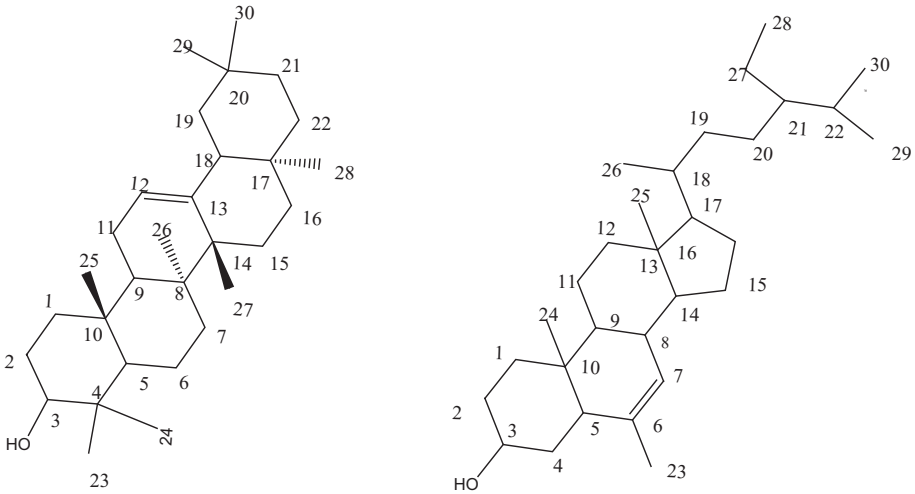

Fig. 1. The isolated compounds from *Cyperus conglomeratus* Rottb.

Table 4
Effect of *Cyperus conglomeratus* Rottb on liver and kidney functions.

Treatment	Liver function (U/l)		Kidney function (mg/dl)	
	AST	ALT	Blood urea	Serum Creatinine
Control	65.11 ± 2.62	144.62 ± 5.39	32.16 ± 1.83	0.36 ± 0.03
Alcohol extract (500 mg/kg)	67.39 ± 4.26	146.50 ± 4.27	33.40 ± 1.96	0.37 ± 0.03

Table 5
The anticandidal activity of *Cyperus conglomeratus* extract and isolated compounds.

Candida species		*Candida albicans*	*C. dubliniensis*	*C. famata*	*C. glabrata*	*C. inconspicua*
Diameter of inhibition zone (mm)	Total extract	22	11	18	13	15
	Chloroform extract	23	16	20	15	17
	Comp. C1	18	14	–	14	–
	Comp. C2	–	–	19	11	8
	Amphotericin B	26	24	21	18	20
Minimum Inhibitory Concentration (MIC) (μg/ml)	Total extract	3.9	–	125	–	–
	Chloroform extract	3.9	125	15.6	–	125
	Comp. S1	250	–	–	–	–
	Comp. S2	–	–	250	–	–
	Amphotericin B	0.12	0.12	0.24	0.48	0.24

activity (23 mm, 3.9 μg/ml) and (22 mm, 3.9 μg/ml) was obtained by chloroform and total extract, respectively against *Candida albicans*. The chloroform extract of *C. conglomeratus* showed anticandidal activity (20 mm, 15.6 μg/ml), (17 mm, 125 μg/ml), and (16 mm, 125 μg/ml) against *C. famata*, *C. inconspica*, and *C. dubliniensis*, respectively, higher than the total extract (Table 5). On the other hand, the highest activity of the isolated compounds was obtained by β-sitosterol (19 mm, 250 μg/ml) and α-amyrin (18 mm, 250 μg/ml) against *Candida famata* and *C. albicans*, respectively. It seems the highest anticandidal activity of the chloroform extract is related to the phytochemical contents (Akinyeye et al., 2014).

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