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Chemical Constituents and Biological Activities of the Aerial Parts of *Cyperus rotundus* (Cypereaceae)

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The present study reports the chemical constituents, antioxidant, anticonvulsant and α-amylase activities of the aerial part of *Cyperus rotundus* collected in Cameroon. Phytochemical study leads to the isolation of six known compounds alongwith lupeol (**1**), stigmasterol (**2**), tetracosanoic acid (**3**), a mixture of β-sitosterol (**4a**) and stigmasterol (**4b**), ursolic acid (**5**) and saikogenin F (**6**). Compounds **3**, **5** and **6** were isolated for the first time from this species. The structures of these compounds were determined using spectroscopic analysis including 1D and 2D NMR, mass spectrometry and comparison with the literature data. Biological activities carried out on the extracts showed that the methanol extract exhibited good antiradical scavenging activity against DPPH radical (IC_{50} = 2.873 µg/mL) and very good ferric reducing antioxidant power (FRAP) (IC_{50} = 7.535 µg/mL). It appeared that the mixture of hexane and ethyl acetate extract at the dose 100 mg/kg protected 66% of mice against convulsion induced by the pentylenetetrazol and 50% of protection when using picrotoxin at the same dose. All the extracts and compounds from this plant showed no inhibition against α-amylase related to antidiabetic activity.

Keywords: *Cyperus rotundus*, Aerial parts, Antioxidant activities, Anticonvulsant, α-Amylase.

INTRODUCTION

Man uses numerous plants as a source of medicines for the prevention and treatment of varied ailments as they are considered to be the main precursors of many drugs [1]. According to the WHO report, more than 80 % of the population in Africa continent are using plants for their primary health care [2], hence a renewed interest of many scientists in such plants. The uses of conventional drugs in the treatment of various diseases encounter today a problem of efficiency or selectivity due to the resistance of microorganisms to these drugs. Plants have been at the origin of the isolation of new bioactive compounds to deal with the diverse health problems encountered in chemotherapy or by the use of molecules in the treatment of several other pathologies. The discovery of many drugs from plants such as artemisinin isolated from *Artemisia annua* [3] and taxol from *Taxus brevifolia* [4] are some few examples. *Cyperus rotundus*, is an herbaceous species belonging to the *Cyperus* genus and Cyperaceae family which includes approximately 5500 species [5]. Commonly known as nut grass, it is a weed that is well distributed in the temperate tropical and subtropical regions of the world [6]. In Cameroon, *Cyperus rotundus* is found in the wetlands and sandy area [7]. It's a traditional herbal medicine used widely as analgesic, sedative, antispasmodic, antimalarial, stomach disorders and to relieve diarrhea. Infusion of *C. rotundus* rhizome has been used in pain, fever, dysentery and other intestinal problems [6-8]. Previous pharmacological studies have reported that rhizomes and aerial parts of *Cyperus rotundus* possess anti-diarrheal, antioxidant, anti-inflammatory, antimutagenic, anti-

periodic, anticonvulsant, anti saturative, antipyretic, antifungal, antidiabetic, antimalarial, antilipidemic, antibacterial, antiviral, antitumoral and cardioprotective activities [9-13]. This plant has been the object of several chemical studies which have led to the isolation of many compounds belonging to the following families of compounds: terpenes, flavonoids, mono and sesquiterpenes [14-17] and lot of essential oils. Despite the well documented benefits and studies on *Cyperus rotundus*, the plant originated from Cameroon has not been the subject of any phytochemical and pharmacological study. This study consists to bring out our contribution to the phytochemical study of *Cyperus rotundus* from Cameroon and to evaluate its antioxidant, anticonvulsant and α-amylase activities.

EXPERIMENTAL

The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were scanned on Bruker AV-500 spectrometer, with $CDCl_3$ and DMSO as NMR solvents and coupling constants (*J*) are expressed in Hertz (Hz) and chemical shifts (δ) were given in ppm. Mass spectra were recorded by TOF-MS-ES+, in positive mode on a SL512 16 mass spectrometer. The *m/z* values peaks are mentioned and the figures in bracket attached to each *m/z* values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh) and thin layer chromatograpy (TLC) on silica gel aluminum plates 400 mesh ASTM, 13 × 20 cm). Spots were visualized under UV light (254 and 365 nm), sprayed with 20% sulfuric acid then heated at 105 ºC for 15 min.

Plant materials: The aerial part of *Cyperus rotundus* were collected in the south river of Benoue (Garoua) in the North region of Cameroon in December 2015. Identification of the plant was made by Prof. Mapongmetsem Pierre-Marie, a botanist at the Department of Biological Sciences of the Faculty of Science of the University of Ngaoundere, Cameroon and then been authenticated under the voucher number: No. 8175/SRF/Cam at the National Herbarium of Cameroon by Dr. Ghogue Jean Paul.

Extraction and isolation: Air-dried powder (1 kg) of *Cyperus rotundus* was extracted by maceration with intermittent stirring at room temperature using *n*-hexane, ethyl acetate and methanol. For each solvent used (3 L) the extraction was repeated three times after 72 h. The solutions obtained were filtered and evaporated using rotatory evaporator (Büchi Rotavapor R-300). The hexane and ethyl acetate extracts were combined (12 g) according to their TLC profile and subjected to column chromatography on silica gel and eluted with the gradient of hexane/ethyl acetate and MeOH. A total of 133 fractions of 300 mL each were collected and grouped according to their TLC profile to give seven series (A-G). Column chromatography of fractions A; C; E and F lead to the isolation of six known compounds including lupeol (**1**; 30 mg), stigmasterol (**2**; 5 mg), tetracosanoic acid (**3**; 10 mg), a mixture stigmasterol and β-sitosterol (**4**; 8 mg); ursolic acid (**5**; 13 mg) and saikogenin F (**6**; 11 mg). The spectroscopic data of the isolated compounds is discussed as follows:

Lupeol (1): White powder, *m/z*: 426.3 g/mol $C_{30}H_{50}O$. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 4.67 (1H, s, H-29b), 4.55 (1H, s, H-29a), 3.19 (1H, dd, *J* = 2.0; 6.0 Hz, H-3), 1.01 (3H, s, H-28), 0.95 (3H, s, H-26), 0.93 (3H, s, H-23), 0.81 (3H, s, H-27), 0.77 (3H, s, H-24). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 150.9 (C-20), 109.3 (C-29), 79.0 (C-3), 55.3 (C-5), 50.4 (C-9), 48.3 (C-18), 48.0 (C-19), 43.0 (C-17), 40.0 (C-4), 38.6 (C-1), 38.0 (C-13), 35.6 (C-16), 27.9 (C-23), 27.4 (C-2), 20.9 (C-11), 18.3 (C-6), 18.0 (C-28), 16.1 (C-25), 16.0 (C-26), 15.4 (C-24), 14.5 (C-27).

Stigmasterol (2): TOF-MS-ES+: (*m/z*) $[M+H]^+$ 413.3 for $C_{29}H_{48}O$. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 5.39 (1H, t, *J* = 6.1Hz, H-5), 5.16 (1H, m, H-21), 3.52 (1H, m, H-20), 3.51 (1H, tdd, *J* = 4.5, 4.2, 3.8 Hz, H-3), 1.04 (3H, s, H-29), 0.91 (3H, d, *J* = 6.2 Hz, H-19), 0.86 (3H, d, *J* = 6.6 Hz, H-27), 0.84 (3H, t, *J* = 7.1 Hz, H-24), 0.71(3H, s, H-28).^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 140.8 (C-5), 138.3(C-20), 129.3 (C-21), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.1 (C-17), 50.2 (C-9), 45.9 (C-22), 42.3 (C-4), 42.2 (C-13), 40.5 (C-18), 39.7 (C-12), 37.3 (C-1), 36.5 (C-10), 31.9 (C-2), 31.7 (C-7, C-8), 29.2 (C-16), 28.9 (C-25), 25.4 (C-23), 24.3 (C-15), 21.1 (C-11, C-19), 19.80 (C-26), 19.40 (C-27), 18.98 (C-28), 12.22 (C-29), 12.05 (C-24).

Tetracosanoic acid (3): TOF-MS-ES+: (*m/z*) $[M+Na^++2H]^+$ 393,3 for $C_{24}H_{48}O_2$. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 2.35 (t, *J* = 7.5 Hz, 2H), 1.65-1.61 (m, 2H), 1.25 (s, 44H), 0.88 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (125 MHz, $CDCl_3$) δ_C 178.2 (C-1), 34.0 (C-2), 31.9 (C-22), 29.7 (C-6 to C-20), 29.4 (C-5, C-21); 23.1 (C-3), 22.7 (C-23), 14.1 (C-24).

β-sitosterol (4a): *m.f.*: $C_{29}H_{50}O_2$. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.39 (1H, t, *J* = 6.4 Hz, H-5), 3.52 (1H, tdd, *J* = 4.5, 4.2, 3.8 Hz, H-3), 1.04 (3H, s, H-29), 0.91 (3H, d, *J* = 6.5 Hz, H-19), 0.86 (3H, d, *J* = 6.4 Hz, H-27), 0.85 (t, 3H, *J* = 7.2 Hz, H-24), 0.84 (3H, d, *J* = 6.4 Hz, H-26), 0.71(3H, s, H-28). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 140.8 (C-5), 121.6 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.1 (C-9), 45.8 (C-22), 42.3 (C-4), 42.2 (C-12), 37.3 (C-1), 36.5 (C-10), 36.2 (C-18), 33.0 (C-8), 32.0 (C-7), 31.7 (C-2), 29.4 (C-25), 29.1 (C-16), 24.3 (C-15), 23.1 (C-23), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 12.0 (C-29), 11.9 (C-24).

Stigmasterol (4b): *m.f.*: $C_{29}H_{48}O$. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 5.39 (1H, t, *J* = 6.4 Hz, H-5), 5.16 (1H, m, H-21), 4.96 (1H, m, H-20), 3.52 (1H, tdd, *J* = 4.5, 4.2, 3.8 Hz, H-3), 1.04 (3H, s, H-29), 0.91 (3H, d, *J* = 6.5 Hz H-19), 0.86 (3H, d, H-27), 0.85 (t, 3H, *J* = 7.2 Hz, H-24), 0.84 (3H, d, *J* = 6.4 Hz, H-26), 0.71 (3H, s, H-28). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 140.7 (C-5), 138.3 (C-20), 129.3 (C-21), 121.6 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.1 (C-9), 45.8 (C-22), 42.3 (C-4), 42.2 (C-12), 37.3 (C-1), 36.5 (C-10), 36.2 (C-18), 33.0 (C-8), 32.0 (C-7), 31.7 (C-2), 29.4 (C-25), 29.1 (C-16), 24.3 (C-15), 23.0 (C-23), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 12.0 (C-29), 11.9 (C-24).

Ursolic acid (5): TOF-MS-ES+: (*m/z*) $[2M+Na]^+$ 935.7 for $C_{30}H_{48}O_3$. ^{1}H NMR (500 MHz, DMSO) δ ppm: 11.90 (s, 1H), 5.13 (s, 1H), 3.33 (s, 1H), 3.0 (m, 6H), 1.04 (s, 3H), 1.04 (s, 9H), 0.81 (d, *J* = 6.4 Hz, 3H), 0.75 (s, 3H), 0.68 (s, 3H).^{13}C NMR (125 MHz, DMSO) δ ppm: 178.2 (C-28), 138.2 (C-13), 124.6 (C-12), 76.8 (C-3), 54.8 (C-5, C-18), 47.0 (C-9,), 41.6 (C-14, C-17), 40.1 (C-8, C-19), 39.0 (C-20), 38.5 (C-1, C-4),

36.5 (C-22), 32.7 (C-7, C-10, C-21), 28.3 (C-15 C-23), 27.0 (C-2 C-15), 23.8 (C-16, C-28), 23.3 (C-11), 18.0 (C-6, C-29), 16.1 (C-24), 15.2 (C-25).

Saikogenin F: (6) TOF-MS-ES+: (*m/z*) $[2M+K]^+$ 472.3 for $C_{30}H_{48}O_4$. ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 5.99 (1H dd, *J* = 10.4, 1.4 Hz H-12), 5.57 (7H, dd, *J* = 10.3, 3.2 Hz, H-11), 3.25 (1H, d, *J* = 6.9 Hz, H-28b), 1.22-1.06 (3H, s, H-27), 1.03 (3H, s, H-25), 0.96 (3H, s, H-30), 0.82 (3H, s, H-29). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 133.5 (C-11), 129.1 (C-12), 85.4 (C-13), 77.2 (C-3, C-28), 60.8 (C-16, C-23), 53.2 (C-9, C-18), 45.2 (C-5, C14, C-17), 42.1 (C-4), 41.9 (C-8), 38.3 (C-1, C-19), 36.5 (C-10, C-15, C-21), 30.9 (C-7, C-20), 29.9 (C-22), 23.2 (C-2, C-30), 19.6 (C-26, C-27), 17.7 (C-6, C-25), 15.1 (C-24).

Total phenolics content: Total phenolics content was evaluated using Folin-Ciocalteu reagent according to the method described by Talla *et al*. [18] and López-Mejía *et al*. [19] with slight modifications. In fact, 100 µL of extract and 200 µL of Folin-Ciocalteu (1:10) were mixed, then 2000 µL of distilled water where added. The mixture was homogenized and incubated for 3 min. After this time, 1000 µL of Na_2CO_3 20% was added. The final solution was incubated for 60 min in darkness at room temperature and then the absorbance read at 760 nm with a methanol solution used as negative control. Gallic acid was used as a standard and prepared in the same conditions as extract with concentrations ranging from 0.1 to 1.0 mg/mL. The blank was made of 2100 µL of water and 200 µL of Folin-Ciocalteu reagent and was incubated for 3 min, then 1000 µL of Na_2CO_3 20%, were added and absorbance was measured at 760 nm in a UV-vis spectrophotometer. Results are reported as µg equivalent gallic acid (EGA)/100 g EX (extract).

Total triterpenoid content: Total triterpenoid content was determined by the methods described by Wei *et al*. [20] with a slight modification. A stock solution of ursolic acid was prepared in methanol at the concentration of 0.5 mg/mL. From this solution, different dilutions were prepared (1-10 µg/mL). A quantity of 150-148 µL vanillin solution (acetic glacial acid 5%) was added to 100 µL of extract or standard range. Then, add 500 µL of perchloric acid 70 % to the mixture. Heat the solution to 60 ºC during 45 min. The cooled mixture was soaked in an ice bath by adding 2.25 mL acetic glacial acid. The absorbance was measured at 573 nm against blank using UV-vis spectrophotometer Genesys 10S 1100501 series. The tests were triplicate. Results are reported as µg equivalent ursolic acid (EUA)/100 g EX (extract).

Antioxidant activities

DPPH radical scavenging activity: The method is based on the ability of extracts to reduce the DPPH radical (1,1-diphenyl-2-picrylhydrazyl). DPPH radical scavenging activity of all extracts was determined by the method described by Talla *et al.* [18] with slight modifications. The DPPH solution was prepared in methanol at a concentration of 0.4 mM. The solution was conserved in a closed bottle away from light and any heat source before usage. The extracts were prepared at the different concentrations ranging from 0.05 to 1.0 mg/mL. A quantity of 1 mL of DPPH solution was added in each sample containing the positive control and different extracts concentration prepared. After 30 min of incubation, the absorbance of the mixtures was measured at 517 nm against the blank. The butylhydroxytoluene (BHT) and ascorbic acid was used as references. The tests were performed in triplicate. The results were expressed in percentage inhibition.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} \times 100$$

where $\text{Absorbance}_{\text{control}}$ is optical density value of the control and $\text{Absorbance}_{\text{extract}}$ is optical density value of the extract.

Evaluation of the ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power (FRAP) is based on the formation of a complex $K_3Fe(CN)_6$ with Fe^{2+} ion. The reducing power of sample extracts was determined according to the method described by Talla *et al*. [18] with minor modifications. To 100 µL of the extract at different concentration (0.2 to 1 mg/mL) were added a solution of phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated in a water bath at 50 ºC for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture then the tubes are centrifuged using a vortex at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance of the solution was measured at 700 nm. The results are expressed in percentage of inhibition.

$$\text{Ferrous ion chelating power (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} \times 100$$

where $\text{Absorbance}_{\text{control}}$ is optical density value of the control and $\text{Absorbance}_{\text{extract}}$ is optical density value of the extract.

Animal's material: The mice *Mus musculus* swiss male or female (23 ± 3g) were used for induction of convulsion. The mice were provided by the animal house of the University of Ngaoundere, Cameroon. Animal's houses were standard cages at 25 ºC on a 12/12 h light-dark cycle and they animal have free access to food and water. The experiments were conformed to the ethical guidelines of Cameroon. Bioethics Committee (Ref No. FW-IRB00001954) and the US National Institutes of Health (NIH No. 85-23, revised 1996). Before starting the experiments, animals were acclimated to laboratory conditions. In each test, mice were divided into three groups. One negative control group received distilled water, one positive control group received a diazepam injection (10 mg/2mL) substance as a reference and one groups received 100 mg/kg dose of the extract.

Anticonvulsant activity

Induction convulsion by pentylenetetrazol: Three groups of five mice or four mice were treated. The positive control group received 0.1 mg/kg of diazepam intraperitoneally. Clonic seizures were induced in mice by intraperitoneal injection of 70 mg/kg of pentylenetetrazol. After 1 h, mice receive 100 mg/kg by gavage the treatment. Animals that did not convulse within the 10 min of observation were qualied as protected [21,22]. The negative control group received distilled water.

Induction of convulsion by picrotoxin: This test focuses on inducing clonic convulsions by intraperitoneal adminis-

tration and the observations of the mice. Three groups of five mice received different treatments as described below except the positive control group receiving 0.4 mg/kg diazepam. One group of mice received by gavage 100 mg/kg dose of extract. 1 h after gavage of the different dose, we induced in mice by the intraperitoneal injection of 7.5 mg/kg of picrotoxin. The mice were observed for 15 min. Animals that did not convulse within the 15 min of observation were qualied as protected. The negative control received distilled water [23,24].

α-Amylase inhibitory activity: The activity was measured using the method reported by Tran *et al.* [25] with slight modifications. The substrate solution was obtained by mixing 200 mg of potato starch in 20 mL of phosphate buffer (pH 6.9) for 5 min. After cooling at room temperature, the solution was adjusted HCl to (2M, pH 7) and adjust the volume to 100 mL with H_2O. Extract (20 µL) and substrate (40 µL) were mixed in 30 mL of phosphate buffer (pH 6.9, 0.1 M) in a microplate and pre-incubated at 37 ºC for 5 min. After pre-incubation, 20 µL of α-amylase solution (50 µg/mL) was added to each well and incubated in microplate for 15 min. Addition of HCl (40 µL, 0.1 M) and iodine (100 µL, 1 mM) to the solution showed the end of the reaction. The absorbances were measured at 650 nm. Percentage of inhibition was calculated (%):

$$= \frac{(Abs_4 - Abs_3) - (Abs_2 - Abs_1)}{(Abs_4 - Abs_3)} \times 100$$

where Abs_1 is the absorbance of incubated solution containing sample starch and amylase, Abs_2 is the absorbance of incubated solution containing sample and starch, Abs_3 is the absorbance of incubated solution containing starch and amylase, Abs_4 is the absorbance of incubated solution containing starch.

RESULTS AND DISCUSSION

Compounds isolated: The phytochemical study using column chromatography separation on silica gel led to the isolation of six known compounds such as lupeol (**1**) [26], β-stigmasterol (**2**) [27], tetracosanoic acid (**3**) [28], a mixture of β-stigmasterol and β-sitosterol (**4**) [27,29], ursolic acid (**5**) [30,31] and saikogenin F (**6**) [30,32]. The spectroscopic data of these compounds are provided in the experimental section. Their structures were elucidated using NMR data and comparison made with the existing literature data. To the best of our knowledge, this is the first phytochemical study carry out on *Cyperus rotundus* from Cameroon. Except compounds **3**, **5** and **6**, other compounds are reported here for the first time in this specie. This result showed that there is a significant variability in the chemical constituents of *Cyperus rotundus* according to the geographical localization. Despite the extensive phytochemical studies on *Cyperus rotundus,* this study enriches the chemotaxonomy of this species.

Phytochemical screening test: The qualitative phytochemical screening test was carried out to determine the presence of various classes of secondary metabolites by using the method reported by Talla *et al.* [27] in the obtained extracts. The test is based on the physico-chemical properties including colouration or appearance of the precipitates. The phytochemical screening test carried out on the *n*-hexane, ethyl acetate and methanol extracts of *Cyperus rutundus* are presented in Table-1. This qualitative screening test reveal the presence of various class of secondary metabolites including alkaloids, terpenoids, phenolic compounds, saponins and tannins. The anthraquinone where absent in all extracts.

TABLE-1
PHYTOCHEMICAL SCREENING TEST OF EXTRACTS OF *Cyperus rotundus*

Phytochemical constituents	Extracts		
	Hexane	Ethyl acetate	Methanol
Alkaloids	+	+	–
Triterpenes	+	+	+
Phenolic compound	–	+	+
Flavonoids	–	+	+
Tannins	–	+	+
Anthraquinones	–	–	–
Saponins	–	+	+

+ indicate presence and-indicate absence.

Total phenolic and terpenoid contents: The phenolic and terpenoid contents of *C. rotundus* were quantified for the methanol and *n*-hexane/ethyl acetate extracts. The methanol extract was found to be rich in phenolic compounds (490.385 ± 0.001 µg gallic acid equivalent per 100 g of extract) and poor in triterpenoid content (12.123 ± 0.006 µg ursolic acid equivalent per 100 g of extract). Therefore, *n*-hexane/ethyl acetate extract has a lower content in phenolic compounds (123.077 ± 0.002 µg gallic acid equivalent per 100 g of extract) and the higher content in triterpenoid content (21.399 ± 0.003 µg ursolic acid equivalent per 100 g of extract).

Antioxidant activity: The antioxidant activity of extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and ferric reduction antioxidant power (FRAP) assay. The results are presented in Figs. 1 and 2. The methanol extract was the most active extract and inhibits significatively the DPPH radical (76.12 %). The extract also showed a good power reduction of Fe^{2+} ion (89.41%) using FRAP method. The IC_{50} values are reported in Table-2.

Anticonvulsant activity: The anticonvulsant proprieties were carried out on the combined mixture of *n*-hexane/ethyl acetate extract and on lupeol using the mice gavage. The convulsion was induced by picrotoxin and pentylenetetrazol. The combined mixture of extract hexane/ethyl acetate at the dose of 100 mg/kg protects 66% of mice from convulsion using

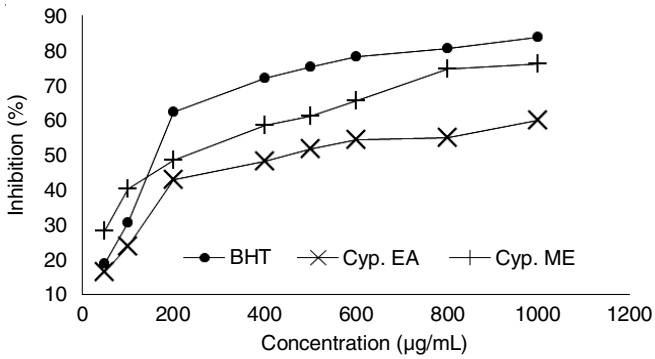

Fig. 1. DPPH inhibition percentage of *Cyperus rotundus* extract

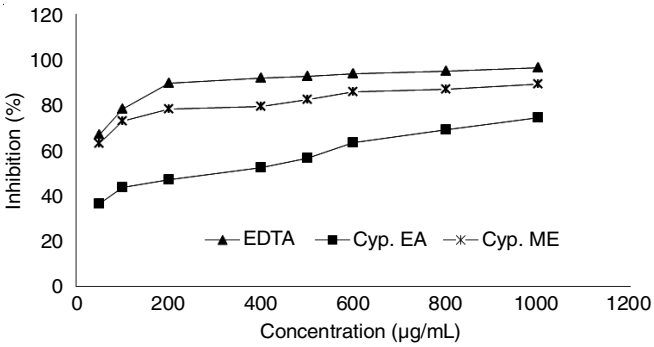

Fig. 2. Ferric reducing antioxidant power of extracts of *Cyperus rotundus*

TABLE-2
INHIBITION PERCENTAGE (IP) AND IC_{50} OF EXTRACT OF *Cyperus rotundus*

	Extracts	IP (%)	IC_{50} (µg/mL)
DPPH	BHT	83.784 ± 1.802	–
	Ethyl acetate	60.135 ± 1.351	1.296 ± 0.069
	Methanol	76.126 ± 1.952	2.873 ± 2.856
FRAP	EDTA	96.460 ± 0.050	–
	Ethyle acetate	72.072 ± 3.003	18.468 ± 25.752
	Methanol	89.416 ± 0.100	7.535 ± 0.369

pentylenetetrazol, while 50% of mice were protected when using picrotoxin at the same dose. Lupeol showed no protection against convulsions induced by picrotoxin. In the case of convulsion induced by pentylenetetrazol, lupeol protects 33% of mice from convulsion at the dose of 100 mg/kg. All samples tested showed no significant protection against convulsion compared to that of diazepam used as reference antiepileptic drugs which protected respectively 80% and 100% of mice against convulsions induced by picrotoxin and pentylenetetrazol at the dose of 0.4 mg/kg for picrotoxin and 0.1 mg/kg for the pentylenetetrazol.

Inhibition of α-amylase: The results of the inhibition of α-amylase by extracts and some isolated compounds are shown in Table-3. The methanol extract and the mixture of extract *n*-hexane/ethyl acetate showed very low inhibition against alpha amylase. The percentage of inhibition at the dose of 400 µg/mL was 30.6% for methanol extract and 36.3% for *n*-hexane/ethyl acetate extract. Among tested compounds, saikogenin F was the most active and showed a low inhibition of α-amylase.

The phytochemical screening test showed that *n*-hexane, ethyl acetate and methanol extracts revealed the presence of various classes of secondary metabolites including alkaloids,

TABLE-3
INHIBITION OF α-AMYLASE OF EXTRACTS AND COMPOUNDS FOR THE AERIAL PART OF *Cyperus rotundus*

Extracts and compounds	400 µg/mL
Methanol	30.6 ± 1.7
Hexane/ethyl acetate	36.3 ± 4.3
β-Sitosterol and stigmasterol	7.65 ± 0.4
β-Stigmasterol	Not active
Ursolic acid	17.8 ± 1.6
Tetracosanoic acid	22.3 ± 0.9
Saikogenin F	39.7 ± 1.1

triterpenoids, phenolic compounds, flavonoids, saponins and tannins. Previous phytochemical screening test on the extracts of *Cyperus rotundus* revealed the presence of tannins, flavonoids, coumarins and sterols [33] indicating some similarities with the obtained results. Wangila [34] also detected the presence of steroids, saponins, alkaloids, glycosides and tannins in *C. rotundus*. The anthraquinones families were not detected in different extracts, while Masfria & Yade [35] and Omeman *et al.* [36] have detected anthraquinones in *C. rotundus* from Indonesia and Lybia, respectively. These results showed that some differences observed in the phytochemical profile of *C. rotundus* suggest that its chemical composition vary according to geographical position of the plant.

The antioxidant activities have been carried out on the ethyl acetate and methanol extracts using the DPPH radical and FRAP method. The methanol extract showed the higher inhibition percentage of the DPPH radical and the Fe^{2+} ion from FRAP method. This activity is justified by the higher content in phenolic compounds quantified in this extract. The antioxidant activity is related to the presence of phenolic compounds which influence positively the antioxidant activity [37]. The results obtained correlate with those of Wangila [34], who demonstrated the antioxidant activity of extracts from *C. rotundus* with good percentage of inhibition. Many studied demonstrated the antioxidant activity of *C. rotundus*, but this is the first report of this plant collected in Cameroon.

α-Amylase, secreted directly from salivary glands and pancreas, is an enzyme that catalyzes the hydrolysis of α-glucose polymer at the C_1-C_4 connection and hydrolysis of starch present in food, generating glucose, maltose and dextrin [38]. α-Amylase inhibitor is an oral antidiabetic used for type 2 diabetes mellitus [39]. All extracts and compounds isolated were tested for the inhibition of α-amylase. No significant result was obtained for extracts and compounds on the inhibition of α-amylase. This result is in opposition to those obtained by Tran *et al*. [25], where extracts of *Cyperus rotundus* from Vietnam showed a significant inhibition of α-amylase and the authors justify this result by the fact that *Cyperus rotundus* contains polyphenol compounds which are the inhibitors of carbohydrate digestive enzymes.

The anticonvulsant activities were performed on the mixture of *n*-hexane/ethyl acetate extract and lupeol. A mixture of *n*-hexane/ethyl acetate extract exhibits good anticonvulsant activity by protecting 66% of mice from convulsion when using pentylenetetrazol, while the extract protects 50% of mice from convulsion when using picrotoxin. The results obtained with this extract is more active than those previously obtained by Shivakumar *et al*. [40] of *C. rotundus* from India, where the ethanol extract protects 50% of mice from mortality at the dose of 100 mg/kg using pentylenetetrazol induced seizures in rats. Moreover, flavonoids present in the ethanol extract could be responsible for this activity [40]. Another compound tested, lupeol, showed no anticonvulsant activity and a 100% mice dead was observed.

Conclusion

The aim of this study was to identify chemical constituents and evaluate the antioxidant, anticonvulsant and α-amylase

activity of the aerial part of *Cyperus rotundus* from Cameroon, which is the first report on this plant originated from Cameroon. The phytochemical study of the mixture of extract *n*-hexane/ethyl acetate led to the isolation six known compounds (**1-6**). Compounds **3**, **5** and **6** are isolated for the first time from this plant species. The phytochemical screening test on extracts revealed the presence of many secondary metabolites. The methanol extract was found to be richer in phenolic compounds while ethyl acetate extract was richer in terpenoids content. The methanol extract also showed the best antioxidant activity related to its high content in phenolic compounds. The mixture of *n*-hexane/ethyl acetate extract exhibits a good anticonvulsant activity induced by pentylenetetrazol, while lupeol was inactive. All extracts and compounds studied here were not active to inhibit α-amylase for antidiabetic activity. Some differences observed in the chemical constituents and phytochemical screening of *Cyperus rotundus* from Cameroon and those of other area of the world showed that the chemical composition of this plant vary considerably according to the geographical position. These results contribute significantly to the chemotaxonomy of *Cyperus rotundus.*

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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