British Journal of Pharmaceutical Research
7(1): 52-62, 2015, Article no.BJPR.2015.091
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antioxidant Activity, Total Phenolic and Flavonoid Contents of Essential Oils of Three *Cyperus* Species (Cyperaceae)

Oladipupo A. Lawal[1,2*], Anthony B. Ojekale[3], Olugbenga S. Oladimeji[3], Titilola S. Osinaike[4], Adetayo A. Sanni[4], Mthokozisi B. C. Simelane[5], Rebamang A. Mosa[6] and Andy R. Opoku[6]

[1]Department of Chemistry, University of Zululand, Kwa Dlangezwa 3886, South Africa.
[2]Department of Chemistry, Lagos State University, PMB 001, LASU Post Office, Ojo, Lagos, Nigeria.
[3]Department of Biochemistry, Lagos State University, PMB 001, LASU Post Office, Ojo, Lagos, Nigeria.
[4]Department of Botany, Lagos State University, PMB 001, LASU Post Office, Ojo, Lagos, Nigeria.
[5]School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Scottville 3209, South Africa.
[6]Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa.

Authors' contributions

This work was carried out in collaboration between all authors. Author OAL designed the study, isolation of the oils and wrote part of the manuscript. Authors ABO and OSO managed the literature searches. Authors TSO and AAS wrote the final draft of the manuscript. Authors MBCS and RAM carried out the polyphenol contents and antioxidant activity. Author ARO supply the chemicals and supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/18631
Editor(s):
(1) Abdelwahab Omri, Department of Chemistry and Biochemistry and Departments of Biomolecular Sciences, Laurentian University, Canada.
Reviewers:
(1) Daniela Hanganu, Department of Pharmacognosy, "Iuliu Hatieganu"University of Medicine and Pharmacy, Romania.
(2) Anonymous, Medical University of Bialystok, Poland.
(3) Rodrigo Crespo Mosca, São Paulo University, Brazil.
(4) Petigrosso, Lucas Ricardo, National University of Mar del Plata, Argentina.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1176&id=14&aid=9464

Original Research Article

Received 2nd May 2015
Accepted 16th May 2015
Published 28th May 2015

ABSTRACT

Aims: To determine the *in vitro* antioxidant activity, total phenolic and flavonoid contents of essential oils from the rhizomes of *Cyperus distans*, *Cyperus papyrus* and *Cyperus rotundus*

**Corresponding author: Email: jumobi.lawal@lasu.edu.ng;*

collected from different locations.
Study Design: The study was design to investigate the total phenolic and flavonoid contents and antioxidant properties of essential oils from the rhizomes of *Cyperus distans*, *Cyperus papyrus* and *Cyperus rotundus* in order to assess their medicinal values.
Place and Duration of Study: Fresh plant materials of *Cyperus distans*, *Cyperus papyrus* and *Cyperus rotundus* were randomly collected at the flowering stage between October, 2006 and September, 2007 from different locations in KwaZulu-Natal Province, South Africa.
Methodology: The antioxidant potential of the oils of *C. distans*, *C. papyrus* and *C. rotundus* was evaluated by 1,1-diphenylpicryl-hydrazyl (DPPH), hydroxyl radical, nitric oxide, metal chelating and reducing potential methods. The total phenolic and flavonoid contents of the oils were determined by spectrophotometer using Gallic acid and Quercetin equivalents, respectively.
Results: The essential oils of *C. distans*, *C. papyrus* and *C. rotundus* showed no metal chelating activity and poor aptitude to scavenge 1,1-diphenyl-2-picrylhydrazyl radical with inhibitory concentration (IC_{50}) >173 µg/mL, when compared to standard antioxidants; butyl hydroxyl anisole (BHA), butyl hydroxyl toluene (BHT), ascorbic acid (Vitamin C) and α-tocopherol with IC_{50} ≤10.70 µg/ mL. But, displayed significant antioxidant effects, when tested with other methodologies. The total phenolic content and the concentrations of flavonoid in the oils varied from (0.032 to 0.085) mg (Gallic acid)/100 µL and (0.027 to 0.072) mg (Quercetin)/ 100 µL equivalents, respectively.
Conclusion: The observed difference in antioxidant activities of the essential oils of *C. distans*, *C. papyrus* and *C. rotundus* may be due to climatic, seasonal and geographic conditions, harvest period, distillation technique, plant maturity and the presence of some major components or synergy between compounds present in the essential oils.

Keywords: Cyperus species; Cyperaceae; essential oils; polyphenol contents; antioxidant activity.

1. INTRODUCTION

Reactive species (RS) including reactive oxygen (ROS), nitrogen (RNS), bromine (RBS), chlorine (RCS) and sulphur (RSS) species, such as peroxyl ($ROO^{\cdot}$), singlet ($O_2^1 \Sigma g^+$), hydroxyl ($HO^{\cdot}$), superoxide anion (O_2^-), nitro oxide ($NO^{\cdot}$), atomic chlorine (Cl), hydrogen peroxide (H_2O_2), dinitrogen tetroxide (N_2O_4) and hypobromous acid (HOBr) are various forms of activated chemical species generated as by-product of biological reactions [1-5]. They roles in many exogenous and endogenous pathways including enzymatic and non-enzymatic reactions are important in many organic synthetic reactions [6]. However, these species were lethal to cells causing diversity of disorders such as membrane fragmentation, disruption of membrane-bound enzyme activity, disintegration and swelling of mitochondria, atherosclerosis, diabetes, ischemia, hypertension, genotoxicity, gastroenterology and hormonal/metabolic diseases [1,7]. In addition, reactive oxygen species causes the effects of other processes in the fields of nutrition, cosmetic, pharmaceutical, food safety and allergy [8]. Furthermore, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), *tert*-butylhydroquinone (TBHQ) and propylgallate (PG) that were widely used as food additives against oxidative degradation of foods by free radicals have been suspected of being responsible for several disorders [9,10]. As a result, there has been growing interest in research concerning the beneficial potentials of medicinal plant extracts including essential oils as a source of natural antioxidant in the prevention of cancers, cardiovascular and Alzheimer's diseases [11-14].

Essential oils are large range of plant oils, which are highly aromatic and mostly found in the flowers, buds, seeds, fruits, leaves, twigs, bark, wood and roots of plants, while, some are obtained from animal or produced by microorganisms [15,16]. They are complex mixtures of over 3,000 compounds with well-defined odor, which could be detected at a very low concentration [15]. Most essential oils consist of hydrocarbons, monoterpenoids, sesqiterpenoids, phenylpropanoids, carboxylic acids and benzyl alcohols [17,18]. Essential oils like spices have been used in the food, perfumery, cosmetic, sanitary and pharmaceutical industries as well as in medicine [19]. Some have also been reportedly used in aromatherapy, while, few have valuable alternatives to synthetic compounds [20,21]. Furthermore, several essential oils and their components are known to have multifunctional biological properties and many have been confirmed to possess antioxidant activities that

are relatively less damaging to the living cells [22-24].

The species *Cyperus distans* L. f., *Cyperus papyrus* L. and *Cyperus rotundus* L. (belongs to the genus *Cyperus*, family: Cyperaceae), were annual or perennial plants, mostly found in wet habitats and moist areas [25,26]. Investigation on these plants shows that some have been extensively studied, while many others have not been screened and little is known scientifically about them [26-28]. Although, several species of the genus *Cyperus* have been reportedly used as food, natural drug, building materials, traditional mats and ornamental plants, while, some hold significance uses in land management [27-29]. While, numerous studies have reported the chemical compositions, biological and pharmaceutical activities of the volatile oils of several species of the genus *Cyperus* [30-39]. In addition, the chemical composition of the essential oils of *C. distans* and *C. rotundus* from South Africa has been previously reported [40,41]. The major components of *C. distans* essential oil were cyperene (47.6%), α-pinene (18.8%), 1,8-cineole (14.5%) and caryophyllene oxide (7.3%). While, the oils of *C. rotundus* had β-pinene (5.3-11.3%), α-cyperone (7.9-11.0%), α-pinene (3.0-10.8%), myrtenol (7.1-7.9%), α-selinene (2.7-6.6%), caryophyllene oxide (2.6-5.4%) and β-selinene (4.6-5.1%) as the main constituents. Furthermore, the main compounds of *C. papyrus* essential oils were di-isobutyl phthalate, humulene epoxide II, aristolene and aromadendrene epoxide II (personal communication). Ethnomedicinal information on *Cyperus* species regarded as weeds indicates that some of the plants have strong healing power against diseases [27,28], though no previous studies on the antioxidant activities of essential oils of *Cyperus* species have been reported, apart from the few reports on the antioxidant activity of essential oil of *C. rotundus* [36,38]. However, to the best our knowledge, there is no report on the antioxidant activity and polyphenol contents of essential oils of these *Cyperus* species from South Africa, and no study have previously reported the total phenolic content of the essential oils of *C. distans*, *C. papyrus* and *C. rotundus*, and antioxidant activity of the volatile oils of *C. distans* and *C. papyrus*. In continuation of our studies on the essential oils of *Cyperus* species from South Africa [40,41], this paper reports for the first time, the antioxidant activity, total phenolic and flavonoids contents of essential oils of *Cyperus distans*, *Cyperus papyrus* and *Cyperus rotundus* growing wild in different locations in the city of uMhlathuze district, KwaZulu-Natal Province, South Africa.

2. METHODOLOGY

2.1 Chemicals

2,2-Diphenyl-1-picryl-hydrazyl (DPPH), 2-dexyribose, sodium nitroprusside, trichloro acetic acid (TCA), thiobarbituric acid (TBA), naphthylethylenediamine dihydrochloride and 4,4- [3-(2-pyridinyl)-1,2,4-triazine-5,6-dryl] bisbenzene sulphonic acid (ferrozine), Folin-Ciocalteau reagent, 2-dexyribose, sodium nitroprusside, Ethylenediaminetetraacetic acid (EDTA), Dimethylsulfoxide (DMSO), Ferrous ammonium sulfate, Quercetin, Gallic, Ammonium molybdate and Hydrogen peroxide were obtained from Sigma-Aldrich Co., Ltd (Steinheim, Germany). All other chemicals and solvents are of analytical grade.

2.2 Plant Materials and Extraction of Oils

Fresh plant materials of *Cyperus distans* (CD), *Cyperus papyrus* (CP) and *Cyperus rotundus* (CR) were randomly collected at the flowering stage between October, 2006 and September, 2007 from University of Zululand, KwaDlangezwa campus, Owen Sithole College of Agriculture in Empangeni, Richard's bay town, uThukela River, KwaDlangezwa and Empangeni towns in KwaZulu-Natal Province, South Africa. Identification of each plant material is given in Table 1. Leaves of each plant sample were separately subjected to hydrodistillation using Clevenger-type apparatus. The distillate isolated was preserved in a sealed sample tube and stored under refrigeration until further analysis.

2.3 Polyphenol Contents

2.3.1 Total phenolic content

The total phenolic content of essential oils of *C. distans*, *C. papyrus* and *C. rotundus* was determined using Folin-Ciocalteu reagent according to the method of Singleton and Rossi [42]. One hundred milligram of the essential oil was leached with 250 ml of methanol/water (60:40, v/v, 0.3% HCl) and filtered through a 0.45 µm Millipore filter. To 100 mL filtrate, 100 µL of 50% Folin-Ciocalteau reagent, and 2 mL of 2.5% sodium carbonate were added and mixed completely. After 2 h, the absorbance of the

solution at 750 nm was measured with a spectrophotometer. Quantitation was based on the standard curve of Gallic acid (0-0.5 mg/mL), which was dissolved in methanol/water (60:40, v/v, 0.3% HCl).

2.3.2 Total flavonoid content

The method of Kim et al. [43] was used for the estimation of total flavonoid content of the oils. An aliquot of 100 µl of the oil (100 µg/ml) was added individually to equal volumes of solution of 500 µl of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 ml methanol). The mixture was vigorously shaken, and after 10 min of incubation at room temperature, absorbance was taken at 430 nm. Total flavonoid content was calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg /ml in methanol. The result was expressed as mg Quercetin (mg QU/100 mg essential oil/ml methanol).

2.4 Antioxidant Activity

2.4.1 1,1-diphenylpicryl-hydrazyl radical scavenging activity

The free radical scavenging ability of the essential oils was evaluated as described by Pyo et al [10] and modified by Han et al [11]. 0.2 mL of different concentrations (10 - 250 µg/mL) of essential oils in methanol was mixed with 2.7 mL of 1.0 x 10^{-4}M methanol solution of DPPH. The absorbance at 517 nm was measured using UV-Visible Genesys 20 spectrophotometer; after the solution had been allowed to stand in the dark for 60 min. The absorbance of the samples, the control and the blank were measured in comparison with methanol. Lower absorbance of the reaction mixture indicates higher DPPH scavenging activity.

DPPH scavenging activity was calculated using the following formula:

DPPH scavenging activity (%)
= {1- (S-SB)/(C-CB)} X 100%

Where S, SB, C and CB were the absorbances of the sample, the blank sample (2.0 mL of methanol plus 0.2 mL of sample at different concentrations), the control (2.0 mL of DPPH solution plus 0.2 mL of methanol), and the blank control (methanol) respectively. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentrations.

2.4.2 Hydroxyl radical scavenging activity

The ability of the different concentrations (10 – 250 µg/mL) of the essential oil samples in methanol to scavenge the hydroxyl radical generated by Fenton reaction was measured according to the modified methods of Nagai et al. [13]. The Fenton reaction mixture containing 200 uL of 10 mM 2-dexyribose was mixed with 1.2mL of 0.1 M phosphate buffer (pH 7.4) containing 200 uL of the essential oils. Thereafter, 200 µL of 10 mM H_2O_2 was added to the mixture before incubation for 4 h at 37°C. Later, 1 mL of 2.8% trichloro acetic acid (TCA) and 1 mL of 1% thiobarbituric acid (TBA) were added and placed in a boiling water bath for 10 min. The resultant mixture was allowed to cool to room temperature and centrifuged at 300 X g for 5 min. Absorbance was recorded at 532 nm in a UV-VIS spectrophotometer. The percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the essential oils. The IC_{50} value represented the concentration that caused 50 % inhibition of radical formation.

2.4.3 Nitric Oxide Radical (NO·) scavenging activity

Sodium nitroprus side solution at physiological pH, spontaneously produce nitric oxide, which reacts with oxygen to produce nitrite ions according to the Griess Illosvoy reaction as described by Badami et al. [14]. The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4, 0.01 M) and 0.5 mL of different concentrations of essential oils were incubated at 25°C for 150 min. Thereafter, 0.5 mL of the reaction mixture containing nitrite was pipette and mixed with 1 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotisation. Then, 1 mL of naphthylethylenediamine dihydrochloride (0.1%) was added, and allowed to stand for 30 min in diffused light. The absorbance of the pink coloured chromophore was measured using UV-Visible Genesys 20 spectrophotometer at 540 nm against the corresponding blank solution.

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the

essential oils. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentrations.

2.4.4 Metal chelating activity

The Fe^{2+} chelating effect of the oil was measured according to the method of Senevirathne et al. [44]. To 0.5 mL of various concentrations (0-50 mg/L) of *Cyperus* species essential oil in methanol, 1.6mL of deionized water and 0.05 mL of $FeCl_2$ (2 mM) were added. After 30 s, the reaction was initiated by the addition of 5 Mm ferrozine (0.1 mL). Then, the mixture was shaken and left at room temperature for 10 min. Absorbance of the mixture was measured spectrophotometrically at 562 nm. Ascorbic acid and α-tocopherol were used as standard. The inhibitory effect of the oils was calculated as:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

Where, A_0 is the absorbance value of the fully oxidized control and A_1 is the absorbance of the oil.

2.4.5 Reducing power

The reducing power of the essential oils was determined according to the method of Oyaizu [45]. 2.5 mL of various concentrations of the essential oils (10 – 250 µg/mL) in methanol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1% w/v). The mixture was incubated at 50°C for 20 min. After 2.5 mL of 10% tricloroacetic acid (w/v) were added, the mixture was then centrifuged for 10 min at 1000 rpm. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of 0.1% ferric chloride, $FeCl_3$ (w/v), and the absorbance was measured at 700 nm using UV-Visible Genesys 20 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

2.5 Statistical Analysis

The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated as means±standard deviation (SD) of three independent measurement using Microsoft excel program, 2003 and Origin 6.0 for IC_{50}. Data were subjected to one way analysis of variance (ANOVA). *P* values ≤ 0.05 were regarded as significant and *P* values ≤ 0.01 as very significant.

3. RESULTS AND DISCUSSION

Table 1 showed some properties of the water-distilled essential oils from the rhizomes of *C. distans*, *C. papyrus* and *C. rotundus* according to the British Pharmacopoeia methods [46,47]. The essential oils yields ranged from 0.07% to 0.09% for *C. distans*, 0.10% to 0.20% for *C. papyrus* and 0.16% to 0.20% for *C. rotundus*. The colour of the oils were pale yellow and the characteristic odour reveals that *C. rotundus* was sweet-smelling, while, *C. distans* and *C. papyrus* are aromatic. The refractive index of the oils varied between (1.5112 -1.5180 and 1.5385-1.5389) for *C. distans*, (1.5112 -1.5180 and 1.5385-1.5389) for *C. distans* and *C. rotundus*, respectively.

Table 1. Properties of essential oils of three *Cyperus* species

Plant Code	CDS1	CDS2	CPS1	CPS2	CRS1	CRS2
Location	OSCA	Unizulu	Richard's Bay	uThukela River	Empangeni	Unizulu
Latitude	28°44´57.58"S	28°28´26.88"S	28°44´47.10"S	28°50´25.72"S	28°44´47.10"S	28°50´25.72"S
Longitude	31°54´00.00"E	31°50´27.24"E	31°53´10.81"E	31°49´34.38"E	31°53´10.81"E	31°49´34.38"E
Voucher specimen	Lawal, OA 03 (UZULU)	Lawal, OA 02 (ZULU)	Lawal, OA 07 (ZULU)	Lawal, OA 04 (ZULU)	Lawal, OA 05 (ZULU)	Lawal, OA 06 (ZULU)
% yield[a]	0.20	0.16	0.07	0.09	0.07	0.09
Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Odour	Sweet smelling	Sweet smelling	Aromatic	Aromatic	Aromatic	Aromatic
RI[b]	1.5385	1.5389	1.5180	1.5112	1.5180	1.5112

CD - C. distans, CP - C. papyrus, CR - C. rotundus; OSCA - Owen Sithole College of Agriculture, Empangeni; Unizulu - University of Zululand, KwaDlangezwa Campus; [a]% yield - based on the fresh weight of each plant; [b]RI - Refractive index

The total phenolic (TPC) and total flavonoid contents (TFC) of *C. distans*, *C. papyrus* and *C. rotundus* essential oils were summary in Table 2 as gallic acid equivalents (GAE/10 µL of essential oil/ml methanol) for total phenolic content and quercetin equivalents (QE/10 µL essential oil/ml methanol) for total flavonoid content, respectively. As shown in Table 2, all the essential oils were found to have different phenolic levels, ranging from (0.132 to 0.295 µg GAE/10 µL of essential oil/ml methanol) for total phenolic content and (0.107 to 0.177 µg Qu/10 µL essential oil/ml methanol) for total flavonoid content. For each 10 µL of essential oil, *C. papyrus* has the highest contents of total phenolic and total flavonoid of (0.191±0.002 - 0.295±0.003 µg GAE/ml methanol) and (0.107 to 0.177 µg Qu/ml methanol) in that order, followed by *C. rotundus* (0.168±0.002 - 0.197±0.a µg GAE/ml methanol) and (0.107 to 0.177 µg Qu/ml methanol), and *C. distans* (0.132±0.002 - 0.135±0.003 µg GAE) and (0.107 to 0.177 µg Qu/ml methanol). The results show that the essential oils possessed some quantity of secondary metabolites, which can play a vital role in scavenging of free radicals, chelating transitional metals and redox effects [48].

3.1 Antioxidant Activity

3.1.1 Free radical scavenging assay

1,1-diphenylpicryl-hydrazyl (DPPH) is a free radical compound that has been widely used to test the free radical scavenging ability of various plants extracts including essential oils [13,24,38]. It is considered to be a model for lipophilic radical in which a chain reaction is initiated by the lipid autoxidation. Table 2 shows the inhibitory concentration (IC_{50}) of the scavenging activity of the essential oils of *C. distans*, *C. papyrus*, *C. rotundus* and the standard antioxidants on DPPH radicals at different concentrations. The standard antioxidants (BHA, BHT, ascorbic acid and α-tocopherol) demonstrated the highest inhibitory activities with IC_{50} values ranging between 10.0-10.70 µg/mL, when compared to the essential oils studied. Although, the scavenging effects of the essential oils displayed concentration dependent, however, the concentration of the oils resulting in a 50% inhibition of the free radical scavenging (IC_{50}) was greater than 200 µg/mL for all the essential oils, expect for *C. rotundus* essential oils with IC_{50} values of 173.10 and 193.90 µg/mL respectively.

Hydroxyl radical is reactive oxygen formed by the reaction of various hydroperoxides with transition metal ions. It attacks proteins, polyunsaturated fatty acid in membranes and most biological molecules. It is also known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxidic reaction of lipids [1]. Hydroxyl radical scavenging activity of the essential oils as well as BHA, BHT and α-tocopherol were calculated as the inhibitory concentration (IC_{50}) of the hydroxyl radicals generated in the Fenton reaction mixture (Table 2). The results depicts that some of the oils exhibited strong concentration-dependent inhibition of hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity of the oils of *C. rotundus* were comparable to the commercial antioxidants (IC_{50} < 10.0 µg/mL). Also, was the oil of *C. papyrus* 1 with inhibitory concentration of 14.06 µg/mL. While, the oils of *C. distans* and *C. papyrus* 2 displayed poor aptitude to scavenge the hydroxyl radical.

Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or superoxides are very reactive. These compounds/radicals are liable for the changes in the structural and functional responses of many cellular components, causing serious toxic reactions with proteins, lipids, nucleic acids among others [1]. The inhibitory concentration (IC_{50}) generated from the essential oils of *C. distans*, *C. rotundus* and *C. papyrus,* and the standard antioxidants (BHA, BHT and α-tocopherol) at different concentrations by nitric oxide radical from sodium nitropruside at the physiological pH are given in Table 2. The results of the scavenging activity of nitric oxide radical by the essential oils of *Cyperus* species and the standard antioxidants shows that, it is inversely proportional to concentration. The concentrations of the IC_{50} of the essential oil samples and the standard antioxidants were found to be very active. From the results, it can be perceived that the essential oils have nitric oxide scavenging activity, which may help in preventing chains of reactions and diseases caused by excess nitric oxide radical generation [1].

3.1.2 Metal chelating activity

Transition metals capable of stimulating lipid peroxidation during Fenton reaction by generating hydroxyl radicals can accelerate lipid peroxidation into peroxyl and alkoxyl radicals by lipid hydroperoxides [49]. Also, chelating agents which form σ-bond with metal are valuable

secondary antioxidants because they decrease redox potential, thereby stabilizing the oxidized form of the metal ion [50]. Fig. 1 show that the essential oils have no remarkable chelating ability for the formation of Fe^{2+} complex and cannot act as peroxidation protector, which is related to iron binding capacity. At 250 µg/mL, the essential oils of *C. distans*, *C. papyrus* and *C. rotundus* chelated between (1.34-1.48%), (1.48-1.67%) and (1.33-1.34%) of ferrous ions respectively, while, citric acid (7.42%) and EDTA (14.81%) showed moderate ferrous ion chelating activity. Although, the results obtained indicate poor chelating activities of the essential oils, however, a high amount of chelated ferrous ion by *C. papyrus* essential oils may suggest that *C. papyrus* possesses antioxidant activity.

3.2 Reducing Power

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of antioxidant action. In addition, reducing properties have been associated with the presence of reductants, which exert antioxidant action by breaking the free radical chains through hydrogen atom donation [50,51]. Furthermore, reductones have also been reported to prevent peroxide formation by reacting with certain precursors of peroxide [52]. Table 3 showed the reducing potential of the essential oils and standard antioxidants for Fe^{3+} in a dose dependent concentration. A strong reducing potential was observed for the essential oils of *C. papyrus*, ascorbic acid, BHA, BHT and α-tocopherol, while, lower reducing capacity was displayed by the essential oils of C*. distans* and *C. rotundus*. At 250 µg/mL, the reducing power of the essential oils and standard antioxidants decreased in the following manner: *C. papyrus* > ascorbic acid > BHA > α-tocopherol > BHT > *C. rotundus* > *C. distans*. Based on the data obtained from this study, *Cyperus papyrus* may serve as an indicator of its ability to inhibit antioxidant activity and may act as primary and secondary reductants thereby inhibiting lipid peroxidation.

Table 2. Total phenolic content and free radical scavenging activities of essential oils of three *Cyperus* species[a]

	TPC[b]	TFC[c]	Free radical scavenging activities (IC_{50})[d]		
			DPPH	($^{\cdot}OH$)	($NO^{\cdot}$)
CDS1	0.132±0.005	0.110±0.001	> 250	> 250	40.60
CDS2	0.135±0.002	0.107±0.001	> 250	> 250	20.20
CPS1	0.295±0.003	0.177±0.000	> 250	14.06	32.70
CPS2	0.191±0.002	0.152±0.002	> 250	169.80	128.70
CRS1	0.197±0.004	0.144±0.004	173.10	< 10	< 10
CRS2	0.168±0.002	0.172±0.005	193.90	< 10	13.30
BHA	-	-	10.70	< 10	< 10
BHT	-	-	< 10	< 10	< 10
Vitamin C	-	-	< 10	ND	15.19
α-tocopherol	-	-	< 10	< 10	11.96

[a](n = 3, X ± SEM), [b]Expressed as mg GAE/10 µL of essential oil/ml methanol, [c]Expressed as mg QE/10 µL essential oil/ml methanol, [d]IC_{50} -inhibitory concentration, ($^{\cdot}OH$) - Hydroxyl radical scavenging; ($NO^{\cdot}$)- Nitric oxide radical scavenging

Table 3. Reducing power of *Cyperus* species essential oils and synthetic antioxidants[a]

	10	20	50	100	250
CDS1	0.046±0.002	0.053±0.001	0.057±0.001	0.061±0.000	0.070±0.002
CDS2	0.055±0.003	0.060±0.001	0.063±0.001	0.067±0.001	0.077±0.003
CPS1	0.140±0.002	0.146±0.002	0.166 ±0.001	0.173±0.002	0.186±0.003
CPS2	0.096±0.003	0.104±0.002	0.119 ±0.002	0.127±0.003	0.141±0.01
CRS1	0.061±0.001	0.065±0.001	0.070±0.001	0.080±0.001	0.115±0.102
CRS2	0.051±0.002	0.057±0.001	0.069±0.002	0.077±0.003	0.101±0.002
BHA	0.058±0.002	0.072±0.004	0.081±0.003	0.096±0.003	0.174±0.003
BHT	0.057±0.000	0.069±0.001	0.075±0.001	0.081±0.001	0.154±0.002
Vitamin C	0.159±0.002	0.171±0.003	0.188±0.002	0.194±0.003	0.242±0.003
α-tocopherol	0.058±0.003	0.066±0.003	0.075±0.002	0.091±0.002	0.166±0.002

[a]Concentration - µg/mL

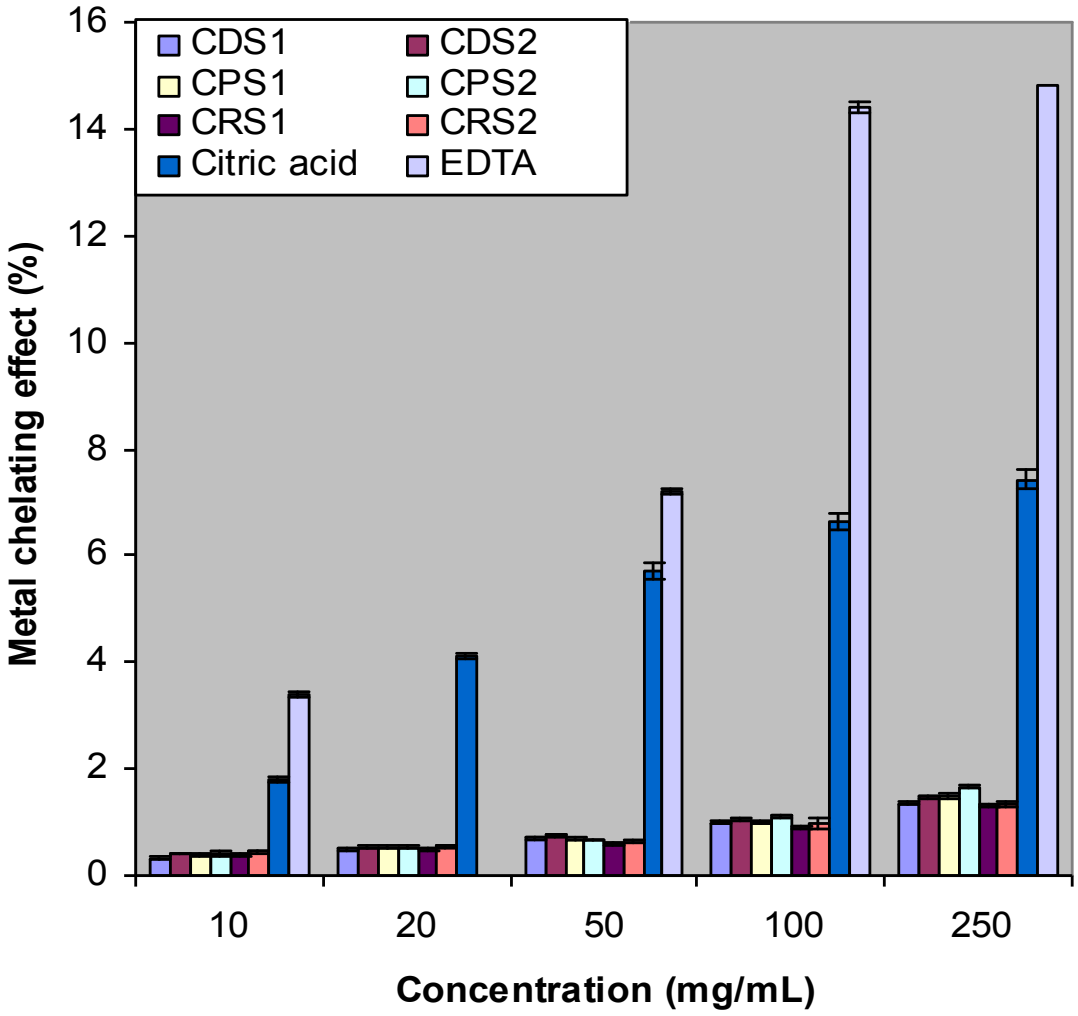

Fig. 1. Metal chelating activity of essential oils of *C. distans*, *C. rotundus* and *C. papyrus*, Citric acid and Ethylenediaminetetraacetic acid. Each value is mean±S.E (*n* = 3)

4. CONCLUSION

The observed difference in the antioxidant activities of the essential oils of *C. distans*, *C. papyrus* and *C. rotundus* may be due to climatic, seasonal and geographic conditions, harvest period, distillation technique and plant maturity, among others. In addition, the presence of some major components or synergy between compounds present in the essential oils might have contributed to their antioxidant properties. Furthermore, some oils showed noticeably radical scavengers than standard antioxidants in some methods suggesting the oils could be use for treatment of diseases, additives in food preservation and domesticated plants to provide continuous supply of medicinal products. Finally, the findings of this study support the view that no single method is sufficient in evaluating the antioxidant activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ceruitti PA. Oxidant stress and carcinogenesis. Eur. J. Clin. Invest. 1991;21:1-11.
2. Davies KJA. Oxidative stress: The paradox of aerobic life. Biochem. Soc. Symp. 1994;61:1-34
3. Halliwell B. Reactive oxygen species in living systems: Source, biochemistry and role in human disease. Am. J. Med. 1991;91:14–22.
4. Regano N, Iorio EL, Guglielmi A, Mazzuoli S, Francavilla A, Fregnan S, et al. The assessment of oxidative stress in clinical practice and its importance in nutrition. Nutr. Ther. Metabol. 2008;26:149-162.
5. Singh PP, Mahadi F, Roy A, Sharma P. Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type-2. Indian J.Clin. Biochem. 2009;24:324-342.
6. Cui K, Luo XL, Xu KY, Murthy MRV. Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants. Prog Neuropsychopharmacol Biol Psychiatry. 2004;28:771-799.
7. Jonas NG, Elias SJA. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Rad. Biol. Med. 2001;31:1287-1312.
8. Favier A. Oxidative stress in human diseases. Ann Pharm Fr. 2006;64:390-396.
9. Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenecity of buthylated hydroxyanisole in F344 rats. J Natl Cancer Inst. 1983;70:343–347.
10. Pyo YH, Lee TC, Logendrac L, Rosen RT. Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies cycla) extracts. Food chem. 2004;85;19-26.
11. Han J, Weng X, Bi K. Antioxidants from a Chinese medicinal herb – *Lithospermum erythrorhizon*. Food chem. 2008;106:2-10.
12. Bounatirou S, Smiti S, Miguel MG, Faleiro L, Rejeb MN, Neffati M, et al. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. Food Chem. 2007;105:146-155.
13. Nagai T, Myoda T, Nagashima T. Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) *Equisetum arvense* L. Food Chem. 2005;91:389-394.
14. Badami S, Rai SR, Suresh B. Antioxidant activity of *Aporosa lindleyana* root. J. Ethnopharmacol. 2005;101:180-184.
15. Baser KHC. Analysis and assessment of essential oils: In a manual on the essential oil industry. UNIDO, Vienna, Austria; 1995.
16. Guenther E. The Essential Oils. D. Van Nostrand Company, New York, U.S.A. 1949;3.
17. Ernest JP. The chemistry of essential oils and artificial perfumes. The Aberdeen University Press. Aberdeen, U.K; 1921.
18. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - A review. Food Chem. Toxicol. 2008;46:446-475.
19. Boyle W. Spices and essential oils as preservatives. Am. Perfum. Essent. Oil Rev. 1955;66:25-28.
20. Ilmberger J, Heuberger E, Mahrhofer C, Dessovic H, Kowarik D, Buchbauer G. The influence of essential oils on human attention. I. Alertness. Chem. Senses. 2001;26:239-245.
21. Bharkatiya M, Nema RK, Rathore KS, Panchawat S. Aromatherapy: Short overview. Int. J. Green Pharm. 2008;2:13-16.
22. Juteau F, Masotti V, Bessiere JM, Dherbomez M, Viano J. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. Fitoterapia. 2002;73:532-535.
23. Dorman HJD, Figueiredo AC, Barroso JG, Deans SG. *In vitro* evaluation of antioxidant activity of essential oils and their components. Flavour Frag. J. 2000; 15:12-16.
24. Kulisica T, Radonicb A, Katalinicc V, Milosa M. Use of different methods for testing antioxidative activity of *oregano* essential oil. Food Chem. 2004;85:633.
25. Gordon-Gray KD. Cyperaceae in Natal. National Botanical Institute: Pretoria, South Africa; 1995.
26. Goetghebeur P. Cyperaceae. In The families and genera of vascular plants IV: Alismataneae and Commelinanae (except Gramineae). Springer Verlag, Berlin, Germany; 1998.

27. El-Kamali HH, El-Khalifa KF. Folk medicinal plants of riverside forests of the Southern Blue Nile district, Sudan. Fitoterapia. 1999;70:493-497.
28. Roberts CH. The Greek Papyri. In: The Legacy of Egypt. Clarendon Press, Oxford, U.K; 1963.
29. Cunningham AB. People, park and plant use. UNESCO, Paris, France; 1996.
30. Sonwa MM, Koenig WA. Constituents of the essential oil of *Cyperus alopecuroides*. Phytochemistry. 2001;56:321-326.
31. Xu Y, Zhang H, Yu C, Lu Y, Chang Y, Zou Z. Norcyperone, a novel skeleton norsesquiterpene from *Cyperus rotundus* L. Molecules. 2008;13:2474-2481.
32. Olawore NO, Usman A, Ogunwande IA, Adeleke KA. Constituents of rhizome essential oils of two types of *Cyperus articulatus* L. grown in Nigeria. J. Essen. Oil Res. 2006;18:604-606.
33. Zoghbi MDGB, Andrade EHA, Carreira LMM, Rocha EAS. Comparison of the main components of the essential oils of "priprioca": *Cyperus articulatus* var. *articulatus* L., *C. articulatus* var. *nodosus* L., *C. prolixus* Kunth and *C. rotundus* L. J. Essen. Oil Res. 2008;20:42-46.
34. Jirovetz L, Wobus A, Buchbauer G, Shafi MP, Thampi PT. Comparative analysis of the essential oil and SPME-headspace aroma compounds of *Cyperus rotundus* L. roots/tubers from South-India using GC, GC-MS and olfactometry. J. Essent. Oil-Bearing Pl. 2004;7:100-106.
35. Mahmout Y, Bessiere JM, Dolmazon R. Constituent of the essential of *Cyperus maculates* Boeck. J. Essent. Oil Res. 2002;14:263-265.
36. Kilani-Jaziri S, Neffati A, Limem I, Boubaker J, Skandrani I, Sghair MB, et al. Relationship correlation of antioxidant and antiproliferative capacity of *Cyperus rotundus* products towards K562 erythroleukemia cells. Chem.-Biolo. Interact. 2009;181:85-94.
37. Kubmarawa D, Ogunwande IA Okorie DA, Olawore NO, Kasali AA. Chemical constituents of the volatile oil of *Cyperus esculentus* L. from Nigeria. Flavours frag. J. 2005;20:640-641.
38. Kilani S, Ledauphin J, Bouhlel I, Ben Sghaier M, Boubaker J Skandrani I, et al. Comparative study of *Cyperus rotundus* essential oil by a modified GC/MS analysis method, evaluation of its antioxidant, cytotoxic, and apoptotic effects. Chem. Biodivers. 2008;5:729-742.
39. Zoghbi MDGB, Andrade EHA, Oliveira J, Carreira LMM, Guilhon GMS. Analysis of the essential oil of the rhizome of *Cyperus giganteus* Vahl. (Cyperaceae) cultivated in the north of Brazil. J. Essent. Oil Res. 2006;18:408-410.
40. Lawal OA, Oyedeji AO. The composition of the essential oil from *Cyperus distans* Rhizome. Nat. Prod. Commun. 2009;4: 1099-1102.
41. Lawal OA, Oyedeji AO. Chemical composition of the essential oils of *Cyperus rotundus* L. from South Africa. Molecules. 2009;14:2909-2917.
42. Singleton VL, Rossi JA. Colorimetry of total phenolic with phosphomolybdic phosphotungestic acid reagents. Am. J. Enol. Vitic. 1965;16:144-158.
43. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. 2003;516:509-6515.
44. Senevirathne M, Kin SH, Siriwardhana N, Ha JH, Lel KW, Jeon YJ. Antioxidant potential of *Ecklonia cava* on reactive oxygen species, scavenging, metal chelating, reducing power and lipid peroxidation inhibition. Food Sci. Technol. Int. 2006;12:27-38.
45. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Jpn. J. Nutr. 1986;44:307-315.
46. Guenther E, Gilbertson G, Koenig RT. Essential oils and related products. Anal. Chem.1971;43:45-64.
47. Guenther E, Gilbertson G, Koenig RT. Essential oils and related products. Anal. Chem. 1975;47:39-157.
48. Mohamed AA, Khalil AA, El-Beltagi HES. Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). Grasas Y Aceites. 2010;61:67-75.
49. Zhao G, Xiang Z, Ye T, Yuan Y, Guo Z. Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem. 2006;99:767-774.

50. Gordon MH. The mechanism of antioxidant action *in vitro*. In Food antioxidants. B. J. F. Hudson (Ed.), Elsevier. London. 1990; 1-18.

51. Pin-Der-Duh X. Antioxidant activity of burdock (*Arctium lappa* Linne): Its scavenging effect on free-radical and active oxygen. J. Am. Oil Chem. Soc. 1998;75:455-461.

52. Rathee JS, Hassarajani SA, Chattopadhyay S. Antioxidant activity of *Nyctanthes arbor-tristis* leaf extract. Food Chem. 2007;103:1350-1357.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1176&id=14&aid=9464