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Antioxidant activity of bioactive extracts obtained from rhizomes of *Cyperus digitatus* Roxb.

[Actividad antioxidante de extractos obtenidos a partir de rizomas de *Cyperus digitatus* Roxb.]

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Abstract: Members of the family Cyperaceae such as *Cyperus alopecuroides*, *Cyperus articulatus*, *Cyperus scariosus* and *Cyperus rotundus* possess significant amount of studies about their antioxidant activities and other properties. Nevertheless, the plant *Cyperus digitatus* belonging to the genus Cyperus lacks of studied about any kind of intrinsic activity. Different extracts and fractions were obtained from the rhizomes of *Cyperus digitatus*, and a Phytochemical screening and the content of phenols and flavonoids and the antioxidant properties (FRAP, DPPH and β-Carotene bleaching) were quantified in each of theextracts and fractions. Of all the extracts obtained, the BE and AqE extracts showed the best antioxidant potential, meanwhile, none of the fractions obtained from the EAE extract show a relevant activity.

Keywords: *Cyperus digitatus*, antioxidant activity, oxidative stress

Resumen: Los miembros de la familia Cyperaceae, tales como *Cyperus alopecuroides*, *Cyperus articulatus*, *Cyperus scariosus* y *Cyperus rotundus* poseen una cantidad significativa de estudios sobre sus actividades antioxidantes y otras propiedades. Sin embargo, la planta *Cyperus digitatus* perteneciente al género Cyperus carece de estudio de cualquier tipo de actividad intrínseca. Razón por la cual se estudió sus propiedades antioxidantes (FRAP, DPPH y blanqueamiento del β-caroteno), cuantificación de contenido fenolico y flavonoides totales en extractos y fracciones obtenidos de los rizomas de *Cyperus digitatus*, y un perfil fitoquímico. De todos los extractos obtenidos, BE y AqE mostraron el mejor potencial antioxidante, por otra parte ninguna de las fracciones obtenidas a partir del extracto EAE mostro una actividad relevante.

Palabras clave: *Cyperus digitatus*, actividad antioxidante, estres oxidativo

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INTRODUCTION

Oxidative stress is defined in general as excess formation and/or incomplete removal of highly reactive molecules such as reactive oxygen species (ROS). The most deleterious effect caused by ROS is the peroxidation of membrane lipids, proteins and DNA, (Selvi & Chinnaswamy, 2007) leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans. (Farber, 1994)

Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, they are quite large and diverse and their main function is to oppose the process of oxidation largely by neutralizing free radicals. (Sohal & Weindruch, 1996)

The growing interest in the substitution of synthetic antioxidant by natural ones has fostered research on plant sources and the screening of raw materials for identifying new antioxidants or intrinsic antioxidant activities. (Priya & Padmakumari, 2012)

The family of the Cyperaceae conformed by 70 genus and about 3700 species, is abundant in wetlands and in the waters edge from Ecuador to the poles. (Vare & Kukkonen, 2005) The genus *Cyperus* has a great importance in terms of their use in traditional medicine due to its broad spectrum of biological activities, from the estrogenic activity of the ethanolic extract of the inflorescences of *C. alopecuroides*, (Nassar *et al.,* 2002) analgesic activity of the decoction of the rhizomes of *C. articulatus*, (Dalziel, 1937) hepatoprotective activity of the methanol extract of *C. scariosus* (Gilani & Janbaz, 1995) and antioxidant activity of the DPPH radical in extracts of *C. rotundus*, (Kilani *et al*., 2005) being the latter (*C. rotundus*) the most common specie of this family, known as weeds and with more bibliographic information. (Holm *et al*., 1997)

However, *Cyperus digitatus* (Family: Cyperaceae; *C. digitatus*) is a herb of this family that has not been investigated, it is distributed in pantropics and subtropics (Bangladesh, Bhutan, India, Myanmar, Sri Lanka, Nepal, Pakistan, Mexico, Central America, South America and Africa). It is a perennial herb, found in swamps or seasonally flooded areas, ditches and river banks. (Kumar, 2011). The whole plant is used in Pakistan as a skin anti-allergic (Ikram *et al.,* 2014). In view of the no chemical information regarding *C. digitatus*, the present study was designed to examine the phytocheminal composition, the total polyphenol and flavonoid content and the antioxidant activity of different extracts and fractions obtained from the *C. digitatus*, that can explain at least in part the ethnopharmacological use of *C. digitatus* as a skin anti-allergic (Kawai *et al.,* 2007)

MATERIAL AND METHODS

Chemicals and reagents

Sodium sulfate anhydrous, thin layer chromatography (TLC) plates, methanol, ethanol, ethyl acetate and butanol were obtained from Arquimed (Santiago, Chile), whereas gallic acid, quercetin, Folin-Ciocalteu, sodium carbonate, sodium nitrate, aluminum chloride, β-Carotene, hydrochloric acid, sodium hidroxide, linoleic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH),2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), tween 40 and hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, Missouri/MO, U.S.A).

Plant Material

The plant material (rhizomes) of *C. digitatus* was collected from the research center MACAGUAL of the University of the Amazon (Colombia) with coordinates, North: 01° 29' 51.1" West: 75° 39' 35.5", at a height of 254 meters and classified taxonomically in the Venezuelan Institute of Scientific Research (IVIC) and a voucher specimen of the plant is kept for reference (Nº 010104) in the Herbarium of the University of the Amazon (Colombia)

Extraction and isolation

The air-dried plant material (310 g), was extracted with ethanol (EtOH) at 95% at room temperature, obtaining 16.6 g of crude extracts (EE). Subsequently, was performed a liquid-liquid partition of 10.7 g of the EE with ethyl acetate (EtOAc): water (1: 1, v/v) to obtain two phases. The ethyl acetate extract (EAE), at which anhydrous sodium sulfate (Na_2SO_4) was added to remove any residual water, obtaining 4.6 g of crude extracts, and the aqueous residue (AR) was added butanol (BuOH) to obtain two phases. The butanol extract (BE) was concentrated under reduced pressure to obtain 0.3 g, and the aqueous extract (AqE) obtained was lyophilized and 1.6 g were extracted.

The EAE (1.8 g) was fractionated by column chromatography using silica gel and eluting with a gradient of increasing polarity with Petroleum ether, EtOAc and EtOAC : MeOH (98:2, 95:5 and 7:3, v/v), collecting a total of 33 fractions (10 mL / tube), of which, according to the behavior obtained by the monitoring made by thin layer chromatography (TLC) and revealed with a UV lamp (254-366 nm)

and iodine chamber, 3 fractions were made (EAF1, EAF2, EAF3).

Phytochemical screening

The preliminary phytochemical analysis to identify the presence of secondary metabolites in the different extracts and fractions of *C. digitatus*, was performed by the methods of revealed on TLC, previously described by Wagner *et al*. (1984).

Total Phenolic Content (TPC) and ToTal Flavonoid Content (TFC)

The TPC of the extracts and fractions was determined according to the Folin-Ciocalteu method. (Singleton & Joseph, 1965) the total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per g of extract or fraction. The TFC were determined spectrophotometrically using the method of Zhishen *et al*. 1999, based on the formation of a flavonoid-aluminum complex. The results are reported as quercetin equivalents (QE) in milligrams per g of extract or fraction.

Determination of antioxidant activity

The scavenging activity of the extract and fractions were estimated using DPPH as the free radical model according to the method adapted from Brand-Williams *et al*., 1995, and Molyneux, 2004. Quercetin was used as reference compounds. The FRAP analysis was introduced by Benzie and Strain, 1996, to measure the total antioxidant activity and is based on the ability of polyphenols to reduce the Fe^{+3} to Fe^{+2}. At a low pH the complex ferric-tripyridyltriazine (Fe-TPTZ) is reduced to the ferrous form (Fe^{+2}) to form a blue complex with an absorption maximum of 593 nm.The percentage of Fe^{+3} scavenging (reduction to Fe^{+2}) was calculated by comparison with the standard curve (mmol Fe^{+2} per g of extract or fraction). The β-Carotene bleaching assay was evaluated according to Miller, 1971. The results were expressed as the percentage of bleaching inhibition at 90 min.

Statistical analysis

Each measurement was performed in triplicate. All data are expressed as mean ± standard error of mean (SEM). The statistical analysis t test was used with the software SPSS 15.0 (Statistical Product and Service Solutions). The statistical significance level was set up at $p < 0.05$**.**

RESULTS AND DISCUSSION

Free radicals are highly reactive molecules with an unpaired electron and are produced by radiation or as byproducts of metabolic processes. Antioxidant compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus reduce the level of oxidative stress and slow/prevent the development of complications associated with oxidative stress-related diseases. (Wu & Hansen, 2008) Many synthetic antioxidants have shown toxic and mutagenic effects, which have shifted attention towards naturally occurring antioxidants (Devi *et al.*, 2008). Extracts of plants could replace those synthetic antioxidants which have been questioned due to possible undesirable side effects. That is why, in this study we demonstrated the antioxidant activity of a plant which has not been studied as the *C. digitatus* which is traditionally used as an anti-allergenic (Ikram *et al.,* 2014) and study of its antioxidant properties and preliminary composition may partly explain this traditional use.

Phytochemical study

The EE, EAE, BE, AqE, and the fractions obtained from column chromatography of the EAE (EAF1, EAF2 and EAF3) were analyzed by TLC using as a mobile phase a mixture of EtOAc:MeOH (98:2, v/v) and different revelators to identify phytochemical constituents, evaluating the presence of flavonoids, alkaloids, anthrones, anthraquinones, coumarins, terpenes and steroids, the general fractionation is described in Figure 1.

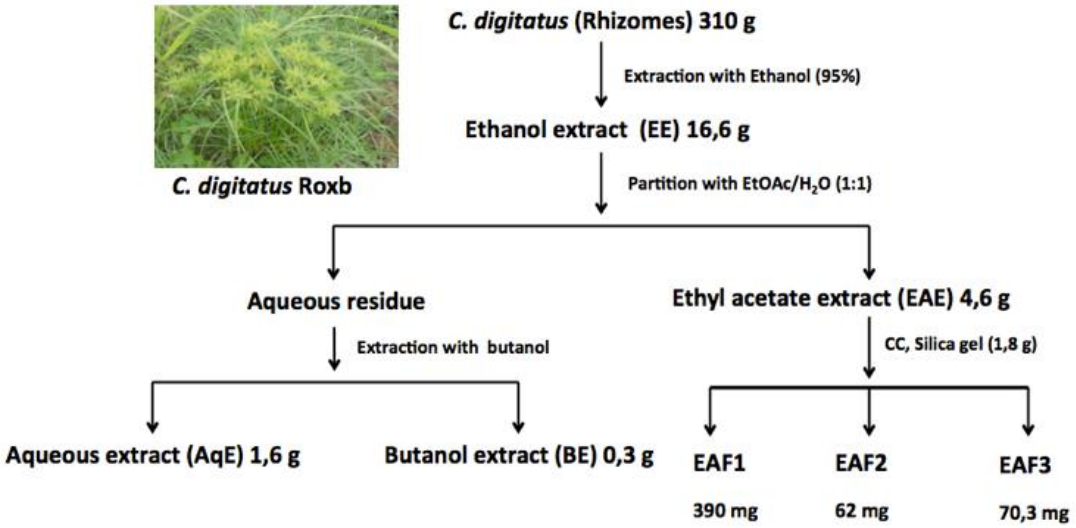

Figure 1
Extracts obtained by liquid-liquid separation and fractionation by column chromatography of the *Cyperus digitatus*.

The phytochemical study of *C. digitatus* showed the presence of various quantities of flavonoids in BE and EAE, whereas all extracts obtained showed the presence of terpenes and steroids. Meanwhile, all fractions obtained from EAE showed the presence of flavonoids and terpenes, while in EAF1 was also observed the presence of alkaloids and coumarins (Table 1). Like in other *Cyperus* species such as *C. articulatus* and *C. rotundus*, it was observed the presence of secondary metabolites such as coumarins, flavonoids, terpenes and steroids, demonstrating good chemotaxonomic relationship among the species of the genus. (Schultes & Raffaud, 1990) In turn, the use of different solvents showed an immediate effect in the various extraction yields.

Table 1
Phytochemical screening and yield obtained from the extracts and fractions of *Cyperus digitatus*

Extracts	Flavonoids	Alkaloids	Antrona	Antraquinone	Coumarins	Terpenes	Steroids	Yield (%)
AqE	-	-	-	-	-	+	+	0.52
EE	-	-	-	-	-	+	+	5.35
BE	+	+	-	-	-	+	+	0.10
EAE	+	+	+	+	-	+	+	1.48
EAF1	+	+	-	-	+	+	+	0.02
EAF2	+	-	-	-	-	+	+	0.02
EAF3	+	-	-	-	-	+	+	0.02

Total Phenolic and Flavonoid Content
All extracts were determined total phenolic and flavonoid content using colorimetric methods. The results expresed as concentration and antioxidant activity expresed as inhibitory concentration of 50% (IC_{50}) are sumarized in Table 2.

Table 2

Sample	TPC mg GAE/g	TFC mg QE/ g	DPPH IC_{50} (µg/mL)	β-Carotene IC_{50} (µg/mL)
AqE	10.8 ± 1.0	2.9 ± 0.3	15.2 ± 0.7	48.1 ± 1.0
EE	18.6 ± 0.9	6.8 ± 0.9	54.4 ± 0.5	>100
BE	103.0 ± 2	13.2 ± 1.0	23.6 ± 0.8	46.3 ± 0.7
EAE	52.0 ± 0.8	9.0 ± 1.0	18.3 ± 0.6	>100
EAF1	7.0 ± 0.9	3.0 ± 0.6	>100	>100
EAF2	19.0 ± 1.0	11.0 ± 0.9	>100	>100
EAF3	20.0 ± 0.7	3.4 ± 1.0	>100	>100

Total phenolic and flavonoids content of extracts and fraction of *C. digitatus* and antioxidant activity. Values are presented as means ± S.E.M (n = 3)

The TFC of the extracts were ranged from 2.9 to 13.2 mg QE/g of extract. The differences were significant ($p < 0.05$) among the extracts of different polarities, with the highest value for the BE. The TFC among the fractions were significant ($p < 0.05$), the EAF2 presented the highest value followed by fractions EAF3 and EAF1. The highest concentration of phenols and flavonoids was found in the extracts obtained with medium and high-medium polarity solvents (ethyl acetate and butanol), similar results have been found in other studies, where the use of butanol results in greater extraction of phenols compared to ethyl acetate (Brand-Williams *et al*. 1995). For its part, the fractions EAF3 and EAF2 showed a total phenol concentration greater than the fraction EAF1 ($p < 0.001$), however, none of the fractions obtained showed antioxidant activity at a concentration of 100 µg/mL.

Antioxidant activity

The DPPH, is a stable nitrogen centered free radical with a characteristic absorption at 517 nm, and it has been widely used for rapid evaluation of the antioxidant activity of plant extracts relative to other methods due to their simple, rapid, sensitive and reproducible procedures. (Villano *et al*., 2007; Hu & Kitts, 2000). The results indicate a good hydrogen donating ability of the AqE and BE, since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability.

All extracts and fractions wetre evaluated as β-carotene bleanching, the BE and the AqE showed activity with an IC_{50} of 46.3 and 48.1 µg/mL, respectively (Table 2). The bleaching mechanism of *β*-carotene is a free radical mediated phenomenon resulting from the formation of hydroperoxides from linoleic acid and the presence of antioxidants hinders the extent of bleaching by neutralizing the linolichydroperoxyl radical formed. (Jayaprakasha *et al.,* 2001; Ismail & Tan, 2002) Based on the foregoing, the BE and AqE, were the only in show a significant effect neutralizing the linolichydroperoxyl radical. These results demonstrate the antioxidant potential of the butanol and aqueous extracts in protect the extent of *β*-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

FRAP (ferric reducing antioxidant power) assay

Of all the extracts obtained from the *C. digitatus* only the BE showed the ability to reduce the iron statistically significant compared with the rest (Figure 5), with a capacity of 1.9 mmol Fe^{+2} / g of extract ($p < 0.05$). Meanwhile, the fractions obtained from the EAE behaved similarly to the extract of which were obtained (data not shown).

For the measurement of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of an extract or fraction of *C. digitatus*. Of the extracts, only BE showed a significant reducing ability which greatly depends on the presence of antioxidant with a reduction potential, which exhibit antioxidant potential by breaking the free radical chain by donating a hydrogen atom (Duh, 1998). The reducing capacity of BE is a significant indicator of its potential antioxidant activity.

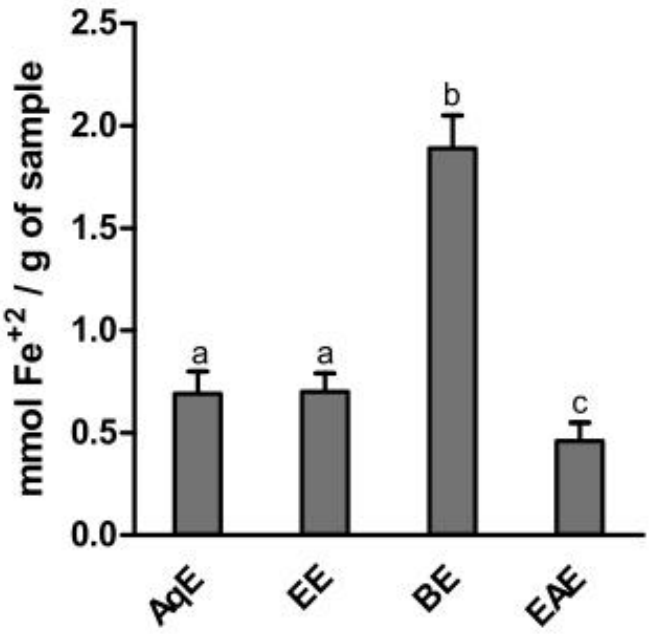

Figure 5
Antioxidant activity of the different extracts. Quercetin (10 µg/mL) presents 323 ± 2.2 mmol Fe^{+2}/g. AqE: aqueous extract; EE: ethanol extract; BE: butanol extract; EAE: ethyl acetate extract. $^{a}P < 0.05$. Statistically significant differences between AqE, EE and BE, EAE. $^{b}P < 0.001$. Statistically significant differences between BE and AqE, EE, EAE. $^{c}P < 0.05$. Statistically significant differences between EAE and AqE, EE, BE. Values are presented as mean ± S.E.M (n = 3).

Conclusion

Since the role of free radicals has been implicated in a large number of diseases, the antioxidant activity of different plants is of significant importance in exploiting their therapeutic potential. The present study elucidated the antioxidant property of *C. digitatus*. This study suggested that, among the different extracts and fractions obtained, the Butanol extracts possesses the higher antioxidant activity followed by the Aqueous extract, this might be helpful in preventing the progress of various oxidative stress related disorders. Holding a good prospective as a nutraceutical or chemotherapeutic agent because of their radical scavenging ability.

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