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Phytochemical screening, total phenolic content and antioxidant activity of some plants from Brazilian flora

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The present study evaluated the total phenolic and flavonoid content as well as the antioxidant activity of methanolic leaf extracts of five plants from Brazilian flora: *Abarema cochliacarpos*, *Croton corchoropsis*, *Myroxylon peruiferum*, *Stryphnodendron pulcherrimum* and *Tanaecium cyrtanthum* by 2,2'-azino-bis- (3-ethylbenzothiazoline-6-sulfonate) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total antioxidant capacity assays. A thin layer chromatography analysis of all plant extracts has also been performed and it showed the presence of different types of secondary metabolites, namely saponins, phenylpropanoids and flavonoids. Among the studied plants, *A. cochliacarpos* and *S. pulcherrimum* showed considerable antioxidant radical scavenging activity on all the tested assays and they also exhibited substantial amounts of phenolic compounds. In addition, a positive correlation was found between total phenols and both ABTS radical scavenging activity and total antioxidant capacity assays, thus indicating the major role of phenols on the antioxidant activity of these plants. To the best of the authors' knowledge, this is the first approach where the phenolic content and antioxidant activity of *A. cochliacarpos, C. corchoropsis, M. peruiferum, S. pulcherrimum* and *T. xanthophyllum* were explored.

Key words: Brazilian medicinal plants, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), flavonoid content, phenolic content.

INTRODUCTION

Oxidation and reduction of molecules are essential to life; they represent normal phenomena that occur in cell metabolism. Among substances involved in oxidation-reduction reactions of molecules are free radicals, which are organic or inorganic compounds having one or more unpaired electrons on their valance shell, they are chemically unstable and very reactive (Lushchak, 2014).

In organism, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in metabolic processes such as energy production, regulation of cell

growth, intercellular signaling, phagocytosis and synthesis of important biological molecules. For many years, chemists have known that free radicals cause oxidation, which can be controlled or prevented by a range of antioxidants substances (Bild et al., 2013; Rahal et al., 2014).

The amount of free radicals in the body is counterbalanced by the availability of antioxidants, which are compounds capable of either preventing formation of free radicals or by reacting with them directly. The imbalance of free radicals/antioxidants in favor of free radicals can lead to establishment of oxidative stress, a situation characterized by biomolecule impairment and consequently to human health peril (Rajendran et al., 2014).

Antioxidants are believed to play an important role in the prevention of several diseases (Ngo et al., 2011). The therapeutic effects of various natural plant-derived medicines are correlated with their antioxidant activity (Ezhilarasan et al., 2014). Among molecules produced by plants, the polyphenols are one of the most widely studied classes whose remarkable antioxidant capacity are credited primarily due to their reducing properties and chemical structure (Barreiros et al., 2006). Studies indicate that consumption of fruits and vegetables containing phenolic antioxidants and other phytochemicals is advantageous for health (Almeida et al., 2011).

Antioxidants are applied in food industry as agents that prevent autoxidation of meat, fruit and oils, where several compounds such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butyl hydroquinone (TBHQ) are produced for this purpose (Karpińska-Tymoszczyk, 2014). However, there is growing concern over the possible carcinogenic effects of synthetic antioxidants in foods (Juntachote et al., 2006). Yet, the potential health risks by the use of synthetic antioxidants have triggered the interest in the search for natural antioxidants (Mohamed et al., 2011).

Abarema cochliocarpos (Gomes) Barneby & Grimes (Fabaceae) is a plant popularly known as "barbatimão", "bordão-de-velho", "ingá-negro" and "barbimão" (Iganci and Morin, 2012). This plant is mainly found in the Brazilian Atlantic Forest and Caatinga biomes (Watkinson, 2012). Regarding the biological activities of this species, antimicrobial, antinociceptive, healing and antioxidant properties have been described (Santos et al., 2007; Silva et al., 2009; Sánchez-Fidalgo et al., 2013). The decoction from its bark is utilized in folk medicine as wound-healing, antiseptic, analgesic and it is used against dermatosis, leukorrhea, inflammation and gastric ulcer (Araújo et al., 2002; Santos et al., 2007; Silva et al., 2010a).

Myroxylon peruiferum L. f. (Fabaceae) is a tree present all over the Brazilian territory, especially at semideciduous forests and it is locally known as “cabreúva” (Sebbenn et al., 1998). Ohsaki et al. (1999) evaluated its antimicrobial potential against *Helicobacter pylori*, but studies on the biological activity for this species are scarce. From its wood can be extracted an exudate known as “balsam of Peru” or “Tolu”, it is used in folk medicine against coughs, bronchitis, diabetes and sedative in case of urinary problems (Rizzini and Mors, 1995).

Croton corchoropsis Baill (Euphorbiaceae) is a subshrub plant found in Brazilian Cerrado, Campos Rupestres and Caatinga biomes (Silva J et al., 2009). Although, some studies involving biological activities of plants of this genus have been reported in the literature (Morais et al., 2006; Salatino et al., 2007), so far, no studies of antioxidant activity for *C. corchoropsis* were performed. *Stryphnodendron pulcherrimum* Mart. (Fabaceae) is an arboreal species occurring in the Amazonian forest and Atlantic forest in northeast Brazil (Scalon, 2007). Castilho et al. (2013) reported the antibacterial activity of extracts of this plant against oral pathogens.

Tanaecium cyrtanthum (Mart. ex DC.) Bureau & K.Schum. (Bignoniaceae) is a liana that can be found in northeast Brazil (Lohmann and Taylor, 2014). Studies regarding this species are rare. All plants listed in this study are species found in the Caatinga biome, a semi-arid climate region which offers a set of characteristic environment conditions that are believed to promote an augmentation in plant production of secondary metabolites, some of those molecules in turn can have antioxidant properties (Lemos and Zappi, 2012; Chaves et al., 2013; Alamgir et al., 2014).

All these plant species are found in Pernambuco state (northeast Brazil) inside rural community regions where locals make use of folk medicine as their primary healthcare, but studies are lacking to give support for these traditional plant usages as well to promote proper exploitation of new phytotherapics. Overall, *M peruiferum*, *C. corchoropsis*, *S. pulcherrimum* and *T. cyrtanthum* are poorly studied plants and with the exception of *M. peruiferum*, so far no articles were found on leaf extracts of these species.

This study aimed to conduct analyses of antioxidant activity as well as the total phenol and flavonoid contents in methanolic leaf extracts of five plants (*A. cochliacarpos*, *C. corchoropsis*, *M. peruiferum*, *S. pulcherrimum* and *T. cyrtanthum*) located in Pernambuco state, Brazil and to correlate the total phenolic and

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flavonoid content with the antioxidant activities of the extracts.

MATERIALS AND METHODS

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis- (3-ethyl benzothiazoline-6-sulfonate) (ABTS), gallic acid, Folin-ciocalteu's reagent, quercetin and trolox were purchased from Sigma-Aldrich. Sodium phosphate, ammonium molybdate were from Vetec (Brazil); ascorbic acid from Anidrol (Brazil), sulfuric acid from Cinética (Brazil) and potassium persulfate from Labsynth (Brazil). All the chemicals including solvents were of analytical grade.

Plant material

The plants chosen for this study were based on ethnobotanical data available in the literature (Agra et al., 2007, 2008). The plant species, *A. cochliacarpos* (Gomes) Barneby & Grimes (Fabaceae) and *S. pulcherrimum* (Willd.) Hochr. (Fabaceae), were collected at Reserva de Floresta Urbana Mata de Camaçari, Cabo de Santo Agostinho, Pernambuco State, Brazil, a Conservation Unit of the Atlantic Forest biome. *C. corchoropsis* Baill. (Euphorbiaceae), *M. peruiferum* L.f. (Fabaceae) and *T. cyrtanthum* (Mart. ex DC.) Bureau & K.Schum. (Bignoniaceae) were collected at Parque Nacional do Catimbau, Conservation Unit of the Caatinga biome, Municipalities of Buíque, Ibimirim and Tupanatinga, Pernambuco State, Brazil. Leaves were collected in February (2012) and the plants were botanically identified at Herbarium IPA, from Instituto Agronômico de Pernambuco (Agronomic Institut of Pernambuco State), Brazil. Vouchers species: *A. cochliacarpos* (92001), *S. pulcherrimum* (92002), *C. corchoropsis* (92003), *M. peruiferum* (92004) and *T. cyrtanthum* (92005) were deposited at the herbarium IPA.

Preparation of leaf extracts

The leaves were dried in forced-air circulation oven at 40°C for 72 h, pulverized with a grinder, and stored at room temperature in closed containers until use. The powdered leaves of the five species (100 g, each) were extracted in Soxhlet extractor at 65°C containing 1000 mL of methanol. The material was refluxed for about 48 h until exhaustion and the resulting extracts were filtrated through Whatman No.4 filter paper. The extracts were then concentrated in vacuum at 40 °C using a Rotary Evaporator. Then the extracts were preserved in sealed vials at 4°C until further analyses.

Phytochemical screening

The phytochemical screening of the extracts was performed by thin-layer chromatography (TLC) on silica plates (60F254, aluminum backed, 200 µm layer thickness, 10.0 x 5.0 cm, Macherey-Nagel, Ref. 818160, Germany). The presence of flavonoids, phenylpropanoids, alkaloids, terpenes, steroids, coumarins, quinones and proanthocyanidins were investigated using adequate development systems and revealers, as shown in Table 1 (Roberts et al., 1957; Brasseur and Angenot, 1986; Wagner and Bladt, 1996; Harborne, 1998). After development, the plates were air dried and sprayed with the revealers in a fume hood.

Estimation of total phenolic content

The total phenolic content was determined by Folin-Ciocalteu method (Singleton and Rossi, 1965) with minor modifications. 200 µL aliquots of plant extract s at 1 mg/mL were mixed with 1 mL of Folin Ciocalteu reagent (1: 1 v/v) and 2.5 mL of 20% Na_2CO_3 were added. The mixtures were incubated for 30 min at room temperature and protected from light for subsequent reading of absorbance against a blank solution consisting of methanol plus all reagents without extracts. They were read in a 765 nm spectrophotometer and the total phenolic content was calculated using gallic acid as reference in the range of 25-500 mg/mL. The results were expressed in mg of gallic acid equivalents per extract gram (mgGAE.g^{-1} extract).

Estimation of total flavonoid content

The flavonoid contents were measured by aluminum chloride colorimetric method (Woisky and Salatino, 1998). 500 µL of samples (1 mg/mL) were added to 500 µL of 2% methanolic $AlCl_3$. After 1 h incubation at room temperature, the absorbance was measured against a blank of methanol and aluminum chloride in a spectrophotometer at 420 nm. Flavonoid content was estimated using a quercetin standard curve (0.98-7.81 µg/mL) and the results were expressed as mg of quercetin equivalent per extract gram (mg QE. g^{-1} extract).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH free radical scavenging activity of the extracts was performed according to Brand-Williams et al. (1995) with some modifications. A stock solution of DPPH 200 µM in methanol was further diluted in methanol to obtain an absorbance between 0.6 - 0.7 at 517 nm, resulting in the DPPH working solution. Different concentrations of the extracts were mixed with DPPH solution and after 30 min incubation in darkness, the absorbance were read at the same wavelength mentioned above. Then it was plotted a graph of DPPH scavenging activity against different concentrations of extracts to calculate the IC_{50}, which denotes the sample concentration required to decrease the initial DPPH radical concentration by 50%. Gallic acid was used as standard. The measurements were triplicate and their scavenging activities were calculated based on the percentage of DPPH scavenged.

2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging assay

ABTS radical scavenging assay was carried out according to Re et al. (1999) with minor modifications. Briefly, a radical ABTS stock solution was prepared by dissolving ABTS (7 mM) with potassium persulphate ($K_2S_2O_8$, 2.45 mM). The mixture was left to stand for 16 h (time required for the formation of the radical) in the dark at room temperature before use. To perform the assay, the previously made solution was diluted in ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm, thereby obtaining the ABTS radical working solution. 30 µL plant extracts (1 mg/mL) were mixed with the working solution and left to rest for 6 min before measuring the absorbance at 734 nm against a blank (working solution plus methanol) and it was applied as standard.

Evaluation of total antioxidant capacity

The total antioxidant capacity (TAC) was based on the method of Prieto et al. (1999). 0.1 mL of the extracts (1 mg/mL) were combined with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the solutions were incubated at 95°C for 90 min, then cooled to room temperature and the absorbance of each

Table 1. Development systems and revealers used for phytochemical screening by thin-layer chromatography.

Secondary metabolites	Standard	Development system	Revealer
Flavonoids and phenylpropanoids	Quercetin, rutin and chlorogenic acid	EtOAc-HCOOH-AcOH-H_2O (100:11:11:27 v/v)	Neu's reagent
Alkaloids	Pilocarpine	EtOAc-HCOOH-AcOH-H_2O (100:11:11:27 v/v)	Dragendorff's reagent
Triterpenes and steroids	β-sitosterol and ursolic acid	Toluol:EtOAc (90:12 v/v)	Liebermann-Burchard's reagent
Mono and sesquiterpenes	Thymol	Toluol:EtOAc (97:03 v/v)	Anisaldehyde-sulphuric acid reagent
Coumarins and quinones	Coumarin and lapachol	$CHCl_3$-MeOH (98:2 v/v)	KOH
Proanthocyanidins	Catequin	EtOAc-HCOOH-AcOH-H_2O (100:11:11:27 v/v)	Vanillin- hydrochloric acid reagent

Table 2. Phytochemical screening of plant extracts by thin layer chromatography.

Secondary metabolites	*Abarema cochliacarpos*	*Croton corchoropsis*	*Myroxylum peruiferum*	*Stryphnodendron pulcherrimum*	*Tanaecium cyrtanthum*
Saponins	+++	+	+++	+++	+
Flavonoids	++	+	+	+	+
Phenylpropanoids	++	++	+	+	+
Triterpenes and steroids	*	-	-	-	-
Mono and sesquiterpenes	+	-	*	*	-
Alkaloids	-	+	-	-	-
Proanthocyanidins	+ [(1)]	-	-	+ [(1)]	-
Coumarins	-	-	+	-	-
Quinones	-	-	-	-	-

[1]Polymeric proanthocyanidins, monomeric and dimeric proanthocyanidins absent; *only traces detectable.

sample were measured at 695 nm against a blank (1 mL of reagent plus 0.1 mL of methanol).

Statistical analysis

Assays were performed in triplicate and th results are shown as mean ± standard deviation. Linear regression analysis and Pearson's correlation coefficient were calculated using Microsoft Excel Windows 2013.

RESULTS AND DISCUSSION

Phytochemical screening by TLC indicated the presence of different types of secondary metabolites, namely flavonoids, saponins and phenylpropanoids in all plant extracts. *A. cochliacarpos* and *S. pulcherrimum* also showed the presence of polymeric proanthocyanidins, while *M. peruiferum* showed the presence of coumarins. Alkaloids were detected only in *C. corchoropsis* (Table 2). Preliminary phytochemical screening experiments are commonly performed to promote a guidance of substantial phytochemicals that may be involved in the antioxidant activity of plant extracts (Anandakirouchenane et al., 2013; Basma et al., 2011; Das et al., 2012).

Phenolic compounds are considered important natural antioxidants and represent one of the most abundant compounds in plants. They display several functions such as pigmentation, protection against ultraviolet rays, allelopathic action, defense against microbial attack and predators (Naczk and Shahidi, 2006). The total phenolic content of the extracts ranged from 28.84 to 120.39 $mgGAE.g^{-1}$, it was higher for *A. cochliacarpos* (120.39 ± 2.82 $mgGAE.g^{-1}$), followed by *S. pulcherrimum* (86.67 ± 0.83 $mgGAE.g^{-1}$) (Table 3).

Phenolic compounds exhibit their antioxidant activity by various mechanisms such as donation of hydrogen atoms to free radicals and through connection to transition metal ions resulting in more stable forms (Kumar et al., 2014). Various physiological actions performed by polyphenols were related to the prevention of neurodegenerative and cardiovascular diseases, cancer, among others, mainly because of their high antioxidant capacity (Wootton-Beard et al., 2011).

Table 3. Total phenolic content (mgGAE.g^{-1}) and flavonoid content (mgQE.g^{-1}).

Plant extracts	Phenolic (mgGAE.g^{-1})	Flavonoid (mgQE.g^{-1})
Abarema cochliacarpos	120.39 ± 2.82	5.55 ± 0.13
Stryphnodendron pulcherrimum	86.67 ± 0.84	6.04 ± 0.18
Myroxylum peruiferum	42.18 ± 3.57	10.52 ± 0.55
Tanaecium cyrtanthum	30.16 ± 1.41	3.84 ± 0.03
Croton corchoropsis	28.84 ± 1.17	4.11 ± 0.14

GAE: Gallic acid equivalent; QE: quercetin equivalent.

Table 4. Effect of methanolic extracts on different antioxidant models.

Plant extracts	DPPH IC_{50}	$ABTS^{+}$ (%)	TAC (%)
Abarema cochliacarpos	31.62 ± 2.28	75.69 ± 3.88	43.71 ± 1.66
Stryphnodendron pulcherrimum	35.47 ± 2.48	69.65 ± 5.60	41.91 ± 1.91
Myroxylum peruiferum	37.26 ± 1.54	20.92 ± 3.41	15.55 ± 0.87
Tanaecium cyrtanthum	43.77 ± 2.10	30.83 ± 2.65	21.75 ± 1.66
Croton corchoropsis	87.84 ± 1.74	33.66 ± 1.67	15.13 ± 1.52

Data are expressed as means of three replicates.

Among the polyphenol compounds, the most studied subclass is the flavonoids which in plants are commonly found conjugated to sugars (Wang et al., 2013). The total flavonoid content was quantified by the aluminum chloride method and expressed as quercetin equivalents (QE) per gram of substrate. The total flavonoid content was in the range of 3.84 to 10.52 mg QE.g^{-1} in which *M. peruiferum* had the highest concentration among the five extracts (10.51 ± 0.55 mg QE.g^{-1}) (Table 3).

Silva et al. (2010b) reported the occurrence of polyphenolic compounds highlighting catechins (flavonoids) the major constituent in butanolic extracts of *Abarema cochliocarpos* bark. Mathias et al. (2000) reported the presence of isoflavones, pterocarpans, coumestans, flavanone and isoflavanones from the bark of *Myroxylon peruiferum* and Carvalho et al. (2008) isolated 3 compounds from its leaves, namely 3',4',7-trimethoxyisoflavone (cabreuvina), 6-hydroxy-4',7-dimethoxyisoflavone and germacrene D. Some species of *Stryphnodendron* genus are known for their high polyphenol content of tannins (Lima et al., 2010). Phenolic substances have been reported for several *Croton* species, among which are flavonoids, lignoids and proanthocyanidins predominate (Salatino et al., 2007). As for *Tanaecium cyrtanthum*, there are only few reports in the literature on this species and no phytochemical assessment was found.

The antioxidant activity of the extracts by DPPH, ABTS and total antioxidant capacity (TAC) methods are outlined in Table 4. IC_{50} values for DPPH scavenging activity were smaller for *A. cochliocarpos* (31.62 ± 2.28 µg/mL) and *S. pulcherrimum* (35.47 ± 2.48 µg/mL) followed by *M. peruiferum* (37.26 ± 1.54 µg/mL) (Table 4). The lower IC_{50} value indicates higher antioxidant capacity, therefore *A. cochliacarpus* and *S. pulcherrimum* showed the best results. The ABTS radical scavenging activity was higher for *A. cochliocarpos* (75.69 ± 3.88%); followed by *S. pulcherrimum* (69.65 ± 5.60%) (Table 4). *A. cochliocarpos* and *S. pulcherrimum* extracts obtained the highest antioxidant activities in both DPPH and ABTS assays.

DPPH and ABTS radical scavenging assays are methods based on the sequestration of these radicals by proton donor substance, the reaction is followed by a measurable change in absorbance spectrophotometry (Floegel et al., 2011). Both DPPH and ABTS assays are widely used to assess the antioxidant capacity of natural products (Schaich et al., 2015).

The total antioxidant capacity (TAC) is a spectrophotometric assay based on the reduction of Mo(VI) to Mo(V) by the action of an antioxidant substance with the subsequent formation of a green phosphate/Mo(V) complex with a maximum absorption at 695 nm (Prieto et al., 1999). The total antioxidant capacity (%TAC) of the extracts were higher in *A. cochliocarpos* (43.71 ± 1.66%) and *S. pulcherrimum* (41.91 ± 1.91%) (Table 4). In the same manner of DPPH and ABTS assays, *A. cochliocarpos* and *S. pulcherrimum*

Table 5. Correlation between total phenolic content, total flavonoid content and antioxidant assays

Parameter	Total phenolic content		Total flavonoid content	
	R^2	ρ	R^2	ρ
DPPH $1/IC_{50}$	0.564	0.751	0.182	0.427
$ABTS^+$ (%)	0.858	0.926	0.068	-0.261
TAC (%)	0.872	0.933	0.026	-0.163

R^2: Coefficient of determination, ρ: Pearson coefficient.

had the highest total antioxidant activity, in addition they also were the ones that showed the highest total phenolic content among the studied plants (Table 3). It is worthy to note that the possible antioxidant potentials of plant extracts commonly depend on the phytochemical composition and extraction systems including methods, duration and polarity of organic solvents. For this reason, the antioxidant potentials cannot be completely described using one single method. In this context, more than one antioxidant test system is required to determine the mechanisms of antioxidant actions of plant extracts (Wong et al., 2006).

The total phenolic and flavonoid contents of the extracts were compared with the values of their respective antioxidant activities by using the Pearson coefficient (ρ) and coefficient of determination (R^2), where a positive correlation can be found between total phenols and ABTS radical scavenging activity (ρ = 0.926, R^2 = 0.858) as well as phenolic compounds and TAC (ρ = 0.933, R^2 = 0.872) (Table 5). Whereas flavonoid content of the extracts showed no significant correlation with the antioxidant activities (Table 5). These results suggest the importance of phenolic compounds (not flavonoids) on the antioxidant activity (%$ABTS^+$ and %TAC assays) of these plant extracts, however we cannot neglect the potential influence of other bioactive molecules that may be included in the extracts such as tocopherols, saponins, polysaccharides and ascorbic acid (Ananthi et al., 2010).

Kumar et al. (2014) using methanolic extracts of *Lantana camara* L. leaves obtained similar results when comparing phenolic content with ABTS activity (ρ = 0.998, R^2 = 0.997) and TAC (ρ = 0.946, R^2 = 0.896). In the same way, Basma et al. (2011) assessing antioxidant activities of *Euphorbia hirta* extracts found a relevant correlation between phenol content and IC_{50} DPPH values (R^2 = 0.989) but on the other hand, a moderate correlation was found between flavonoid content and IC_{50} DPPH (R^2 = 0.696). Silva et al. (2011) working with hydroalcoholic extracts of *Anadenanthera colubrina*, *Libidibia ferrea* and *Pityrocarpa moniliformis* fruits noted correlation between polyphenols and TAC (ρ = 0.923, R^2 = 0.862). In fact it is well known that there is a strong relationship between phenolic content and antioxidant activity in plants (Abdelhady et al., 2011; Hossain and Shah, 2015).

Conclusions

Based on the results obtained in the present study, it is concluded that the methanolic leaf extracts of *A. cochliacarpos* and *S. pulcherrimum* exhibit considerable antioxidant radical scavenging activity on all tested assays and they possess substantial amounts of phenolic compounds. Thus, these 2 plants can be considered a good source of antioxidants which might be beneficial for combating oxidative stress. A positive correlation was found between total phenolic content and ABTS radical scavenging activity as well as total antioxidant capacity assays of the plant extracts, thus indicating the key role that phenolic compounds may exert on the antioxidant activity of these plants. Hence more studies are required to isolate and identify these bioactive compounds responsible for such activities so as to assess their antioxidant activity *in vivo*.

Conflict of Interests

The authors have not declared any conflict of interests.

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