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Phytochemical screening, total phenolic content and antiradical activity of *Asplenium africanum* (Aspleniaceae) and fruit of *Megaphrinium macrostachyum* (Marantaceae)

Joseph Privat ONDO[a]*, Louis-Clément OBAME [a], Timoléon ANDZI BARHÉ [a], Gontran NSI AKOUE [a-b], Edouard NSI EMVO [a] and Jacques LEBIBI [a]

[a]Laboratoire de Substances Naturelles et de Synthèse Organométallique-URCHI, Faculté des Sciences, Université des Sciences et Techniques de Masuku, BP 901 Franceville, Gabon.

[b]Laboratoire de Physiologie Animale: Electrophysiologie-Pharmacologie-URAB, Faculté des Sciences, Université des Sciences et Techniques de Masuku, BP 913 Franceville, Gabon.

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ABSTRACT

Extracts from two plants consumed by *Mandrillus sphinx* Lékédi Park of Gabon, *Megaphrinium macrostachyum* (fruit) and *Asplenium africanum*, were screened for their phytochemical and total phenolics contents and their antiradical activity. Total phenolics were determined spectrophotometrically using the Folin-Ciocalteu reagent and expressed as gallic acid equivalent (GAE) and proanthocyanidin contents were expressed as apple procyanidins equivalent (APE). The antiradical activity was determined spectrophotometrically by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. From the results, alkaloids, triterpenoids, polyphenols, reducing sugars and cardiac glycosides were revealed to be present in the two plants. The phenol content varies from 122.83±0.01 to 341.16±0.01 mg GAE/100 g of extract. Proanthocyanidins have ranged between 4.33±0.02 and 55.16±0.03 mg APE/100 g of extract. While antiradical activity (IC_{50}) ranged between 406.65±0.04 and 876.21±0.03 µg/ml of extract. Both the phenolics and DPPH results of extracts were found in good agreement and suggest that these plants are not only interesting sources for phenolic antioxidants but also potential sources of bioactive compounds.

INTRODUCTION

In the world, most of the populations in developing countries still rely on traditional medicine practitioners and local medicinal plants for primary health care (WHO, 1995). Nevertheless, some clinicians remain very sceptical about the use of herbs in traditional medicine in developing countries and this may be due to a number of reasons including lack of proof of efficacy by well controlled clinical trials and the undoubted toxic effects of some herbs (Phillipson, 1999). However, the use of ethnopharmacology has led to the discovery of molecules of therapeutic importance (quinine, vinblastine, artemisinin...). The importance of protective defence systems in living cells, against damages caused by reactive oxygen, is well known. Free radicals and other oxidants are of great importance in the mechanism of action of many toxins. Their involvement in the aging process and diseases has been documented (Bruneton, 2009). Gabon, with an exceptional biodiversity partially described and little or no study constitutes a vast reservoir of unexplored potential active molecules if one considers that a species can produce alone hundreds of molecules. With the aim to study the therapeutic potential of the flora in Gabon, our laboratory uses adjacent to the ethnopharmacological approach, an approach based on zoopharmacology consumption of certain plants by self-medication by animals in search of new therapeutic molecules (Krief *et al*., 2011). Two plants consumed by *Mandrillus sphinx* Lékédi Park (Gabon) have been selected for this study (Nsi, 2012). These fruit *Megaphrinium macrostachyum* (Benth.) Milne-Redhead. (Marantaceae) and an epiphytic fern, *Asplenium africanum* Desv. (Aspleniaceae).

* *Corresponding Author*
Laboratoire de Substances Naturelles et de Synthèse Organométallique-URCHI, Faculté des Sciences, Université des Sciences et Techniques de Masuku, BP 901 Franceville, Gabon.
Tel. +241 07625975 / +241 06123189

A. africanum is an epiphytic fern, growing in tufts on trees, long leaves lanceolate whole, indusium shaped component. It use as fetish luck charm for hunting or fishing. Pygmies move the leaves on the fire to soften them, and then they attach to the legs of children at the breast to help them walk (Raponda-Walker and Sillans, 1961).

M. macrostachyum is an herbaceous, common and formed by starting with a few leaves rhizome mapping, carried on a long petiole than 3 meters long. Halfway flowers appear small, yellowish-white bracts often a bit washed pink. Pulp, tart and slightly sweet of fruits is edible. It is sometimes sucked by infants, we want to wean gradually lose their taste of the milk (Raponda-Walker and Sillans, 1961).

In this study, we seek the presence of certain class of bioactive molecules, the determination of total phenols and antiradical activity in the two plants to evaluate their therapeutic potential.

MATERIALS AND METHODS

Plant material

Authenticated *Asplenium africanum* plant material was collected in October 2012 from Ngouoni city at 30 km from Franceville in Haut Ogooué (Gabon) while *Megaphrinium macrostachyum* plant material (fruits) was collected in April 2012 from Lékédi Park in Bakoumba town at 65 km from Franceville. Their respective voucher specimens are deposited under code FCV.ISSEMBE 07 for *Megaphrinium macrostachyum* and FCV.ISSEMBE 27 for *Asplenium africanum* in the herbarium of the Department of Biology, University of Sciences and Technology of Masuku (Franceville, Gabon).

The fresh plants were dried in the open air and reduced to powder by use of a clean electric blender (Model Phillips 190). The powders were kept at room temperature until required.

Preparation of extracts for phytochemical screening

Air-dried powdered fruits (10 g) of *M. macrostachyum* and *A. africanum* respectively, were separately extracted with 100 ml (of each) of ethanol 50 % (Et-H_2O) and ethanol 100 % (EtOH) by maceration for 24 h. Extracts were filtered and the filtrate was used for photochemical screening.

Preparation of extracts for polyphenols measure and antiradical activity

Air-dried powdered fruits (20 g) of *M. macrostachyum* and *A. africanum* were separately extracted with 150 ml (of each) of ethanol 50 % (Et-H_2O) and ethanol 100 % (EtOH) by maceration for 72 h. Extracts were filtered and dried under reduced pressure at 40° C. Et-H_2O extract (0.211 g, 1.05 % of fruits dries), EtOH extract (0.093 g, 0.46 % of fruits dries) for *M. macrostachyum* and Et-H_2O extract (0.715 g, 3.58 % of plant dry), EtOH extract (0.299 g, 1.49 % of plant dry) for *A. africanum* were stored in freezer at 4°C until further tests.

Phytochemical screening

The two extracts, Et-H_2O and EtOH were screened for their classes of bioactive compounds using standard procedures (Culei, 1982; Harbone, 1984; Sofowora, 1993; Trease and Evans, 2002; Parekh *et al*., 2006). The extracts were tested qualitatively for the presence of chemical constituents such as tannins, terpenes, saponins, flavonoids, cardiac glycosides, coumarins, alkaloids, anthraquinones and reducing sugar.

Determination of total phenol and proanthocyanidin contents

The Folin-Ciocalteu method was used to measure total amount of polyphenol content (Singleton *et al*., 1999). Aliquots of 0.25 ml of extracts (1 mg/ml) were mixed with 1.25 ml Folin–Ciocalteu reagent (0.2 N diluted in MeOH). A reagent blank using MeOH instead of sample was prepared. After 5 min incubation at room temperature, 1 ml sodium carbonate solution (7.5%) was added. Samples were incubated at room temperature for 1 h and the absorbance was measured at 765 nm versus the prepared blank. All tests were carried out in triplicate and total phenol content was expressed as mg of gallic acid equivalents (GAE) per 100 g of extract. Proanthocyanidins (PAs) were quantified with the hydrolysis test of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavans-3-ols constituting the polymers (Prigent, 2005). The heating step destroys the anthocyanidins pigments generated by flavan-4-ols and eliminates part of the chlorophyll pigments. The routine assay was performed by mixing 0.16 ml (1 mg/ml) of the extract with 2.33 ml of 30% HCl-butanol solution (v/v). The mixture was put in tightly closed tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, the absorbances were read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

Radical-scavenging activity assay

Scavenging activity of 2, 2-diphenyl-1-picylhydrazyl (DPPH) radicals of extracts were measured according to the Blois (1958) with minor modifications. Various concentrations of sample extracts (1 ml) were mixed with 1 ml of methanolic solution containing DPPH radicals (20 mg/l). After 15 min incubation at room temperature in the dark, absorbance of the reaction mixtures was measured at 517 nm. The inhibitory effect of DPPH was calculated according to the following formula: [(Acontrol–Asample) / Acontrol]*100. The radical scavenging activity of extracts was expressed as the IC_{50} value (µg/ml), i.e., the concentration necessary to decrease the DPPH concentration by 50 %.

Statistical analysis

Experimental results were expressed as mean ± standard deviation. All measurements were duplicated three times. The IC_{50} values were calculated using linear regression analysis from the graph of scavenging effect percentage against extract concentration.

Table 1. Results of phytochemical screening of extracts from *A. africanum* and *M. macrostachyum* fruit.

Chemical constituents		*A. africanum*		*M. macrostachyum*	
		Ethanolic-water extract	Ethanolic extract	Ethanolic-water extract	Ethanolic extract
Saponins		-	-	++	-
Tannins	Gallic	++	+	-	-
	Catechin	++	+++	-	-
Alkaloids		+++	+++	+++	+++
Triterpenoids		++	+++	+	++
Polyphenols		+++	+++	+++	+
Flavonoids	Flavonols	-	-	-	-
	Flavones	+	-	-	-
	Flavanones	-	-	-	-
Free anthraquinones		+	-	-	-
Coumarine		+++	++	nt	nt
Total flavonoids		nt	nt	+++	+++
Proanthocyane		-	-	+	-
Cardiac glycosides	Digitoxine	-	-	++	-
	Digitoxigenine	-	-	-	-
	Gitoxine	+++	+	-	+++
	Gitoxigenine	++	+	-	-
Reducing sugars		++	-	+++	-

Legend: -: Not detected, +: Rare, ++: Abundant, +++: Very abundant, nt: not tested

Table. 2: Comparison of total phenolic compounds, proanthocyanidins and antioxidant capacity of fruit extracts from M. macrostachyum and A. africanum extracts.

	Plants / Extracts	Total phenols (mg GAE/100 g of extract)	PAs (mg APE/100 g of extract)	Quota of PAs in Total phenols (%)	DPPH: IC50 (µg/ml)
M. macrostachyum	Ethanolic-water extract	122.83 ± 0.01	7.66 ± 0.01	6.24	474.24 ±0.04
	Ethanolic extract	316.16 ± 0.01	55.16 ± 0.03	17.44	406.65 ±0.04
A. africanum	Ethanolic-water extract	341.16 ± 0.01	6 ± 0.03	1.75	433.25 ±0.05
	Ethanolic extract	221.16 ± 0.05	4.33 ± 0.02	1.95	876.21 ±0.03

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. In view of the results in table 1, it appears that two plants studied *A. africanum* and *M. macrostachyum* (fruit) contain alkaloids, triterpenoids, polyphenols, reducing sugars and cardiac glycosides, especially gitoxin. In addition to these compounds, *A. africanum* contains tannins with very abundant catechin tannins in ethanolic extract. There is also a high presence of coumarins, especially in hydro-alcoholic extract. While the fruits of *M. macrostachyum* show a moderate presence of saponins in the hydro-alcoholic extract and a strong presence of total flavonoids, which contrasts with the absence of major flavonoids: flavonols, flavones and flavanones.

All of these bioactive secondary metabolites identified in the various drugs have many pharmacological properties assigned to them (Bruneton, 2009). These properties from compounds found in the extracts of the two plants suggest that they can be used in pharmaceuticals.

Total phenolic and proanthocyanidin Content

Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the righthand side of the proportioning of total phenolic content by the method of Folin-Ciocalteu gave $Y = 0.0012\ X - 0.0004$ with $R^2 = 0.9902$ (Abdoul-latif *et al*., 2012). The total phenolic compound contents in the plant extracts are shown in table 2. It appeare that hydro-ethanolic extract of *A. africanum* had the highest content of phenolic compounds (341.16±0.01 mg GAE/100 g of extract) and fruit hydro-ethanolic extract of *M. macrostachyum* had the lowest content (122.83±0.01 mg GAE/100 g of extract). Ethanolic extract from fruit of *M. macrostachyum* show intermediate phenolic content followed by ethanolic extract of *A. africanum* with 316.16±0.01, 221.16±0.05 mg GAE/100 g of extract, respectively. The phytochemical screening of the extracts had revealed that the main phenols in *A. africanum* were tannins and coumarins. Catechin tannins were much present only in ethanolic extract. While the fruits of *M. macrostachyum* show that the main phenols were flavonoids, which are not flavonols, flavones and flavanones. Phenolic substances have been suggested to play a preventive role in the development of chronic diseases such as cancer and heart disease (Njintang *et al*., 2012). Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods (Rumbaoa *et al.*, 2009), decrease the risk of heart diseases by inhibiting the oxidation of low-density lipoproteins. They are also known to possess antibacterial, antiviral, antimutagenic and anticarcinogenic properties (Moure *et al*., 2001; Manach *et al*., 2004).

Levels of proanthocyanidins were expressed in terms of apple procyanidins equivalent (APE). The equation of the right-hand side of the proportioning of the proanthocyanidins by the HCl-Butanol method gave $Y = 0.0006\ X + 0.0024$ with $R^2 = 0.9869$ (Abdoul-latif *et al*., 2012). Among extracts, proanthocyanidin contents had ranged between 4.33±0.02 and 55.16±0.03 mg APE/100 g of extract (Table 2). Fruit of *M.*

macrostachyum contain the highest amount of proanthocyanidins (55.16±0.03 and 7.66 ± 0.01 mg APE/100 g of extract, respectively for ethanolic and hydro-ethanolic extracts) and *A. africanum* had revealed weak proanthocyanidin contents (4.33±0.02 and 6±0.03 mg APE/100 g of extract, respectively for ethanolic and hydro-ethanolic extracts). Thus, proanthocyanidins in extracts of *M. macrostachyum* fruit represent on average 11.84 % of total phenolic content and 1.85 % of total phenolic content in *A. africanum* extracts. Procyanidins, polymers of flavan-3-ol units, have been reported to exhibit many beneficial health effects such as antioxidant and anti-carcinogenic effects. Thus, Bak *et al*. (2012), have reported that taken together, the procyanidins from wild grape (*Vitis amurensis*) seeds could be used as a potential natural cancer chemopreventive agent through Nrf2/ARE-mediated phase II detoxifying/antioxidant enzymes induction via p38 and PI3K/Akt pathway.

Antiradical activity

Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities. The free radical-scavenging activities of various extracts were evaluated at their initial concentration. All extracts show free radical scavenging activity (Table 2), lower IC_{50} indicating the higher antiradical activity of the extract. The crude ethanolic extract of *M. macrostachyum* fruit show best DPPH free radical-scavenging activities (IC_{50} value = 406.65±0.04 µg/ml) followed by hydro-ethanolic extract of *A. africanum* (IC_{50} value = 433.25±0.05 µg/ml).

Hydro-ethanolic extract of *M. macrostachyum* fruit and ethanolic extract of *A. africanum* which have weak total phenols contents, exhibited weak antiradical activity (IC_{50} values = 474.24±0.04 and 876.21±0.03 µg/ml, respectively). These results show that the extracts of two plants have DPPH radical scavenging activity, which increased with increasing amount of phenolic content.

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress (Dai and Mumper, 2010). The reducing capacity of a compound may serve as indicator of its potential antioxidant capacity (Meir *et al*., 1995). Thus, the extracts of the plants exhibited antioxidant activity in the present study which has potential application to reduce oxidative stress with consequent health benefits. Recently, phenolics have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than vitamins C and E, and carotenoids. The inverse relationship between fruit and vegetable intake, and the risk of oxidative stress associated diseases such as cardiovascular diseases, cancer or osteoporosis has been partially ascribed to phenolics (Dai and Mumper, 2010). The antioxidant capacity of phenolic extracts is very often attributed to their radical scavenging ability mediated by hydroxyl groups (Hatano *et al*., 1989). Phenolic compounds act as free radical acceptors and chain breakers. They interfere with the oxidation of lipids and other molecules by rapid donation of a hydrogen atom to radicals (Dai and Mumper, 2010). In our studied plants we found higher amount of phenolic compounds indicating their strong therapeutic potential as an antioxidant.

CONCLUSION

Our study showed that the analysis of *Megaphrinium macrostachyum* fruit and *Asplenium africanium* extracts were performed to determine levels of phytochemicals content, total phenolic content, proanthocyanidins and antioxidant capacities. The chemical substances present in these plants highlight a beneficial antioxidant capacity of their extracts against the free radicals *in vitro*.

Thus, these plants could be considered as sources of antioxidant, and has been shown according to their IC_{50} with potential anti-cancer and antiathesclerosis properties. This phytochemical study and the significant presence of reducing sugar had shown also dietary values of the plant. In addition, richness alkaloids suggest that these plants may be involved in a large number of therapeutic applications.

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