IN VITRO ANTIOXIDANT POTENTIAL AND PHYTOCHEMICAL CONSTITUENTS OF THREE CAMEROONIAN MEDICINAL PLANTS USED TO MANAGE PARASITIC DISEASES

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Summary

Aqueous and methanol-methylene chloride extracts of *Schumaniophyton magnificum, Rauvolfia vomitoria* and *Pseudospondias microcarpa* were screened for phytochemical constituents. Tests for saponines, phenols, Terpenoids, flavonoids, cardiac glycosides and coumarines were positive in both methanol-methylene chloride and aqueous extracts, while anthraquinons and anthocyanins were absent in *Schumaniophyton magnificum.* The antioxidant potential of these plants were also evaluated using three different methods: FRAP (Ferric reducing antioxidant power), DPPH (1,1-Diphenyl-2- Picrilhydrazyl) and Folin (Folin-Ciocalteu reagent). The aqueous and methanol-methylene chloride extracts of *Pseudospondias microcarpa* had the highest antioxidant activity (P<0.05) follow by *Rauvolfia vomitoria and Schumaniophyton magnificum.*

Key words: Medicinal plants, phytochemicals, antioxidant, Ferric reducing antioxidant power (FRAP), 1,1-Diphenyl-2-Picrilhydrazyl (DPPH), Folin.

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Introduction

Oxidative stress involving enhanced generation of reactive oxygen species (ROS) has been implicated in the etiology of over one hundred human diseases (1). Reactive oxygen species (ROS) such as superoxide anions ($O2^{-}$), hydroxyl radical ($^{\cdot}OH$), ferric ion and nitric oxide (NO) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation, and thus have been shown to augment collagen synthesis and fibrosis. The increased production of toxic oxygen derivatives is considered a universal feature of stress conditions (2). Antioxidants capable of neutralizing ROS and their actions are considered beneficial (1). Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecules and enzymes (2). Thus, medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, phenolic compounds and coumarins (3,4,5). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (6,7).

Schumaniophyton magnificum, Rauvolfia vomitoria and *Pseudospondias microcarpa* are extensively used in herbal medicine in Centre, East, South and Litoral regions of Cameroon. These plants are widely used in the treatment of various diseases like: Hyperglycemia, gonococci, anemia, diarrhea, filaria, angulillulose, rheumatisms, ankylostomia, ascaridiose, cestodose, malaria, typhoid fever, haemorrhoid, oedemas, rheumatisms, stomach ache, icterus, pediculoses, diabetes, trypanosomiasis, leishmaniasis and elephantiasis (8,9,10,11,12,13). A detailed review of literature afforded no information on the antioxidant potential of these plants. The present study investigates the fundamental scientific bases for the use of these plants by defining and quantifying the amount of crude phytochemical constituents present in these plants.

Material and Methods

Collection and identification of plant materials

Schumaniophyton magnificum, Pseudospondias microcarpa and *Rauvolfia vomitoria* were collected between January and March 2008 in Yaoundé, Cameroon. Their identification was done at the Cameroon National Herbarium voucher specimen's N° 01623/HNC, 41437/HNC and 50626/HNC respectively.

Preparation of extracts

The stem-bark of our plants was sun dried till constant weight, and ground to powder consistency. The plant powder (1500 g) was used for aqueous and methanol-methylene chloride extractions. 500 g of this plant powder was decocted in 4 L of distilled water for 15-20 min. This was repeated four times, until the resulting extract gave no further coloration. When cooled to room temperature, the preparation was sieved through four-layers cotton fabric gauze. The filtrate was allowed to stand for 90 to 120 minutes after which the supernatant was filtered through Whatman filter paper N°1. The decoction obtained was evaporated at 40°C till total dryness using a convection air oven. The dry solid material obtained from *Schumaniophyton magnificum, Rauvolfia vomitoria* and *Pseudospondias*

microcarpa (yield: 52.27, 20.80 and 15.20% w/w respectively) was used immediately or stored at 4°C.

The remaining powder (1000 g) was soaked in 5 L of a mixture of methanol-methylene chloride (1:1) for 48 h, and for a further 24 h in the same solvent. This was filtered and concentrated to a small volume to remove the entire solvent using rotavapor. The remaining liquid was later further dried in an oven at 40° C, to obtain an extract (14). The extracts obtained from *Schumaniophyton magnificum, Rauvolfia vomitoria* and *Pseudospondias microcarpa* (yield: 11.89, 13.23 and 11.10% w/w respectively) was used immediately or stored at 4°C.

Phytochemical screening

Phytochemical properties of differents extracts of plants materials were tested using the following chemicals and reagents according to the method of Trease and Evans (15): Alkaloids with Mayer and Dragendoff's reagents, Tannin (FeCl3), Saponins (frohing test), Flavonoids (chip of magnesium and HCl), Glycosids (NaCl, and Felhing's solutions A and B), Sterols and Triterpens (ethylic, sulphuric acid and anhydride acetic), Anthraquinone (ether-chloroform and NaOH), Phenols -FeCl3 and K3Fe(CN6)-, Cardiac glycosids (acetic acetic, FeCl3, concentrate sulphuric acid), Quinones (ether, chloroforme, NaOH), Anthocyanins (HCl), Coumarins (silica plate) and Polyphenols - K3Fe(CN6)-.

***In vitro* Antioxidant Studies**

1. **Reduction of 1, 1- Diphenyl- 2- Picryl Hydrazyl (DPPH) Free Radical**

Scavenging activity against the DPPH (1,1-Diphenyl-2-Picrilhydrazyl) free radical was studied as follows:
20 µL of extract was introduced into 2mL of a methanolic solution of DPPH (0.3mM) and kept in the dark for 30 min. The extract was replaced by methanol for the control and catechin for the standard. The absorbance was then spectrophotometrically read at 517 nm and the antioxidant content were calculated as earlier described (16).

2. **Phenol content**

The phenolic content of both extracts were measured at 750 nm using Folin-ciocalteu reagent diluted 10 times before use with catechin as standard. Optical density was read after 20 min of incubation (17).

3. **Ferric Reducing Antioxidant Power**

The Ferric Reducing Antioxidant Power (FRAP) of extracts was determined using the method of Benzie and Strain (18). The FRAP reagent consisted of ten part acetate buffer (300mM, pH3.6), one part of TPTZ (10 mM in 400 mM of HCl, Sigma) and one part of ferric chloride (10mM). Different dilutions of extracts amounting to 1 mL were added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The mixtures were incubated at room temperature for 20 min, after which 2.5 mL trichloroacetic acid (10%) was added. An aliquot of the mixture (2.5 mL) was taken and mixed with 2.5 mL water and 0.5 mL 1% FeCl3. The absorbance at 593 nm was measured after allowing the solution to stand for 30 min. FRAP of a sample is estimated in terms of catechine equivalents (CE) in mg CE/g of sample.

Statistical analysis

Measurements of absorbance were made in triplicate and the results presented as mean±standard deviation. The homogeneity of data was analysed by ONOVA and the Student-Newman-Keuls was used as posthoc test for comparison between mean (P<0.05). The relation between the methods was established by applying Pearson product moment correlation (P<0.05). We used Sigmastat 3.1 software for this analysis.

Results

Phytochemical screening

The present study carried out on the plant samples revealed the presence of medicinally active constituent. Tests for saponins, phenols, terpenoids, flavonoids, cardiac glycosides and coumarines were positive in both methanol-methylene chloride and aqueous extracts of all the plants tested while anthraquinons and anthocyans were absent in *Schumaniophyton magnificum.* These results are summarized in the **Table 1**.

Table 1: Preliminary phytochemical study of medicinal plants extracts studied.

Familly	Sc. N.	Extracts	A	S	F	T	Q	AQ	GC	P	TT	ST	AT	G	L	C
Rubiaceae	***S. mag***	**A**	+	+	+	+	+	-	+	+	+	-	-	-	-	+
		M-MC	+	+	+	+	+	-	+	+	+	+	-	-	+	+
Anacardiaceae	***P. mic***	**A**	+	+	+	+	+	+	+	+	+	-	+	+	-	+
		M-MC	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Apocynaceae	***R. vom***	**A**	-	+	+	+	-	+	+	+	+	-	+	-	-	+
		M-MC	+	+	+	-	+	+	+	+	+	+	+	+	+	+

A: alcaloids; **S:** saponins; **F:** flavonoids; **T:** tannins; **Q:** quinones; **AQ:** anthraquinons; **GC:** cardiac glycosides; **P:** polyphenols; **TT:** triterpenes; **ST:** sterols; **AT:** anthocyans; **G:** glycosides; **L:** lipids; **C:** coumarines.

A: aqueous; **M-MC:** methanol-methylene chloride

+: present; **-:** absent

Sc. N.: Scientific name

S. mag: *Schumaniophyton magnificum*
P. mic: *Pseudospondias microcarpa*
R. vom: *Rauvolfia vomitoria*

Antioxidant assay

Quantitative estimation of the phenolic compounds with the folin-ciocalteu reagent in these medicinal plants studied shown the significant value (P<0.05) of phenols of *Pseudospondias microcarpa* who is very high compared to *Rauvolfia vomitoria* and *Schumaniophyton magnificum* respectively. Also, for each sample of plant, the methanol-methylene chloride extracts had the highest polyphenolic capacity compared to aqueous extracts. These results are represented by the **Fig. 1**.

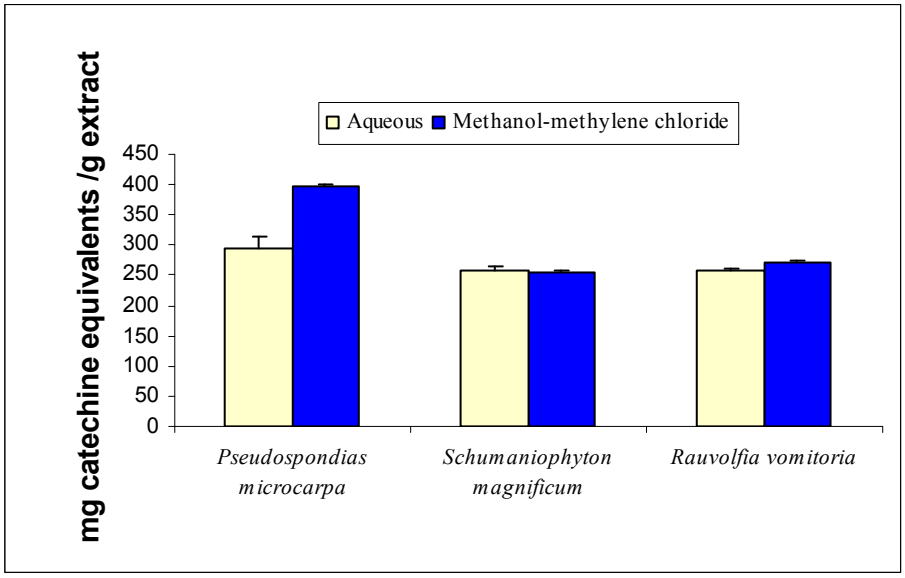

Figure 1. Free polyphenolic concentration of plants extracts as determined using Folin reagent.

The result of the DPPH scavenging assay is shown in **Fig. 2.** From these results, we have observed that *Pseudospondias microcarpa* had the highest activity (P<0.05) follow by *Rauvolfia vomitoria* and *Schumaniophyton magnificum.*

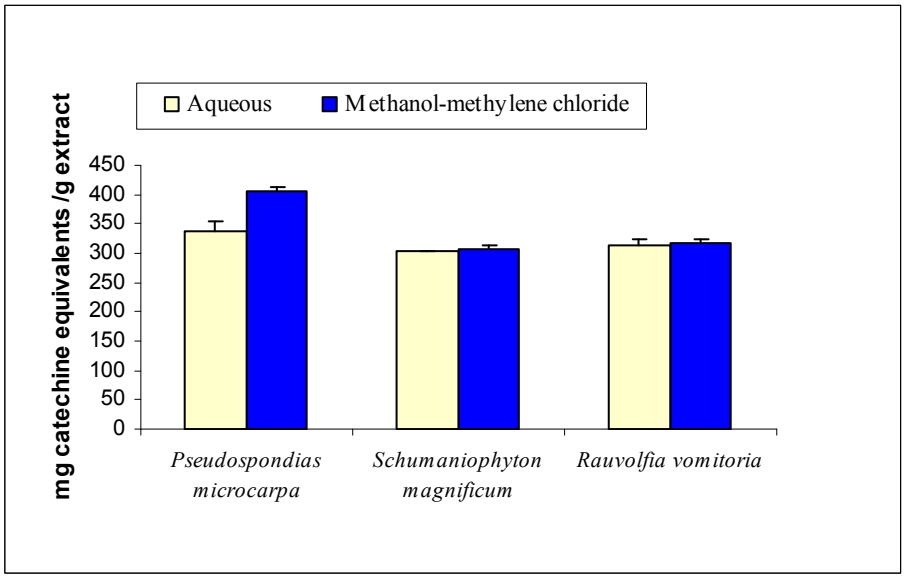

Figure 2. Free radical (DPPH) scavenging activity of plants extracts.

In the FRAP method, the aqueous and methanol-methylene chloride extracts of *Pseudospondias microcarpa* were significantly higher (P<0.05) than the corresponding extracts of the other samples (**Fig. 3**). This makes *Pseudospondias microcarpa* the overall best antioxidant source of the three plants studied. Also, methanol-methylene chloride is the best extraction medium for antioxidants (**Figs 1, 2 and 3**).

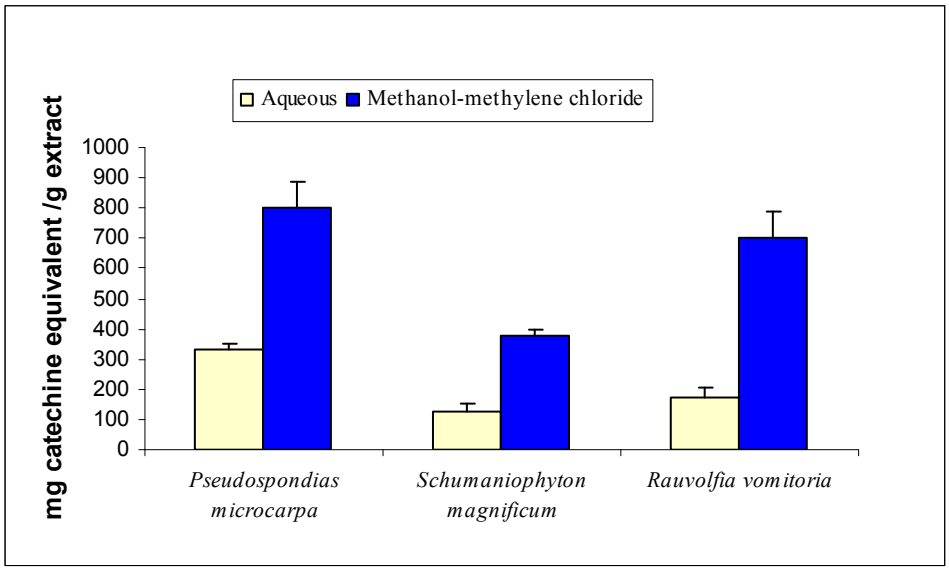

Figure 3. Antioxidant power of plants extracts as determined by FRAP.

We also obtained correlation between the methods used. **Figs 4 to 6** summarise the relationship between the Folin, FRAP and DPPH antioxidant activity. A significant correlation (P<0.05) was observed between Folin and FRAP, DPPH and FRAP antioxidant. Between Folin and DPPH, the correlation was very significant (P<0.01).

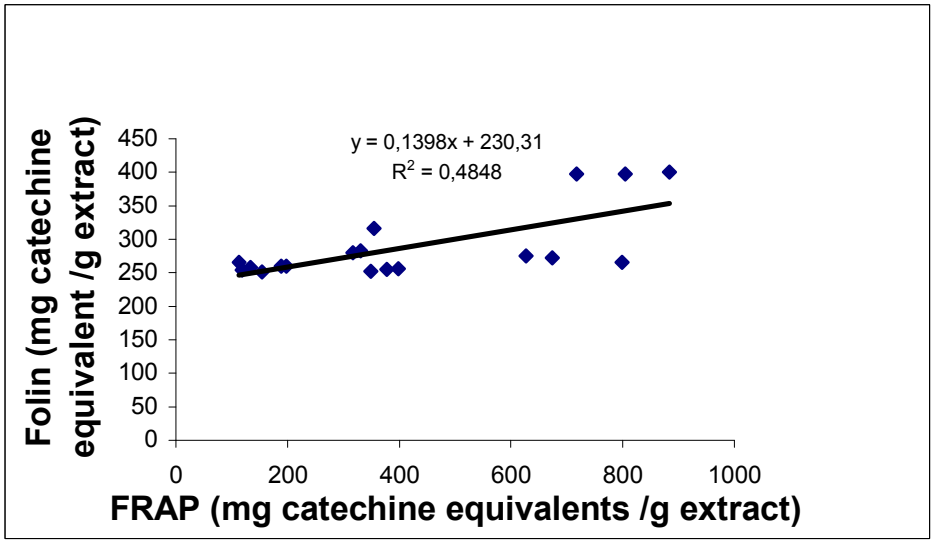

Figure 4. Correlation analysis, between Folin free and FRAP free antioxidant capacities of the studied samples (P<0.05).

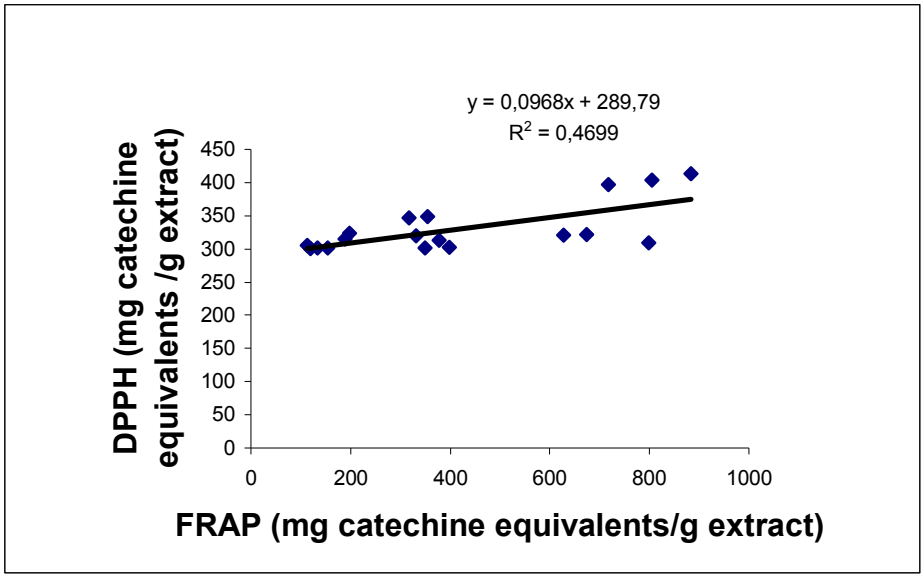

Figure 5: Correlation analysis, between DPPH scavenging activity and FRAP free antioxidant capacities of the studied samples (*P*<0.05).

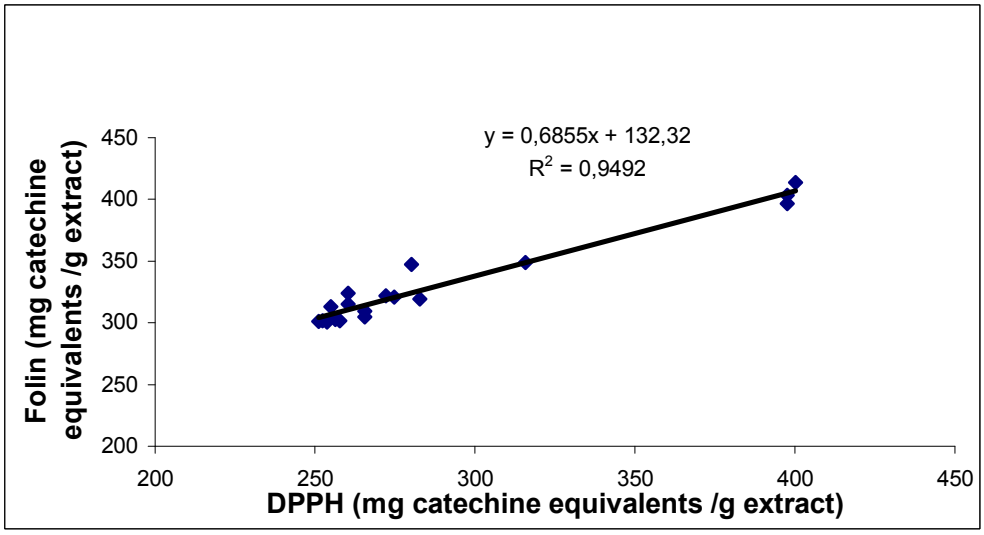

Figure 6: Correlation analysis, between Folin free antioxidant and DPPH scavenging activity of the studied samples (*P*<0.01).

Discussion

Tests for saponins, phenols, terpenoids, flavonoids, cardiac glycosides and coumarines were positive in both methanol-methylene chloride and aqueous extracts while anthraquinons and anthocyanins were absent in *Schumaniophyton magnificum.* Phenols, flavonoids and tannins are good antioxidant substances which have been reported to have anti-diarrhoeal and antidiabetic activities (1,19) and prevent or control oxidative stress related disorders (20,21,22). Also coumarins and tannins found in these plants extracts were known to show antibacterial and antiparasitic activities (4,5).

DPPH is a free radical that forms a stable molecule on accepting an electron or a hydrogen atom. Free radicals induce oxidative stress *in vivo* that may lead to oxidative modification or damage of some biological structures such as lipids, proteins, DNA and may give rise to degenerative diseases (1). The *in vitro* study sounds encouraging as all plants studied have some radical scavenging effect. It has been shown that phenolics, alkaloids, terpenoids and cardiac glycosides detected in the extracts are compounds that have been documented to possess medicinal properties and health-promoting effects (23,24,25,26). Also, these compounds might act as antioxidants or as agents of other mechanisms contributing to cardioprotective action (12,13,27,28). This can justified the important scavenging capacity obtained.

The high antioxidant capacity by the DPPH and FRAP methods of *Pseudospondias microcarpa* may be responsible for its antimalaria and antiparasitic activities earlier reported by the traditional practitioners (4).

Folin measures the polyphenolic concentration of the extract. The principal antioxidant constituents of natural products are phenolic compounds that are comprised of phenolic acids and flavonoids (29). They are potent free radical terminators (30). They donate hydrogen to free radicals, and hence, break the reaction of lipid oxidation at the initiation step (1,31). Thus, high polyphenolic content will mean a strong antioxidant power and a strong scavenging activity. However, this is not always the case since plant tissues are often made up of different matrix that may react differently with change of chemicals/reagent or reaction mechanism.

Conclusion

The plants studied here can be seen as a potential source of useful drugs as determined by three methods. All the medicinal plants studied show some antioxidant activity irrespective of the method used for the analysis. These plants may play an important role in preventing cell destruction, parasitic diseases and other diseases mediated by oxidative stress. It was also shown that the methanol-methylene chloride extracts had higher antioxidant activity than the aqueous extracts. Among all the plants studied, *Pseudospondias microcarpa* had the best antioxidant potential. Nevertheless, further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. Also, the antimicrobial and antiparasitic activities of these plants as claimed by traditional healers are also being investigated.

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