PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT POTENTIAL OF SOME CAMEROONIAN MEDICINAL PLANTS

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Summary

Alkaloids, tannins, saponins, steroid, triterpens, flavonoids, anthraquinones, sterols, carbohydrates, lipids, glycoside and cardiac glycoside distribution in five medicinal plants belonging to different families were assessed. The antioxidant potential of these plants (*Pentaclethra macrophylla* Benth, *Nauclea diderrichii*, *Entandrophragma cylindricum*, *Petersianthus macrocarpus* and *Enantia chlorantha)* were evaluated using three different methods: FRAP (Ferric reducing antioxidant power), DPPH (1,1-Diphenyl-2-Picrilhydrazyl) and Folin (Folin-Ciocalteu reagent). All the plants were found to contain phenols, flavonoids, alkaloids, terpenoids, and glycosides. Concerning the antioxidant potential, the aqueous and hydroethanolic extracts of *Pentaclethra macrophylla* Benth had the highest antioxidant activity ($p<0.05$) follow by *Entandrophragma cylindricum, Nauclea Diderrichii, Petersianthus macrocarpus and Enanthia chlorantha.*

Key words: Medicinal plants, 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. Antioxidants capable of neutralizing ROS and their actions are considered beneficial. [1]. 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. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [2]. 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 [3,4].

Pentaclethra macrophylla Benth, *Nauclea diderrichii*, *Entandrophragma cylindricum*, *Petersianthus macrocarpus* and *Enantia chloranta* are extensively used in herbal medicine in centre and west provinces of Cameroon. These plants are widely used in the treatment of various diseases like: Hyperglycemia, gonococci, anemia, diarrhea, filariose, angulillulose, rheumatisms, ankylostomiase, ascaridiose, cestodose, malaria, typhoid fever, haemorrhoid, oedemas, rheumatisms, stomach ache, icterus, pediculoses and diabetes [5,6,7,8,9,10]. 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

Pentaclethra macrophylla Benth, *Nauclea diderrichii*, *Entandrophragma cylindricum*, *Petersianthus macrocarpus* and *Enantia chlorantha* were collected on September 2006 in yaounde, Cameroon. Their identification were done at the National Herbarium, Yaounde Cameroon,

Preparation of extracts

Plant materials were air-dried for 30 days at room temperature and ground in to powder. The powder plant material was extracted for total and free antioxidant as earlier described [11]

preliminary 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 [12]: Alkaloids with Mayer and Dragendoff's reagents, Tannin ($FeCl_3$), 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), Anthraquine (ether-chloroform and NaOH), Phenols ($FeCl_3$ and $K_3fe(CN_6)$, Cardiac glycosids (acetic acetic, $FeCl_3$ (concentrate sulphuric acid) and Polyphenols - $K_3Fe(CN_6)$-.

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

DPPH scavenging activity: 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 [13].

Ferric Reducing Antioxidant Power: The Ferric Reducing Antioxidant Power (FRAP) of extracts was determined using the method of Benzie and Strain [14]. 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).

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 results showed that all the extracts studied contain: phenols, alkaloids, tannins and flavonoïds excepted hydroethanolic extract of *Petersianthus macrocarpus*, aqueous and hydroethanolic extracts of *Pentaclethra macrophylla* Benth and *Nauclea diderrichii* which contain cardiac glycosides. These results are summarized in the table 1.

Antioxidant capacity

The results of the antioxidant capacity of each sample as analysed by the various methods are presented in figures 1, 2, 3 and 4. The free and total antioxidant capacity were measuring using folin ciocalteu (Folin) while DPPH and FRAP were used to determine free antioxidant. A significant difference ($p<0.05$) was obtained between free and total antioxidants with total antioxidants being comparatively higher than free antioxidants in all of the plants studied. Generally, the hydrolysed extracts had the highest antioxidant capacity; follow by the hydroethanolic and aqueous extracts.

In the antioxidant potential determined by DPPH reagent, *Pentaclethra macrophylla* Benth had the highest activity follow by *Entandrophragma cylincum* and *Nauclea Diderrichii* (Figure 1)

In the FRAP method, it was the aqueous and hydroethanolic extracts of *Pentaclethra macrophylla* Benth that had the highest antioxidant activity follow by *Entandrophragma cylincum*, *Nauclea Diderrichii*, *Petersianthus macrocarpus and Enanthia chlorantha* (Figure 2)

In the Folin method, The hydrolyzed, hydroethanolic and aqueous extracts of *Pentaclethra macrophylla* Benth were significant higher ($p<0.05$) than the corresponding extracts of the other samples. This makes *Pentaclethra macrophylla* Benth the overall best antioxidant source of the five plants studied and 1.2N HCL/M (hydrolyzed extract) the best extraction medium for polyphenols (figures 3 and 4).

Figures 5-8 summarise the relationship between the Folin, FRAP and DPPH antioxidant activity. A significant correlation ($p<0.01$) was observed between free and total Folin antioxidant on one hand; Folin and FRAP, Folin and DPPH, and DPPH and FRAP antioxidant on the other hand.

Table 1: Preliminary phytochemical study of medicinal plants extracts studied

Familly	Sc. Name	Extract	P	T	S	A	SA	TA	AN	G	F	ST	G.C.
Mimosacées	***Pentaclethra***	**A**	+	+	+	+	+	+	+	+	+	-	+
	macrophylla	**H**	+	+	+	+	+	+	+	+	+	+	+
Rubiaceae	***Nauclea diderrichii***	**A**	+	+	+	+	+	+	-	+	+	-	+
		H	+	+	+	+	+	+	+	+	+	-	+
Meliaceae	***Entandrophragma***	**A**	+	+	+	+	+	+	+	+	+	-	-
	cylindricum	**H**	+	+	+	+	+	+	+	+	+	+	-
Lecythidaceae	***Petersianthus***	**A**	+	+	+	+	-	+	-	+	+	-	-
	macrocarpus	**H**	+	+	+	+	-	+	+	+	+	-	+
Annonacées	***Enantia chloranta***	**A**	+	-	+	+	+	+	-	+	+	-	-
		H	+	+	+	+	+	+	-	+	+	-	-

A=Aqueous extract; **H**=Hydroethanolic extract, **Sc**= Scientific name

+ = Positive reaction; **-** = Negative reaction

P=Phenol, **T**=Triterpens, **S**=Sugars, **A**=Alkaloids, **SA**=Saponins, **TA**=Tannins, **AN**=Anthraquinons, **G**=Glycoside, **F**=Flavonoids, **ST**=Sterol, **G.C.** =Cardiac glycoside

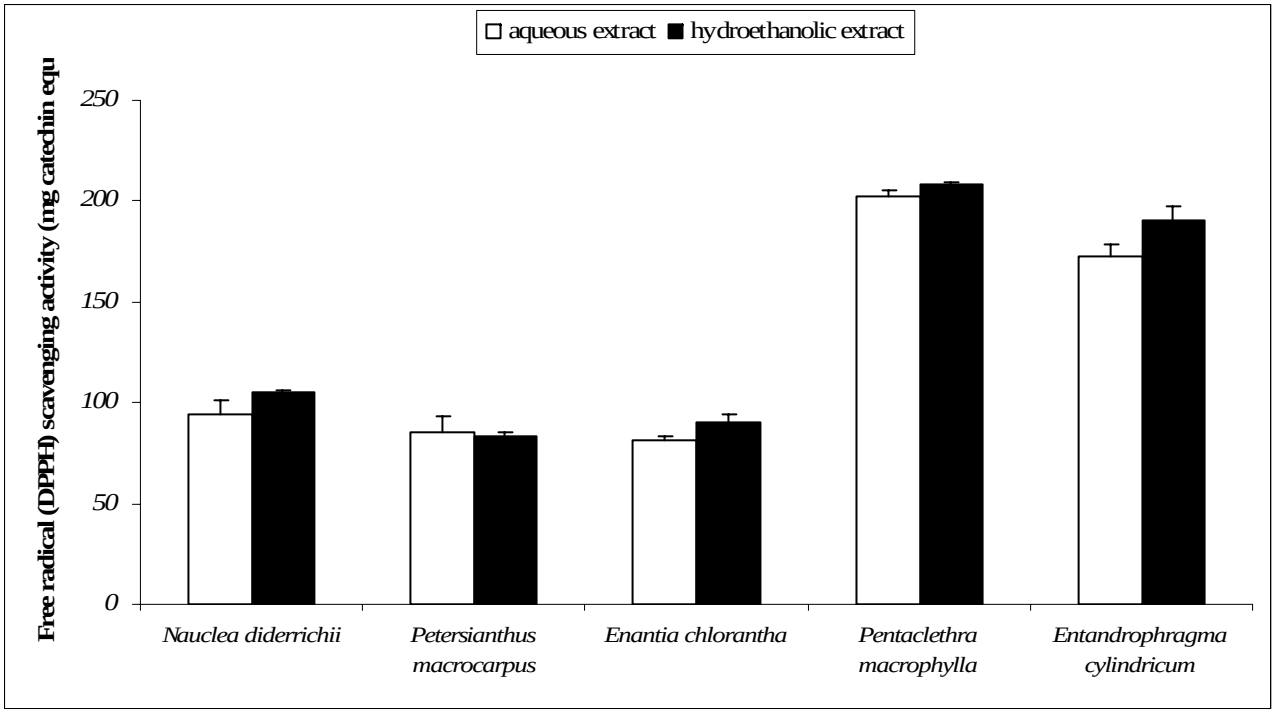

Figure 1: Free radical (DPPH) scavenging activity of plants extracts

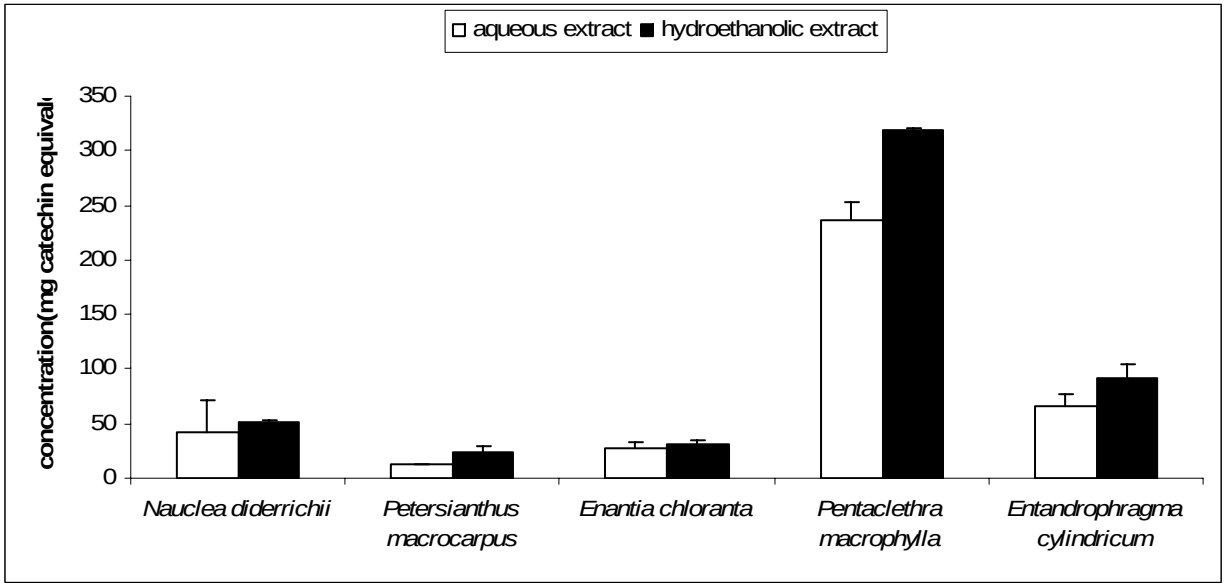

Figure 2: Antioxidant power of plants extracts as determined by FRAP

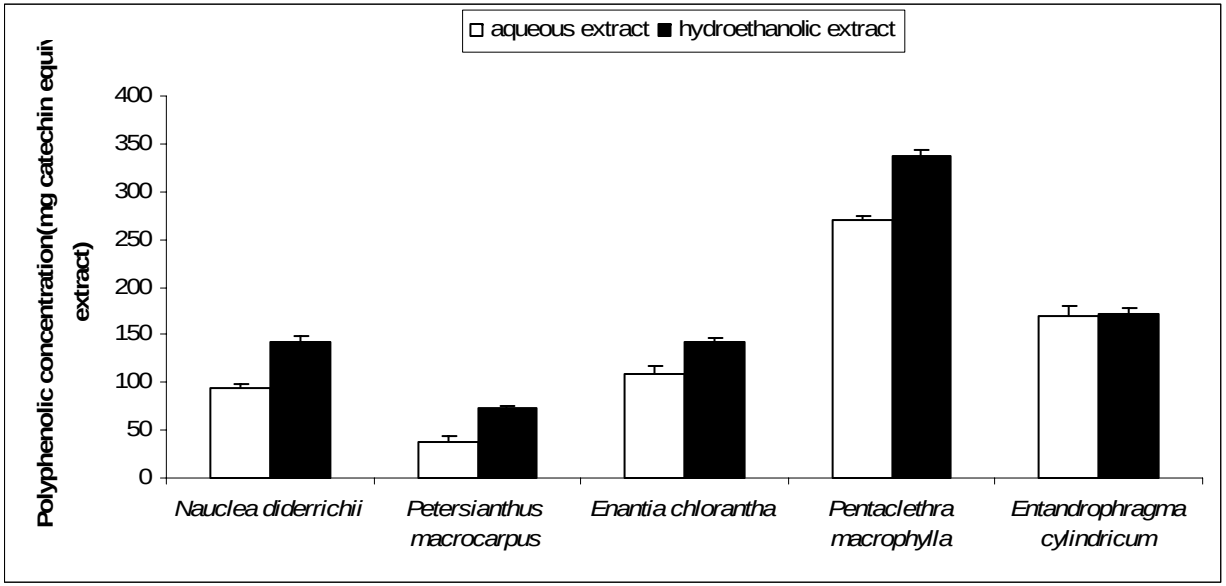

Figure 3: Free polyphenolic concentration of plants extracts as determined using Folin reagent

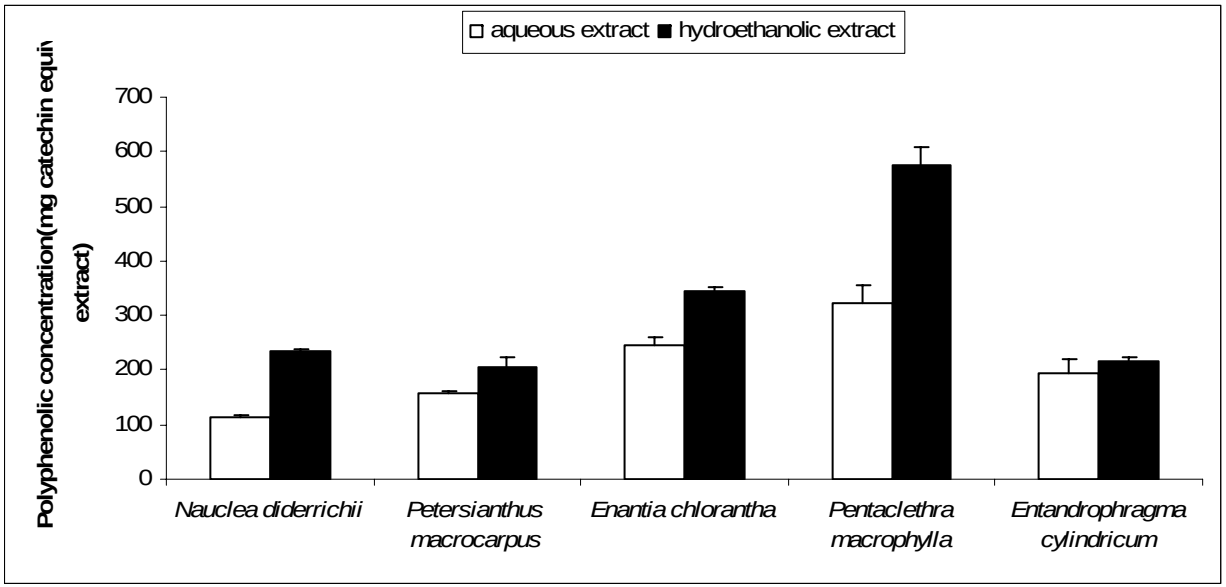

Figure 4: Total polyphenolic concentration of plants extracts as determined using Folin reagent

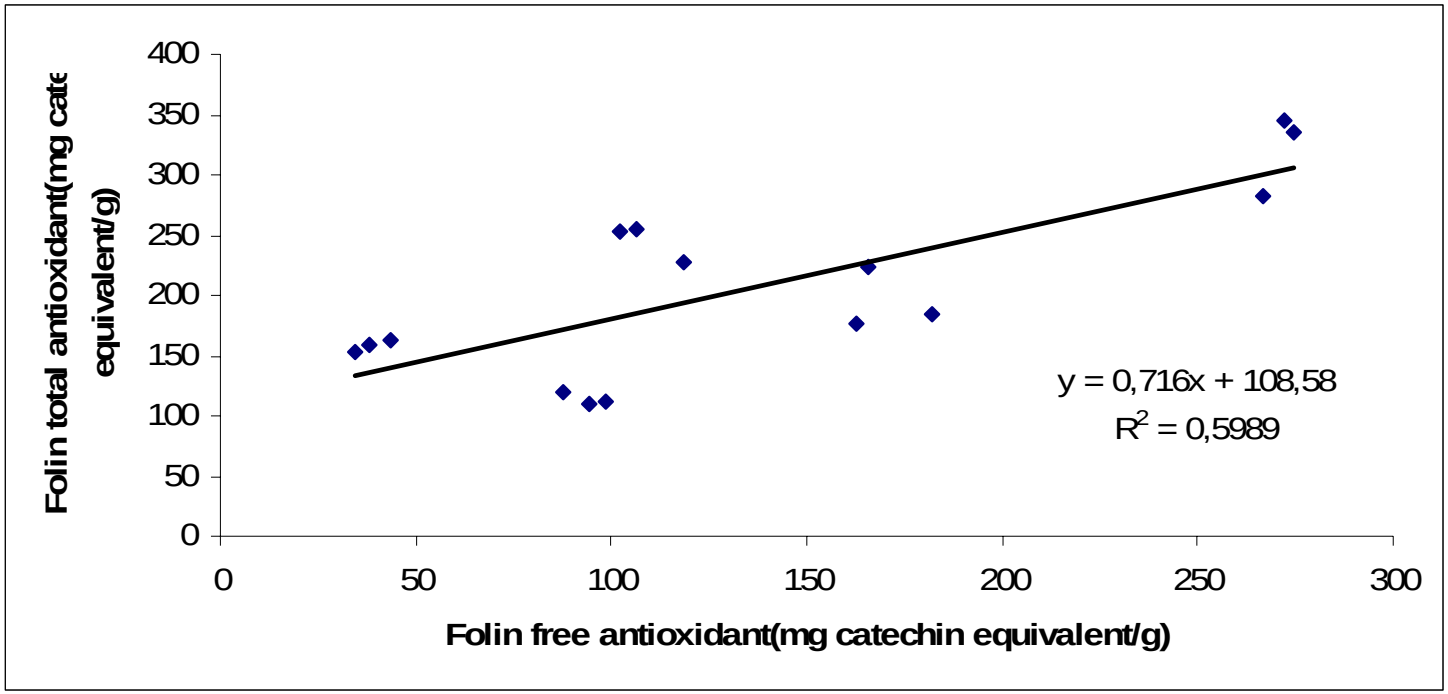

Figure 5: Correlation analysis, between Folin free and total antioxidant capacities of the studied samples ($p<0.001$)

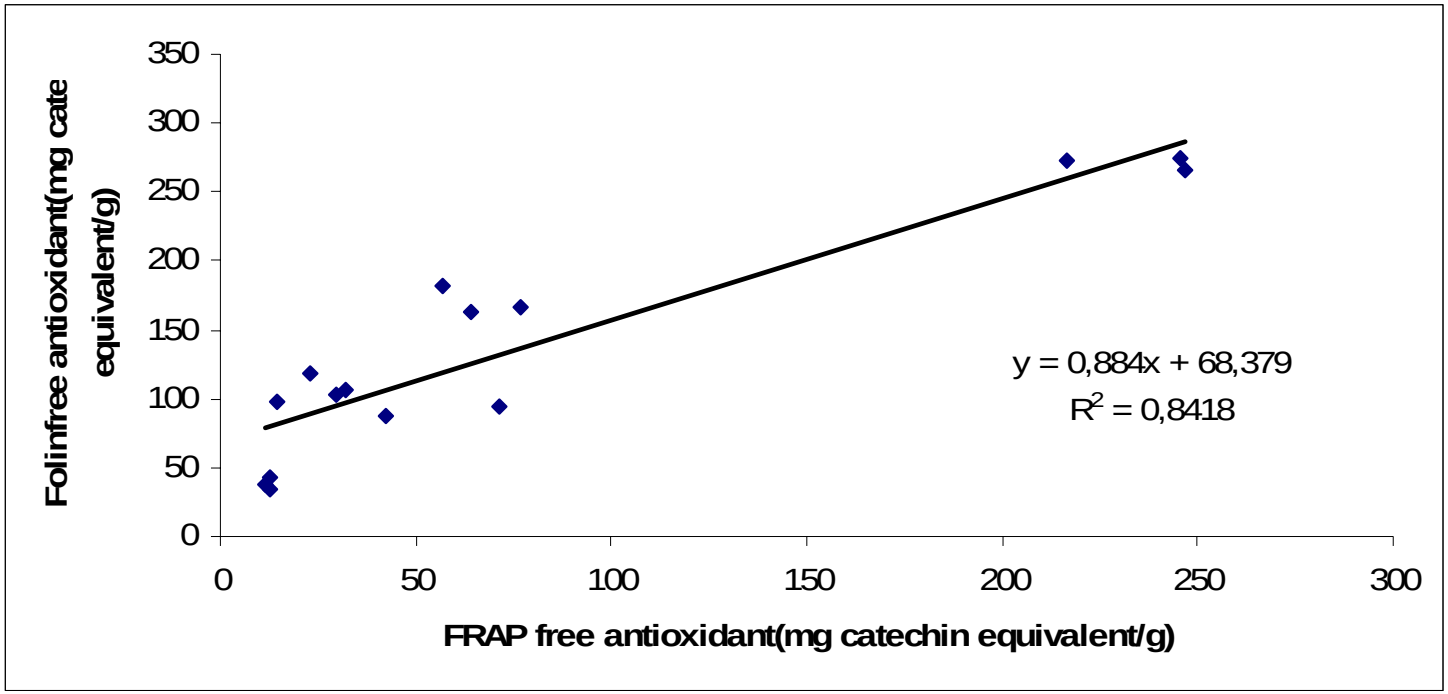

Figure 6: Correlation analysis, between Folin free and FRAP free antioxidant capacities of the studied samples ($p<0.001$)

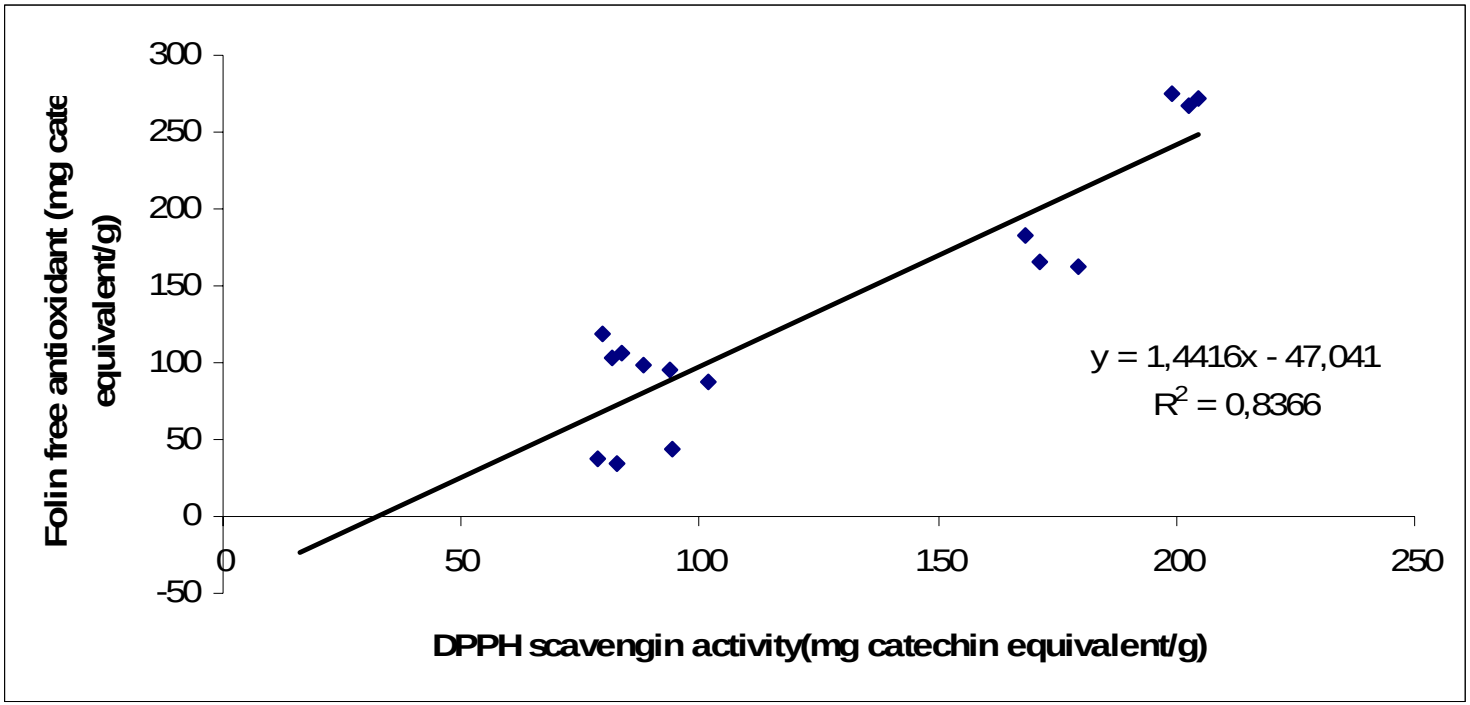

Figure 7: Correlation analysis, between Folin free antioxidant and DPPH scavenging activity of the studied samples ($p<0.001$)

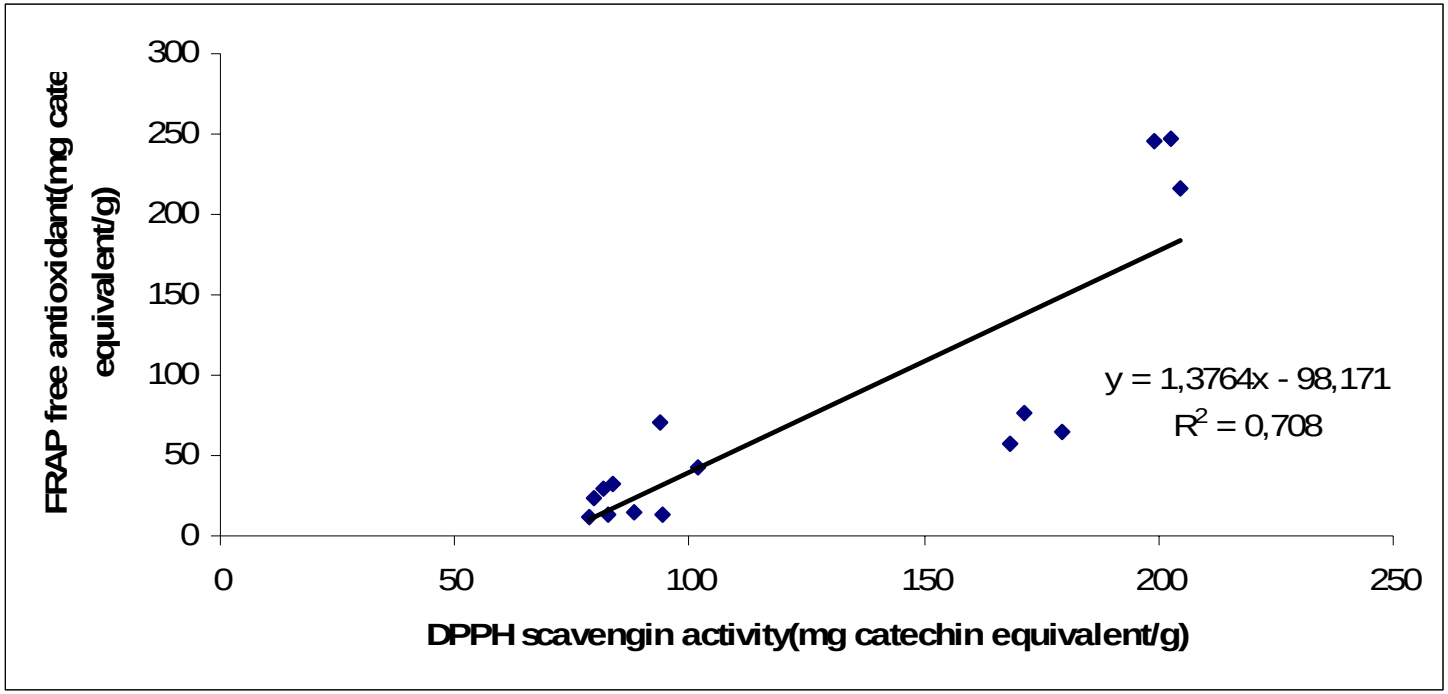

Figure 8: Correlation analysis, between DPPH scavenging activity and FRAP free antioxidant capacities of the studied samples ($p<0.001$)

Discussion

Phenols, flavonoïds and tannins are good antioxidant substances which have been reported to have anti-diarrhoeal and anti diabetic activities [1,15] and prevent or control oxidative stress related disorders [16,17].

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]. There is need for antioxidant intervention which one of the plants studied may be of importance. The *in vitro* study sounds encouraging as all plants studied have some radical scavenging effect. Also, increased consumption of fruits and vegetables is associated with a lower risk of degenerative diseases that come with aging such as cancer, cardiovascular diseases, cataracts and brain and immune dysfunction [18]. These positive influences have been attributed to natural antioxidant phytochemicals. It has been shown that plants phenols such as flavonoïds, anthocyanins and phenylpropanoïds might act as antioxidants or as agents of other mechanisms contributing to cardioprotective action [9,10,19,20].

The high antioxidant capacity by the DPPH and FRAP methods of *Pentaclethra macrophylla* Benth may be responsible for its antimalaria and antidiabetic activities earlier reported [10].

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 flavonoïds [21]. They are potent free radical terminators [22]. They donate hydrogen to free radicals, and hence, break the reaction of lipid oxidation at the initiation step [1,23]. 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.

From results obtained, we can confirm that hydrolysis liberate bound antioxidant substances such as phenols which are bound to sugars as earlier report [11,24,25,26].

Conclusion

The present study has demonstrated that medicinal plants could be a good source of antioxidant substances as determined by three methods. All the medicinal plants studied show some antioxidant activity irrespective of the method used for the analysis. It was also shown that the hydrolyzed extracts had higher antioxidant activity than the non-hydrolysed extracts. *Pentaclethra macrophylla* Benth had the best antioxidant potential.

These plants may play an important role in preventing cell destruction and other diseases mediated by oxidative stress. An *in vivo* antioxidant study of these plants extracts is needed to justify these claims.

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