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ISSN 0189-6016©2007PHYTOCHEMICAL STUDIES AND ANTIOXIDANT PROPERTIES OF FOUR
MEDICINAL PLANTS USED IN CAMEROON**Prosper-Cabral N. Biapa¹, Gabriel A. Agbor^{1,2}, Julius E. Oben¹, Jeanne Y. Ngogang³**¹Department of Biochemistry, University of Yaoundé I, P.O Box 812, Yaoundé, Cameroon,²Institute of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon,³Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon.**E mail:** juliusoben@hotmail.com, **Tel:** 237 774 5087**Abstract**

Four plants (*Amphimas pterocaroides*, *Harungana madagascariensis*, *Myrianthus arboreus*, and *Cussonia barteri*) that are commonly used in Cameroon for the management or reversal of anaemia were screened for their phytochemical content and antioxidant potential. Four extracts (methanolic, hydro-ethanolic, aqueous, and hydrolysed) from each of the plants were prepared and analysed. Qualitative phytochemical tests were used to detect the presence of alkaloids, tannins, saponins, flavonoids, glycosides and phenols, while three quantitative methods; Folin, Ferric Reducing Antioxidant Power (FRAP) and diphenyl -1, 2-picryl hydrazyl (DPPH) were used to determine the antioxidant potential of these extracts. With the exception of the extracts of *Cussonia barteri* (negative for triterpenes and phenols) and the aqueous extract of *Harungana madagascariensis* (negative result for cardiac glycosides and glycosides), all other extracts contained the phytochemicals tested. The highest antioxidant activities were observed in the hydrolysed extracts of each plant, while the aqueous extract showed the least activity irrespective of the method used. The presence of active phytochemical substances with antioxidant activities may provide substantial basis for the use of these plants in ethnomedicine.

Key words: Phytochemicals, Antioxidant, Folin, FRAP, DPPH.**Introduction**

Medicinal plants are plants that have at least one of their parts (leaves, stem, barks or roots) used for therapeutic purposes (Bruneton, 1993). Recently, medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anaemia (Fola, 1993). The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor and Ngogang, 2005; Agbor *et al.*, 2005a). The importance of medicinal plants, and the contribution of phytomedicine to the well-being of a significant number of the world's population, has attracted interest from a variety of disciplines.

Many medicinal plants contain large amounts of antioxidants other than vitamin C, E and carotenoids (Javanmardi *et al.*, 2003). Antioxidants are molecules that can delay or prevent an oxidative reaction (Velioğlu *et al.*, 1998) catalysed by free radicals. This antioxidant effect is mainly due to the presence of phenolic components such as flavonoids (Pietta, 1998), phenolic acids and phenolic diterpenes (Shahidi *et al.*, 1992). Antioxidants such as BHA (Butylated Hydroxy-Anisol), BHT (Butylated Hydroxy-Toluene) protect plants against oxidative assault (Dziezak, 1986) either by binding to metallic ions, eliminating free radicals or by decomposing peroxides (Matook, 2005). Despite the availability of synthetic antioxidants, present research seeks at discovering new natural antioxidant compounds that may play a role in oxidative stress related disorders (Agbor *et al.*, 2005b). Epidemiological studies (Urquiaga and Leighton, 2000; Halliwell, 1994) have showed that

decreases in the incidence of chronic diseases in some populations were related to the consumption of fruits and vegetables.

Four medicinal plants used for the treatment of anaemia are *Harungana madagascariensis*, *Cussonia barteri* and *Myrianthus arboreus*. Earlier studies have shown that *Harungana madagascariensis* have antibacterial, antifungal, and antiviral activities (Moulari and al., 2006). Phytochemical studies of *Cussonia barteri* methanol extract led to the isolation of 1-O-chlorogenoylchlorogenic acid and 1-O-chlorogenoylneochlorogenic acid, a new type of quinic acid esters, in addition to six known quinic acid esters, rutin, and a mixture of saponins (Papajewski et al., 2001). Arjunolic acid and a new triterpene acid, myriantic acid, have also been isolated from the root, wood of *Myrianthus arboreus* (Ojinnaka et al., 1984).

The present study investigates the phytochemical and antioxidant properties of four medicinal plants (*Amphimas pterocaroides*, *Harungana madagascariensis*, *Myrianthus arboreus*, and *Cussonia barteri*) traditionally used in the treatment of anaemia.

Materials and Methods

Collection of plant material and identification

All the plants (*Amphimas pterocaroides*, *Harungana madagascariensis*, *Myrianthus arboreus*, and *Cussonia barteri*) used in this study were harvested in June 2005, from their natural habitat in Okola, situated in the outskirts of Yaoundé. Identification of the plants was confirmed in the National Herbarium of Yaoundé.

Extraction of plants

After harvesting, the bark of each plant was chopped and air-dried in the laboratory for two months. These were later ground into a powder with the aid of a mill blender. The powdered plant material was then extracted as outlined in the schematic presentation (Figure 1).

Phytochemical screening of plants

A qualitative phytochemical test to detect the presence of alkaloids, tannins, saponins, flavonoids, glycosides and phenols was carried out using the method of Trease and Evans, (1983). The intensity of the coloration determines the abundance of the compound present.

Antioxidant potential

Three methods were used for the determination of antioxidant potential: Folin, FRAP (ferric reducing antioxidant power), DPPH (diphenyl -1, 2-picryl hydrazyl).

a) - Folin Ciocalteu reagent (sigma chemical Co., St Louis, MO) is used to determine the concentration of polyphenol as a measure of antioxidant potential. The reagent was diluted 10 times before used as described by Singleton et al, (1999). The absorbance was measured at 750 nm with the aid of a spectrophotometer (Spectronic 20. Genesys).

b) - FRAP was measured as earlier described by Benzie and Strain, (1996). In brief, 2000 µl of freshly prepared FRAP reagent (10 parts of 300 mM acetate buffer (pH 3.6), 1 part of 10 mM TPTZ (Sigma, in 400 mM HCL), and 1 part of 20 mM ferric chloride) was mixed with 75 µl of sample, methanol as appropriate for the reagent blank. The absorbance was read at 593 nm using a spectrophotometer (Spectronic 20. Genesys), after six minutes of incubation.

c) - DPPH measured the ability of the extracts to scavenge free radical. This was estimated using the method described by Hatano et al, (1988).

In all the measurement of antioxidant potential, catechin was used as standard.

Statistical analysis

Measurements were done in triplicates and the results were presented as mean ± standard deviation. The results were analysed using ANOVA one way with Student Newman-Keuls ($p < 0.05$). Correlations between antioxidant capacities as determined by different methods were established using the Pearson product moment correlation. Sigmastat 3.01 was used for this analysis.

Result and Discussion

The results showed that all the extracts studied contain the bioactive compounds glycosides, phenols, alkaloids, flavonoids, tannins, saponins, except aqueous extracts of *H. madagascariensis* which lack cardiac glycoside and glycosides, and *C. barteri* which lack phenols, triterpenes and sterol (Table 1). Phenols, flavonoids and tannins are good antioxidant substances which have been reported to have anti-diarrhoeal activity (Agbor *et al.*, 2004), and prevent or control oxidative stress related disorders (Vinson *et al.*, 1995a&b).

Table 1: Phytochemical study of medicinal plants extracts

Family	Sc. name	Extract	G.C	G	TR	P	S	A	F	TA
Cesalpiniaceae	<i>Amphimas pterocaroides</i>	M	+++	++	++	++	+++	++	+++	++
		H	+++	++	++	++	+++	++	+++	++
		A	++	++	++	++	++	+	++	++
		HCl/M	+++	+++	+++	+++	+++	+++	+++	+++
Hypericaceae	<i>Harungana madagascariensis</i>	M	++	++	+++	++	+++	++	+++	+++
		H	++	++	+++	++	+++	++	+++	+++
		A	-	-	+++	+	+++	++	++	+++
		HCl/M	+++	+++	+++	+++	+++	+++	+++	+++
Moraceae	<i>Myrianthus arboreus</i>	M	+	+	++	+	+	+	+	++
		H	+	+	++	+	+	+	+	++
		A	+	+	++	+	+	+	+	++
		HCl/M	++	++	++	++	++	++	++	++
Araliaceae	<i>Cussonia barteri</i>	M	+	+	-	-	+	+	+	++
		H	+	+	-	-	+	+	+	++
		A	+	+	-	-	+	+	+	++
		HCl/M	++	++	++	++	++	++	++	++

+ = Present, ++ = abundant, +++ = most abundant, - = absent Sc = Scientific M = methanol, H = hydroethanolic, G = Glycosides, A = aqueous, HCl/M = hydrolyzed, TA = Tannins, TR = Triterpenes and sterols, P = Phenols, F = Flavonoides, A = Alkaloides, S = Saponins, GC = cardiac Glycosides

Table 1 also shows that methanolic, hydroethanolic and hydrolysed (1.2N HCL/Methanol) extracts of *H. madagascariensis* and *A. pterocarpoïdes* qualitatively have more dense concentration of alkaloides, cardiac glycosides, flavonoids and saponins than the aqueous extract. *M. arboreus* and *C. barteri* have lower concentrations of all compounds tested. The presence of bioactive compounds in the bark of these plants is an indication of their many possible therapeutic uses.

The antioxidant capacities of the plant extracts as measured by the three different methods are presented in Figures 2, 3 and 4. Generally, the hydrolysed extracts had the highest antioxidant capacity irrespective of the method of assay, followed by the hydroethanolic and the methanolic extracts. This confirms that hydrolysis liberate bound antioxidant substances such as phenols which are bound to sugars as earlier reported (Agbor *et al.*, 2005a; Vinson *et al.*, 1998, 2001). In the antioxidant potential determined by DPPH reagent, *H. madagascariensis* had the highest activity followed by *A. pterocarpoïdes*. No significant difference ($p > 0.05$) was observed between *M. arboreus* and *C. barteri* extracts irrespective of the medium of extraction (Figure 2). 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. There is need for antioxidant intervention which one of the plants studied may be of importance. The *in vitro* study sounds encouraging as all the plants studied have some radical scavenging effect.

In the FRAP method, it was the hydrolyzed and the hydroethanolic extracts of *H. madagascariensis* that had the highest antioxidant activity though its methanolic and aqueous extracts were significantly lower ($p < 0.001$) than corresponding extracts of *A. pterocarpoïdes* (Figure 3).

In the Folin method, the hydrolyzed, hydroethanolic and methanolic extract of *H. madagascariensis* were significantly higher than the corresponding extracts of the other samples. This makes *H. madagascariensis* the overall best antioxidant source of the four plants studied and 1.2N HCL/M (hydrolyzed extract) the best extraction medium for polyphenols (Figure 4). Folin measures the polyphenolic concentration of the extracts. Polyphenols are the principal antioxidant agents in natural products (Kähkönen *et al.*, 1999). They are potent free radical terminators (Shahidi *et al.*, 1992).

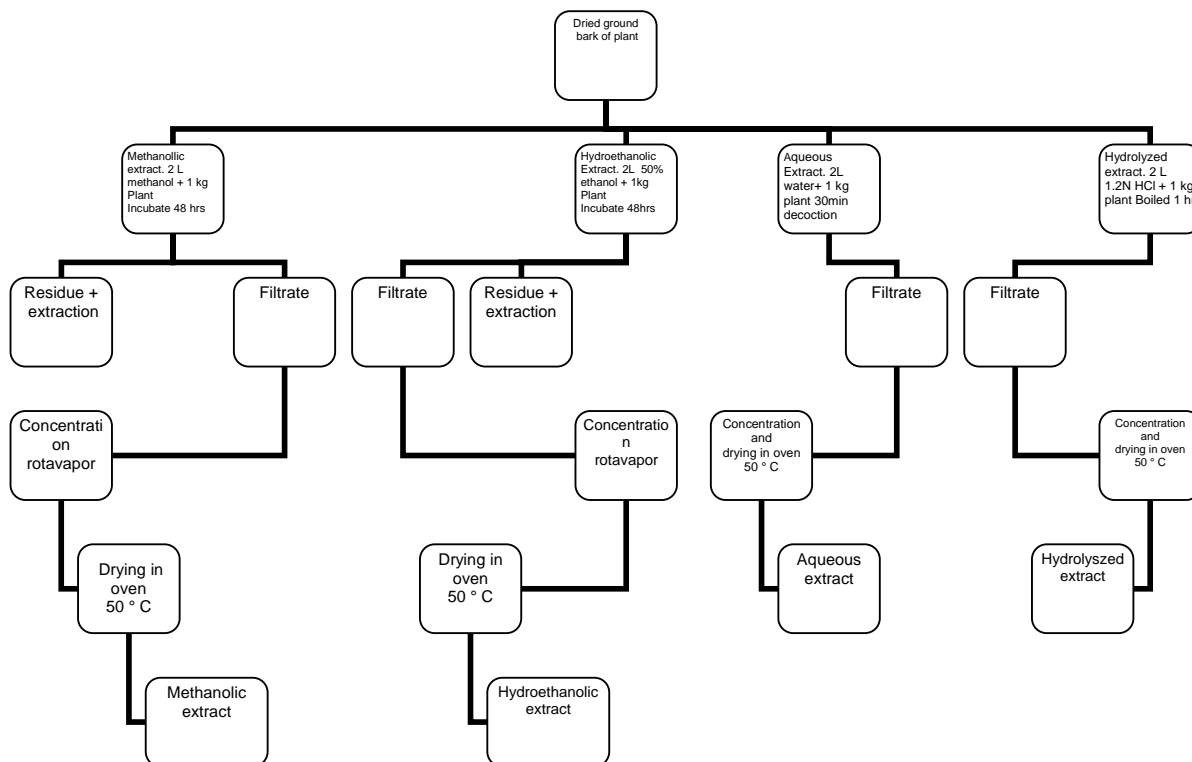
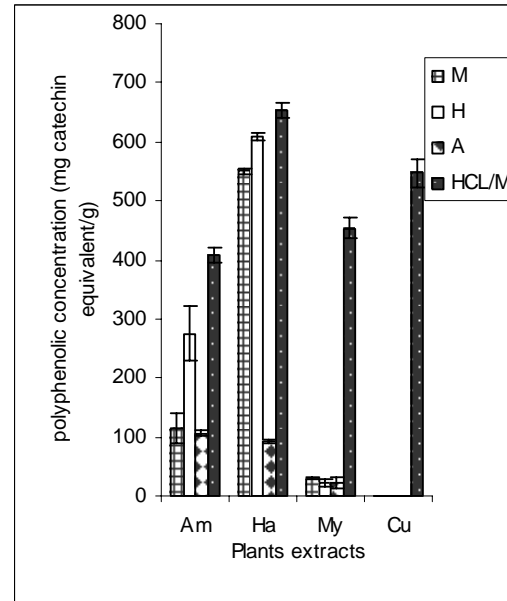
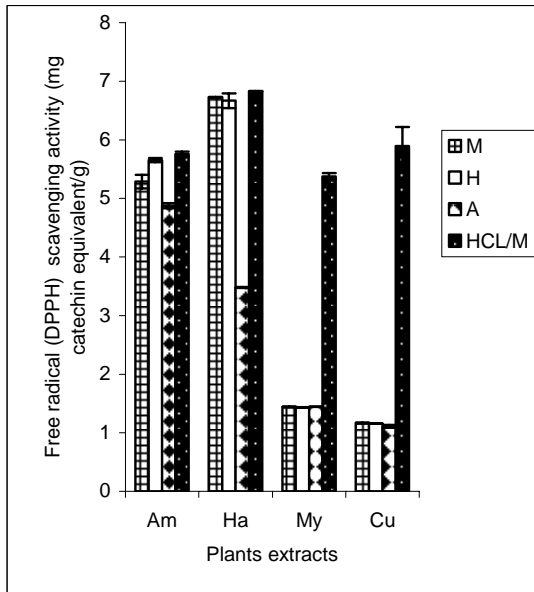


Figure 1: Schematic drawing of the extraction of the plants studied

They donate hydrogen to free radicals, and hence, break the reaction of lipid oxidation at the initiation step (Gülçin *et al.*, 2004). Thus, high polyphenolic content will mean a strong antioxidant power and a strong radical 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.

Of the plants studied, *H. madagascariensis* and *A. pterocarpoides* had significant higher ($p < 0.001$) antioxidant potential than *M. arboreus* and *C. barteri*. These two 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.

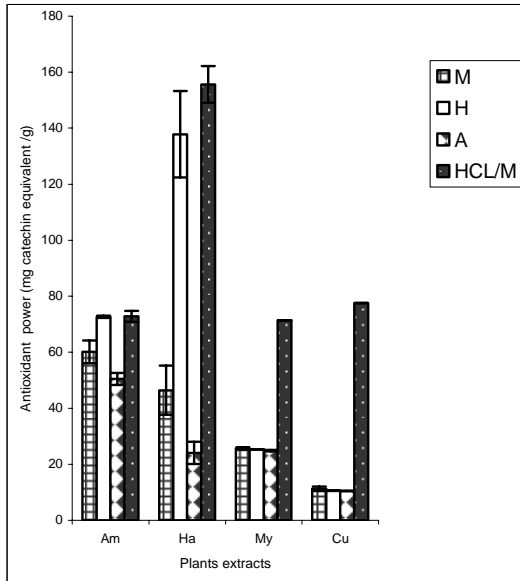


M: Methanolic, H: hydroethanolic, A: aqueous; HCL/M: 1.2N HCl/Methanol
 Am: *Amphimas pterocaroides*, *Harungana madagascariensis*,
 My: *Myrianthus* Ha: *Arboreus*, Cu: *Cussonia barteri*

M: Methanolic, H: hydroethanolic, A: aqueous; HCL/M: 1.2N HCl/Methanol
 Am: *Amphimas pterocaroides*, *Harungana madagascariensis*, My: *Myrianthus*
 Ha: *Arboreus*, Cu: *Cussonia barteri*

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

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



M: Methanolic, H: hydroethanolic, A: aqueous; HCL/M: 1.2N HCl/Methanol
 Am: *Amphimas pterocaroides*, *Harungana madagascariensis*, My: *Myrianthus*
 Ha: *Arboreus*, Cu: *Cussonia barteri*

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

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