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Original Research Article

Antiradical Activity, Total Phenolic and Flavonoid Content of Extracts from the Stem Bark of *Pterocarpus erinaceus*

Paul Toukam Djouonzo[*1, 2], Christelle Flora Ladoh-Yemeda[3], Alembert Tiabou Tchinda [2], Joseph Tanyi Mbafor[1]
[1]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P. O. Box: 812, Yaounde, Cameroon
[2]Institute of Medical Research and Medicinal Plant Studies (IMPM), Centre for Research on Medicinal Plants and Traditional Medicine (CRPMT), P. O. Box: 13033, Yaounde, Cameroon
[3] Laboratory of Biology and Physiology of Plant Organisms, Faculty of Science, University of Douala, P.O.Box: 24157, Douala, Cameroon

***Corresponding author**
Paul Toukam Djouonzo
Email: touks241@yahoo.fr

Abstract: In this study, radical scavenging activity, total phenolic and flavonoid contents of hexane, ethyl acetate, n-butanol and methanol extracts of the stem bark of *Pterocarpus erinaceus* were investigated. Antioxidant activity was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-benzothiazoline-6-sulfonic acid) (ABTS) assay. Total phenolic and flavonoid contents were evaluated with Folin-Ciocalteu and aluminium chloride colorimetric method respectively. Methanol extract showed the best antioxidant activity with the highest total flavonoid content (396.61±3.48 mgE Cat/1g DE) followed by n-butanol extract (263.63±5.44 mgECat/1g DE) and ethyl acetate extract (224.71±7.32 mgECat/1g DE). However, the n-butanol extract showed a highest phenolic content (490.03±4.02 mgEAA/1g DE) than the methanol extract (450.50±1.52 mgEAA/1g DE). These findings showed increase in antioxidant activity directly proportional to increase in extracting solvent's polarity and flavonoid content.
Keywords: *Pterocarpus erinaceus*, total phenolics, total flavonoid, radical scavenging, antioxidant activity

INTRODUCTION

Pterocarpus erinaceus is a deciduous small tree up to 15(-25) m tall and belongs to the family of Fabaceae. It is found in the savanna zone of West to Central Africa [1]. Its stem bark is used in the form of decoction to treat inflammatory disorders and was scientifically proven to bear the activity [2]. Many natural plant products such as terpenoids, flavonoids, polyphenols and alkaloids have demonstrated anti-inflammatory activity. The activity is generally related to the antioxidant power of the molecule. That is its ability to inhibit oxidation. Oxidation occurs when there is oxidative damage caused by ROS/RNS. The oxidative damages caused by ROS on lipids, proteins and nucleic acids may trigger various inflammatory related diseases [3]. The health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting ROS [4]. The factors that generate reactive oxygen species (ROS) exist as products of normal cellular physiology as well as from various exogenous sources [5]. Prolonged state of oxidative stress is involved in the pathogenesis of several diseases, such as cardiovascular disease and cancer [6]. Phenolic compounds are by far the most abundant antioxidants in most human diets. They possess strong antioxidant properties that enable them to scavenge free radicals, donate hydrogen, chelate metal ions, break radical chain reactions, and quench singlet oxygen *in vitro* and *in vivo* [7, 8].

In vitro antioxidant activity of stem bark extract of *P. erinaceus* was earlier reported by some authors. The DPPH radical scavenging assay corresponds to the primary radical scavenging activity of an antioxidant, and ABTS to the ability of an antioxidant to inhibit radical formation. The FRAP on the other hand evaluates the reducing ability of the antioxidant [9]. To the best of our knowledge, no work is yet reported on the ABTS assay. This study reports on the DPPH and ABTS scavenging activity, total flavonoid and phenolic content of the stem bark extracts of *P. erinaceus.*

MATERIAL AND METHODS

Plant material and extraction

Stem barks of *Pterocarpus erinaceus* were harvested in July 2013 at mount *Tinguelin* in the north region of Cameroon and a voucher specimen (N^0 5205/SRFCAM) was deposited at the national herbarium. The outer most layer of the stem was

removed and discarded, the inner layer chopped into small pieces before air- dried and grounded to powder. The vegetal powder was successively extracted by percolation with hexane, ethyl acetate and mixture MeOH/DCM (1:1) in increasing polarity. The methanolic extract was further partitioned with n-butanol/water to give the n-butanol extract (Table 1).

Determination of the total phenolic content

The total phenolic content was determined by the Folin-ciocalteu method [10]. A volume of 23µL at 1mg/mL of each extract was mixed with 1817µl of distilled water, 115µl of reagent of Folin-ciocalteu (diluted to 1:1 v/v) and 345µl solution at 15% of sodium carbonate. The mixture was incubated at dark room temperature for 2hours and absorbance read with spectrophotometer at 765nm. Ascorbic acid (AA) was used as reference and results were expressed in milligram equivalent of AA per gram of dry extract (mgEAA/g DE).

Determination of the total flavonoid content

Total flavonoid content was determined by $AlCl_3$ colorimetric assay [11]. 100µl (1mg/ml) solution of each extract was added to 300µL of distilled water. To this 30µL of 5% $NaNO_2$ was added. After 5minutes 30µL of 10% $AlCl_3$ was added. After 5minutes, 200µL of 1mMNaOH and 1ml of distilled water were added. Absorbance was read at 510nm with a spectrophotometer.

Determination of ABTS scavenging activity

The study of the antioxidant capabilities *via* the decolorization of ABTS (acid 2,2-azinobis-3-ethyl benzothiazoline-6-sulfonic) radical was carried out according to the procedure described by Re *et al*., and Pellegrini *et al*., with some modifications [12, 13]. The ABTS radical cation ($ABTS^{\bullet+}$) was formed by reaction of 7mM stock solution of ABTS with 2.45mM potassium persulfate and allowing the mixture to stand for 16 hours in dark at room temperature. The $ABTS^{\bullet+}$ solution was diluted with methanol until absorbance of 0.7 (±0.002) at 734nm. A volume of 20µL of each extract at different concentrations (7812.50-125000 µg/mL for hexanic extract, 156.25-2500 µg/ml for ethyl acetate extract, 39.06-1250 µg/ml for n-butanol extract and 19.53-1250 µg/ml for methanol extract) were introduced in test tubes containing 2ml of methanolic $ABTS^{\bullet+}$ solution, stirred with a magnetic stirrer; after 6mins, absorbance was measured at 734nm. Ascorbic acid (0.97-500 µg/mL) and butylated hydroxytoluene (15.62-500 µg/ml) were used as references. All tests were performed in triplicate and results expressed in equivalent standard deducted from the equation of line y = ax+b obtained with the calibration curve. The percentage inhibition of radical ABTS is calculated via the formula:

$$\%\ \text{inhibition} = \frac{absorbance\ (control) - absorbance(extract)}{absorbance\ control} \times 100$$

Scavenging concentration at 50% (SC_{50}) or concentration of extract necessary to inhibit 50% of ABTS radicals was determined graphically by linear regression.

Determination of DPPH scavenging activity

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams *et al.,* with slight modifications [14]. 1900µL of 4% DPPH solution in methanol was mixed with 100µL of plant extract solution of varying concentrations 5000-80000 µg/ml for hexanic extract, 19.53-312.50 µg/ml for ethyl acetate extract, 15.62-250 µg/ml for n-butanol extract and 7.81-125.00 µg/ml for methanol extract. Corresponding blank sample were prepared and L-Ascorbic acid (7.81-125.00 µg/ml) was used as reference standard. Mixer of 100µl of solvent used to solubilize extract and 1900µl DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 60 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the above formula. And results are expressed as amount of extract required to decrease the initial DPPH concentration by 50% (SC_{50}), amount extract per amount of DPPH at 50% inhibition point (EC_{50}) and as antiradical power ($ARP=1/EC_{50}$).

Statistical analysis

All data are expressed as mean ± S.D. 50% and above inhibition of DPPH or ABTS radical is considered as significant for scavenging activity, that is SC_{50}<50mg/l and SC_{50}>50mg/ml are respectively considered as high antioxidant capacity and moderate antioxidant capacity [15]. Significance differences between the means were determined by least significant difference (LSD) test at a level $P < 0.05$.

RESULTS AND DISCUSSION

Percentage weight of extracts decrease in the order of decreasing polarity MeOH>EA>Hex. Partition of methanol extract with water and n-butanol leads to a butanolic fraction smaller than the ethyl acetate extract (Table 1). Compounds present in *P. erinaceus* are generally polar. This is in accordance to previous works carried out by Gabriel and Onigbanjo [16].

Total phenolic content of extracts was determined with calibration curve of ascorbic acid ($y=0.0165x - 0.0033$; $R^2=0.9977$) and expressed as milligram equivalent of ascorbic acid per gram of dry extract (mgEqAA/gDE). The n-butanol extract had the

highest phenolic content (490.03±4.02 mgEqAA/gDE) followed by the methanol extract (450.50±1.52 mgEqAA/gDE) and the ethyl acetate extract (312.60±9.97 mgEqAA/gDE). No total phenol was detected in the hexanic extract (Table 2). Phenolic compounds are polar and it is therefore not surprising that they are absent in the hexanic extract.

Table 1: Yield and other physical properties of extracts of *P. erinaceus*

Sample	Yield	Colour	Consistency
Hexane	0.43	yellow	pasty
Ethyl acetate	1.76	brownish	pasty
Methanol	7.31	brownish red	solid hard
n-Butanol	0.89	dark brownish	steaky powder

Table 2: Total phenolic and flavonoid content

Sample	Phenolic (mgEqAA/g DE)	Flavonoid (mgEqCAT/g DE)
Hexane extract	0	0
Ethyl acetate extract	312.60±9.97[a]	224.47±7.32[a]
Methanol extract	450.50±1.52[b]	396.61±3.48[b]
n-Butanol extract	490.03±4.02[c]	263.63±5.44[c]

Values denoted by different letters ([a-c]) in a column are significantly different at $p < 0.05$

Table 3: DPPH and ABTS scavenging capacity results

Sample	DPPH			ABTS
	SC_{50}(mg/l)	CE_{50}(mg/mg DPPH)	ARP	SC_{50}(mg/l)
Hexane extract	4989.34±311.67[a]	65.64±4.10[a]	0.01	2427.70±327.41[a]
Ethyl acetate extract	10.69±1.16[b]	0.14±0.01[b]	7	12.92±0.71[b]
Methanol extract	4.40±0.33[c]	0.05±0.00[c]	20	5.44±0.50[c]
n-Butanol fraction	8.29±0.50[d]	0.10±0.00[d]	10	8.79±0.27[d]
Ascorbic acid	5.50±0.27[e]	0.07±0.00[e]	14	3.65±0.05[e]
Butylated hydroxytoluene	_	_	_	3.82±0.15[e]

Values denoted by different letters ([a-e]) in a column are significantly different at $p < 0.05$.
ARP(Antiradical power)=$1/CE_{50}$

Calibration curve of catechin ($y=0.0191x±0.0488$; $R^2=0.9987$) was used in the determination of total flavonoid content and the results were expressed as milligram equivalent catechin per gramme of dry extract (mgEqCAT/gDE). Methanolic extract had the richest total flavonoid content (396.61±3.48 mgEqCAT/gDE) as compared to butanolic extract (263.63±5.44 mgEqCAT/gDE) and ethyl acetate extract (224.71±7.32 mgEqCAT/gDE). African kinos and stem bark of *P. erinaceus* are rich in catechic tannins [17], and this may explain the high content in polyphenols. Hage *et al*., evaluated the contribution of tannins in Y-secretase inhibitory active of *P. erinaceus* and found that the activity was not only due to proanthocyanidins which is in accordance to work done by Ouedraogo *et al* [18]. *Pterocarpus* species are rich in isoflavonoids [19], terpenoids and related phenolic compounds [20, 21, 22]. Lupeol, friedelin and epicatechin have been isolated from the stem bark of *P. erinaceus* [2] and homopterocarpin from its kino [23]. The presence of the two latter phenolic compounds may contribute to the antioxidant activity of the plant. Hage *et al*., carried out the HPLC-PDA-MS analysis of this plant extracts and found peaks not related to catechin derivatives.

Antioxidant activity assessed by DPPH radical scavenging method showed highest scavenging activity with methanol extract (SC_{50}=4.40±0.33mg/l, CE_{50}=0.05±0.00 mgDE/mg DPPH, ARP=20) followed by butanol fraction (SC_{50}=8.29±0.50 mg/l, CE_{50}=0.10±0.00 mgDE/mg DPPH, ARP=10), ethyl acetate extract (SC_{50}=10.69±1.16 mg/l, CE_{50}=0.14±0.01 mgDE/mg DPPH, ARP=7) and hexanic extract (SC_{50}=4989.34±311.67 mg/l, CE_{50}=65.64±4.10 mgDE/mg DPPH, ARP=0.01). Methanol extract was more active than the reference compound ascorbic acid (Table 3). This result is closed to that earlier described by Ouédraogo *et al*. These authors showed that methanol extract of the stem bark of *P. erinaceus* had comparable antioxidant power using DPPH method with chlorogenic acid and trolox. Patrick *et al* [24] studied the antioxidant activity of crude methanolic and aqueous extracts and ascorbic acid used as standard and found that they had potent antioxidant activity with SC_{50} as 64.00, 80.00 and 10.00 respectively. With FRAP assay, methanolic extract had activity equivalent to that of ascorbic acid whereas aqueous extract showed moderate activity. Crude methanolic extract of *P. marsupium*, a species known for its enormous

pharmacological properties had antioxidant capacity close to ascorbic acid with DPPH method [25].

Scavenging pattern with ABTS assay was the same with that of DPPH. That is MeOH (SC_{50}=5.44±0.50)>nBuOH(SC_{50}=8.79±0.27)>AE(SC_{50} =12.92±0.71)>Hex(SC_{50}=2427.70±327.41); although the scavenging activity of methanol with this assay was lower than that of ascorbic acid (SC_{50}=3.65±0.05) and butylated hydroxytoluene (SC_{50}=3.82±0.15).

The scavenging activity of all samples with both DPPH and ABTS methods increased with concentration which is consistent with the trapping of free radicals due to an increase of the number of antiradical species. Results showed that all samples except hexanic extract have good scavenging activity; however, a discrepancy is observed between butanol extract and methanol extract. Butanol extract had the highest phenolic content and methanol extract the highest flavonoid content with the highest antioxidant capacity. This might suggest flavonoids are responsible of the highest antioxidant potential of methanol extract as compared to other phenolic compounds.

CONCLUSION

Our findings have shown that antioxidant molecules of stem bark extracts are spread over moderately polar to highly polar extracts, with the methanolic extract being the most effective with the highest flavonoid content and less phenolic content in comparison to n-butanol extract. In addition, methanolic extract has an antioxidant power greater than that of ascorbic acid with DPPH method. These suggest that *P. erinaceus* is a rich source of antioxidant molecules and flavonoids might be more responsible as compared to other phenolic compounds of the highest antioxidant activity exhibited by the polar extract.

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