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

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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-picrylhydrazly (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/lg DE) and ethyl acetate extract (224.71±7.32 mgECat/lg 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 (NO 5205/SRFFCAM) 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 AlCl₃ colorimetric assay [11]. 100μl (1mg/ml) solution of each extract was added to 300μL of distilled water. To this 30μL of 5% NaNO₂ was added. After 5minutes 30μL of 10% AlCl₃ was added. After 5minutes, 200μL of 1mMNaOH and 1ml of distilled water were added.

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

Scavenging concentration at 50% (SC₅₀) 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 determined by 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. Mix 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% (SC₅₀), amount extract per amount of DPPH at 50% inhibition point (EC₅₀) and as antiradical power (ARP=1/EC₅₀).

**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 SC₅₀<50mg/l and SC₅₀<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 (γ=0.0165x – 0.0033; R²=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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield</th>
<th>Colour</th>
<th>Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>0.43</td>
<td>yellow</td>
<td>pasty</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1.76</td>
<td>brownish</td>
<td>pasty</td>
</tr>
<tr>
<td>Methanol</td>
<td>7.31</td>
<td>brownish red</td>
<td>solid hard</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>0.89</td>
<td>dark brown</td>
<td>steaky powder</td>
</tr>
</tbody>
</table>

Table 2: Total phenolic and flavonoid content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Phenolic (mgEqAA/g DE)</th>
<th>Flavonoid (mgEqCAT/g DE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane extract</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>312.60±9.97</td>
<td>224.47±7.32</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>450.50±1.52</td>
<td>396.61±3.48</td>
</tr>
<tr>
<td>n-Butanol extract</td>
<td>490.03±4.02</td>
<td>263.63±5.44</td>
</tr>
</tbody>
</table>

Values denoted by different letters (**) in a column are significantly different at p < 0.05

Table 3: DPPH and ABTS scavenging capacity results

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$SC_{50}$ (mg/l)</td>
<td>$CE_{50}$(mg/mg DPPH)</td>
</tr>
<tr>
<td>Hexane extract</td>
<td>4989.34±311.67</td>
<td>65.64±4.10</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>10.69±1.16</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>4.40±0.33</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>n-Butanol fraction</td>
<td>8.29±0.50</td>
<td>0.10±0.00</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>5.50±0.27</td>
<td>0.07±0.00</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

Values denoted by different letters (***) in a column are significantly different at p < 0.05.

Antioxidant activity assessed by DPPH radical scavenging method showed highest scavenging activity with methanol extract ($SC_{50}=4.40±0.33$ mg/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

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 (224.71±7.32 mgEqCAT/gDE) and ethyl acetate extract (224.71±7.32 mgEqCAT/gDE). African kinos and stem bark of P. erinaceus are rich in catechins and related phenolic compounds [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 Ouédraogo et al [18]. Pterocarpus species are rich in isoflavonoids [19], terpenoids and related phenolic compounds [20, 21, 22]. Lupeol, friedelin and epicathechin 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.
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.

REFERENCES