Research Article

ANTIOXIDANT ACTIVITY OF *Croton Penduliflorus* (HUTCH) LEAF EXTRACT AND FRACTIONS

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ABSTRACT

Croton spp. has been used medicinally in the treatment of a variety of ailments, which include cancer, constipation, and diabetes. Various parts of the plant are used in Nigeria as antimicrobial, abortifacient, anti-inflammatory and anti-tumorigenic agents. Dried leaves of *Croton penduliflorus* (Hutch) was powdered, extracted and concentrated, and the aqueous fraction partitioned into dichloromethane, ethylacetate and n-butanol. The following parameters were determined, ABTS and DPPH radical scavenging activity, Free Radical Antioxidant Power, total antioxidant capacity, total phenolic and total flavonoid contents. The aqueous and dichloromethane fractions gave the highest yields, and the crude extracts exhibited a high amount of total phenol and total flavonoids. The crude extract and dichloromethane fractions showed a high scavenging activity for ABTS and DPPH, while the crude extract and its fractions demonstrate significant radical scavenging ability and antioxidant capacity which were due to the presence of phenols and flavonoids.

KEYWORDS: *Croton penduliflorus*; antioxidant; ABTS, DPPH.

INTRODUCTION

*Croton penduliflorus* Hutch is a member of the large genus Croton of the Euphorbiaceae family, found in tropical West Africa, and commonly known as Aworoso (Yoruba) in South Western, and Aki ozara (Igbo) in South Eastern, Nigeria. The genus *Croton* has over 1000 species (trees and shrubs) in tropical regions of the world (Fuentes et al., 2004), and are used medicinally in the treatment of cancer, constipation, diabetes, digestive problems, dysentery, external wounds, intestinal worm, pain, ulcers and weight loss (Salatino et al. 2007). In Nigeria, various parts of the plants are used as antimicrobial, abortifacient, anti-inflammatory, and anti-tumorigenic agents (Ojokuku et al., 2011). Extracts of the seed and whole plant has been used for constipation, general debility, diuretics, arthritis, and purgative (Ojokuku et al., 2011). *Croton spp.* are well known for their toxicity and medicinal properties. *Croton* possesses secondary metabolites such as alkaloids, terpenoids, flavonoids and diterpenoids. The diterpenoid content of the genus *Croton* contains phorbol esters, kaurane, pimarane, and labdane as isolated by Block et al. (2004).

This paper investigates the antioxidant properties of the crude extracts, and the partitioned fractions of the crude extracts of *C. penduliflorus*. The solvents used include dichloromethane, ethylacetate, n-butanol, and water.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study include 2,2′-azono-bis-2-ethylbenzothiazoline-6-sulphenic acid (ABTS), potassium peroxodisulphate, 2,2-diphenyl-1-picryl-hydrazone (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), ferric chloride, sodium acetate, sodium carbonate, Trolox, Folin-Ciocalteau reagent, which were obtained from Sigma Aldrich (St. Louis, MO, USA). Aluminium chloride was from Merck
(Darmstadt, Germany), and ammonium molybdate (BDH Chemicals, Poole, England), gallic acid, ascorbic acid, quercetin, and EDTA. All other reagents used are of analytical grade.

**Plant Materials**

Dr. Iloh of the Department of Botany identified *C. penduliflorus* HUTH leaves obtained from the Botanical Garden, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State. The leaves were oven-dried at 40 °C for 72 h, and powdered.

**Extraction**

Dried leaf samples of *C. penduliflorus* were powdered and subjected to Soxhlet extraction for 6 hrs using 80% methanol and concentrated in a rotary evaporator (####). Aqueous solution of the crude residue was fractionated into dichloromethane, ethylacetate, and n-butanol fractions. Each fraction was then concentrated under vacuum using rotary evaporator.

**Methods**

**Determination of ABTS Radical Scavenging Activity**

A solution of ABTS (7 mmol/l) and potassium peroxodisulphate (7 mmol/l) were mixed in distilled water in the ratio 2:1 v/v, and left in the dark for 12 - 16 hours at room temperature according to the method by Brandt-Williams *et al.* (1995). The solution was diluted with ethanol to give an absorbance of 0.70 ± 0.02. The reaction was started by taking 2 ml of ABTS in a test tube and 20 μl of sample added. After 5 min absorbance was measured at 734 nm. Gallic acid and Trolox were the standard compounds to compare results, and results were expressed as IC$_{50}$.

**Determination of DPPH Radical Scavenging Activity**

The DPPH radical scavenging activity of the extract and fractions was determined using 1 ml of 0.3 mM DPPH in methanol, and 1 ml of extract at varying concentrations. The reaction mixture was then incubated in the dark for 30 min, and the absorbance taken at 517 nm. Ascorbic acid was the standard for comparison.

**Ferric Reducing Antioxidant Power Assay**

This method is based on the ability of the antioxidants to reduce Fe$^{3+}$ to Fe$^{2+}$, while Fe$^{2+}$ reacts with the chromophore 2,4,6-tripyridyl-2-triazine (TPTZ) (Benzie and Strain, 1996). A mixture of 10 mM TPTZ and 20 mM ferric chloride diluted in 300 mM sodium acetate buffer (pH 3.6) in the ratio of 1:1:10 was prepared. Solutions of extract (100 μl) and 1 ml of reagent are added in a test tube and incubated in the dark for 30 min. Absorbance readings of the chromophore (ferrous tripyridyltriazine complex) were measured at 593 nm.

**Determination of Total Antioxidant Capacity**

The total antioxidant capacity of the extract was determined using the phosphomolybdenum method (Prieto *et al.* 1999). Extracts (0.3 ml) were mixed with 3 ml reagent solution containing 0.6 M H$_2$SO$_4$, 28 mM sodium phosphate, 4 mM ammonium molybdate in water. This is reaction mixture was incubated at 95 °C for 90 min. It was allowed to cool and the absorbance readings were taken at 695 nm. Methanol was used to blank the spectrophotometer, and ascorbic acid was the standard used for comparison.

**Determination of Total Phenolic Content**

The total phenolic content of the extract was determined with the method of Singleton and Rossi (1965), using 0.5 ml extract (1 mg/ml) and 0.1 ml of Folin-Ciocalteau reagent (0.5N), thoroughly mixed and incubated for 15 min at RT. Thereafter, 2.5 ml Na$_2$CO$_3$ (saturated) was added and the resulting solution incubated for 30 min at RT. The absorbance of the solution was measured at 760 nm.

**Determination of Total Flavonoid**

Total flavonoid was quantified using the aluminium chloride method (Hazra, et. al., 2008). Extract (1 mg/ml) 0.1 ml was mixed with distilled water (0.3 ml) and 5% Na$_2$SO$_4$ solution (0.03 ml) sequentially, and the resulting mixture incubated at RT for 5 min, after which 10% AlCl$_3$ solution was added and left for 5 min, then 0.2 ml of 1 M NaOH was added. The reaction mixture was diluted to 1 ml using distilled water, and absorbance of the solution was measured at 510 nm. Quercetin was the standard for calibration and the results expressed as quercetin equivalent.

**Statistical Analysis**

The results were expressed as mean ± standard deviations (n = 3), and the IC$_{50}$’s were calculated using GraphPad Prism 5.

**RESULTS**

The yield of the crude extract was 26.80%, from which 1 g in 200 ml distilled water was subjected to fractionation. The dichloromethane (DCM) fraction gave 16.5%, ethylacetate fraction 4.98%, n-butanol fraction 7.49%, and the aqueous fraction gave 28.18% (Table 1). The total phenol and total flavonoids content of the crude extract and the fractions are shown in Table 1. The crude extract had the highest total phenol (17.30 ± 1.16 mgGAE/g) and total flavonoids content (48.76 ± 5.84 mgQE/g), while for total phenol the n-butanol fraction was higher than the ethylacetate fraction. For the total flavonoids content of the fractions, ethylacetate (46.97 ± 2.25 mgQE/g) was higher than the n-butanol fraction (37.75 ± 0.22 mgQE/g). Both the DCM and aqueous fractions were significantly lower. Free radical scavenging activity of the crude extract and fractions was determined. The crude extract and ethylacetate fraction show a high scavenging activity for ABTS and DPPH radicals. The IC$_{50}$ of the crude extract and ethylacetate fraction for ABTS are 0.97 ± 0.09 and 0.88 ± 0.04 mg/ml respectively, while the n-butanol and aqueous fractions gave for ABTS 1.41 ± 0.00 and 5.52 ± 0.08 mg/ml respectively. The IC$_{50}$ for DPPH radical scavenging of crude extracts and ethylacetate fraction showed a high activity, which were 0.05 ± 0.00 and 0.15 ± 0.01 mg/ml, respectively. The DCM and aqueous fractions showed a low activity as 1.26 ± 0.07 and 1.48 ± 0.14 mg/ml, respectively. The n-butanol fraction on the other hand gave 0.17 ± 0.01 mg/ml. Metal chelating activity of the crude extract was measured and was found to be low, with IC$_{50}$ of 8.50 ± 0.03 mg/ml.
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DISCUSSION

In this study the antioxidant activity of a crude extract and the partitioned fractions of *C. penduliflorus* were carried out using radical scavenging assay (ABTS and DPPH), antioxidant capacity (FRAP, TAC), and phenolic content (Total Phenol and Total Flavonoid).

*C. penduliflorus* crude extract gave a 26.8% yield. From the results as shown in Table 1, the aqueous fraction gave the highest yield, and aqueous extract is the most common in traditional medicine in which anthocyanins and soluble phenolics are the important antioxidant molecules present. DCM fraction yield was 16.65% from the reconstituted crude extract. DCM is known to selectively extract terpenoids (Tiwari *et al*., 2011). Ethylacetate and n-butanol fractions gave 4.98% and 7.49% of solids respectively.

Aderogba *et al.* (2013) demonstrated that ethylacetate fraction of *C. penduliflorus* possess the ability to reduce oxidative damage to cells, due to the presence of quercetin, kaempferol and protocatechualdehyde. As the results of this work indicate, the ethylacetate extract exhibits a strong anti-radical and antioxidant ability. Antioxidants from natural sources exhibit a wide range of biological activity which include antimicrobial, anticholinesterase, anti-inflammatory, and anti-mutagenic effects (Koksal and Gulcin, 2008). ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonate) radical is formed using potassium persulphate and incubated in the dark for 12 – 16 hrs. This assay determines the antioxidant activity of the plant extracts with the lowest IC\textsubscript{50} value indicating that the mechanism of action of the extract could possibly be by hydrogen donation, converting the free radical to a stable form (Li *et al*., 2009). The ethylacetate fraction has the lowest IC\textsubscript{50} value of 0.88 ± 0.04 mg/ml. This in contrast to the standards gallic acid and Trolox which gave IC\textsubscript{50} values of 0.05 ± 0.00 and 0.24 ± 0.01 mg/ml respectively, indicates that *C. penduliflorus* is not as strong an antioxidant compared to these standards, therefore its ability to donate hydrogen to quench the radical could be considered as moderate. ABTS quenching ability is related to the presence of polyphenols (Hossai, *et al*., 2009). The polyphenol content of the extracts correlates with the ABTS assay, the crude extract showed the highest total phenol content and a relatively high ABTS scavenging activity, while the ethylacetate fraction has the highest ABTS activity, however ranked third in total phenol content. DCM fraction with the lowest ABTS activity also had the least total phenolic content of the fractions. Phenolics and flavonoids perform antioxidant activities through the following mechanisms – by scavenging radical species in vivo, by the inhibition of enzymatic reactions leading to the formation of radicals, and by influencing the synthesis of protective cellular anti-oxidative systems (Ghasemzadeh and Ghasemzadeh, 2011).

Similarly, DPPH scavenging activity of the crude and ethylacetate fraction showed the highest activity, 0.05 ± 0.00 and 0.15 ± 0.01 mg/ml respectively, while the n-butanol fraction had 0.17 ± 0.01 mg/ml. These activities correlates with the total phenolic and total flavonoid content of the extracts in these fractions.

Ferric ions are reduced by electron transfer mechanism to the ferrous ion. Plant extract with molecules having the ability to perform single electron transfer will reduce ferric to ferrous ions. In this study the potency to reduce ferric ions follows the trend crude extract > ethylacetate fraction > n-butanol fraction. The values for the crude extract and the ethylacetate and n-butanol fractions are significantly different and higher than that of the DCM and aqueous fractions. The exhibited ability could be the result of electron transfer by the components of the extract, with the ability to react with free radicals and convert them to stable compounds, therefore terminating the chain reactions.

In conclusion, the extract and its fractions have demonstrated significant free radical scavenging ability and antioxidant capacity. These activities have been exerted due to the presence of phenols and flavonoids, which are among the active components of plant natural products, with phenolic compounds extensively examined as current knowledge.

**Table 1:** Percent yield of the crude extract and the solvent fractions.

<table>
<thead>
<tr>
<th></th>
<th>Crude</th>
<th>DCM fraction</th>
<th>Ethyl Acetate fraction</th>
<th>n-Butanol fraction</th>
<th>Aqueous fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>YIELD (%w/w)</td>
<td>26.8</td>
<td>16.65</td>
<td>4.98</td>
<td>7.49</td>
<td>28.18</td>
</tr>
</tbody>
</table>

**Table 2:** Results of the various assays of the crude extract and the fractions.

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>Crude</th>
<th>DCM fraction</th>
<th>Ethyl Acetate fraction</th>
<th>n-Butanol fraction</th>
<th>Aqueous fraction</th>
<th>Gallic acid</th>
<th>Trolox</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS (IC50)</td>
<td>0.97±0.09</td>
<td>9.14±0.39</td>
<td>0.88±0.04</td>
<td>1.41±0.00</td>
<td>5.52±0.08</td>
<td>0.05±0.00</td>
<td>0.24±0.01</td>
<td></td>
</tr>
<tr>
<td>DPPH (IC50)</td>
<td>0.05±0.00</td>
<td>1.26±0.07</td>
<td>0.15±0.01</td>
<td>0.17±0.01</td>
<td>1.48±0.14</td>
<td>-</td>
<td>-</td>
<td>0.007±0.00</td>
</tr>
<tr>
<td>FRAP (mgAAE/g)</td>
<td>5.35±0.01</td>
<td>0.82±0.05</td>
<td>5.13±0.06</td>
<td>4.64±0.05</td>
<td>1.01±0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TAC (mgAAE/g)</td>
<td>20.24±1.25</td>
<td>3.57±0.08</td>
<td>15.91±0.12</td>
<td>15.68±0.17</td>
<td>5.27±0.47</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TPC (mgGAE/g)</td>
<td>17.30±1.16</td>
<td>2.87±0.10</td>
<td>13.98±0.84</td>
<td>14.63±0.25</td>
<td>5.32±0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TFC (mgQE/g)</td>
<td>48.76±5.84</td>
<td>9.66±0.90</td>
<td>46.97±2.25</td>
<td>37.75±0.22</td>
<td>10.56±0.00</td>
<td>-</td>
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REFERENCES


