Preliminary Phytochemical Screening and \textit{In vitro} Antioxidant Properties of \textit{Trichilia monadelpha} (Thonn.) J. J. de Wilde (Meliaceae)

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The study evaluated the antioxidant potential and phytochemical constituents in the stem bark extracts of \textit{Trichilia monadelpha} (Thonn) JJ De Wilde (Family: Meliaceae). Petroleum ether (PEE), ethyl acetate (EthE) and ethanol extracts (EAE) of the stem bark of \textit{T. monadelpha} were screened for the presence of phytochemicals. The \textit{in vitro} antioxidant potential of the extracts were also determined using the reducing power and 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) scavenging tests respectively. Total phenol content of the extracts was also estimated. Phytochemical analysis revealed the presence of important secondary metabolites. Alkaloids, terpenoids, phytosterols, reducing sugars and coumarins were present in PEE. EAE had tannins, alkaloids, terpenoids, phytosterols, reducing sugars, flavonoids, cardiac glycosides, anthraquinones and saponins whiles EthE contained tannins, alkaloids, reducing sugars, cardiac glycosides, anthraquinones, terpenoids and phytosterols. Total phenol contents were estimated to be 7.51±0.87 mg tannic acid equivalent/g of petroleum ether extract, 34.14±0.78 mg tannic acid equivalent/g of ethyl acetate extract and 119.30±3.20 mg tannic acid equivalent/g of hydroethanol extract. The extracts showed a concentration-dependent reduction of Fe$^{3+}$ to Fe$^{2+}$ in the reducing power test as well as concentration-dependent DPPH radical scavenging. Of the three extracts, EAE had the most antioxidant activity. Findings of this study suggest that the stem bark of \textit{Trichilia monadelpha} may be a good source of natural antioxidants and might be useful in treating the diseases associated with oxidative stress.

Keywords: Phytochemicals, total phenol content, reducing power, DPPH, antioxidant, free radicals.

INTRODUCTION

Free radicals and reactive oxygen species have received a lot of attention especially in experimental or clinical medicine and biology because of their role in the aetiology of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Halliwell, 2011; Halliwell, 2012; Halliwell \textit{et al.}, 1992). The damaging effects of reactive oxygen species (e.g. singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite) on cells has been shown to be abrogated by plants with antioxidant compounds (Dasgupta \textit{et al.}, 2007). Plants are endowed with antioxidant and free radical scavenging molecules including vitamins, terpenoids, phenolic acids, tannins, flavonoids, coumarins, and other secondary metabolites. The search for compounds, that can protect the human body from oxidative damage and retard the progress of many chronic diseases, has therefore greatly focused on plant sources as they produce significant amount of antioxidants and represent a potential source of new compounds with antioxidant activity.

\textit{Trichilia monadelpha} (Thonn) JJ De Wilde (Family: Meliaceae), known locally as Otanduro (Twi) or Tenuba (Nzema), is a tree that grows 12-20 m high and establishes itself well in the lowland high forest and evergreen semi-deciduous secondary jungles, often near river banks (Abbiw, 1990). Preparations

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ORIGINAL ARTICLE
(decoctions, infusions and tinctures) of the stem bark of the plant have been used in Ghanaian traditional medicine to treat pain, psychoses, epilepsy and inflammation for many years and their efficacies are widely acclaimed in different communities in Ghana (Abbiw, 1990; Dokosi, 1998). Pharmacological studies have revealed that the ethanolic stem bark extract of *T. monadelpha* has anti-trypanosomal and antiplasmodial activities (Kamanzi Atindehou *et al.*, 2004). Also, various stem bark extracts of the plant have been shown to have anti-inflammatory (Ainooson *et al.*, 2012) and analgesic (Woode *et al.*, 2012) properties. In the present study, the phytochemical constituents and the *in vitro* antioxidant and free radical scavenging potential of stem bark extracts of *Trichilia monadelpha* are determined.

**MATERIALS AND METHODS**

**Chemicals**

Acetic anhydride, ammonia, chloroform, Dragendorff’s reagent, ethanol, ferric chloride, gelatin, hydrochloric acid, lead acetate, magnesium metal strips, methanol, *n*-propyl gallate, potassium ferricyanide, sodium chloride, sodium carbonate, sulphuric acid, sodium hydroxide and tannic acid were obtained from British Drug House (BDH) Ltd (Poole, England) while 2, 2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), Folin-Ciocalteau reagent and Wagner’s reagent were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All chemicals were of highest purity (≥ 99.0%).

**Plant collection**

The stem bark of *Trichilia monadelpha* was obtained from Bomaa (7°05’06.82” N; 2°10’01.63” W) in the Tano North District of the Brong Ahafo Region of Ghana between August and October, 2010. The leaves of the plant were authenticated by Dr. Kofi Annan of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. A voucher specimen was kept in the Faculty of Pharmacy Herbarium (No. FP/079/10).

**Preparation of stem bark extracts**

The plant bark was chopped into pieces, sun dried for fourteen days and pulverized into fine powder. The powdered plant bark was serially extracted with 40-60°C petroleum ether, ethyl acetate and 70% ethanol over a 24-hour period using the Soxhlet apparatus. The extracts obtained were labelled as follows: EAE (ethanol extract), PEE (petroleum ether extract) and EthE (ethyl acetate extract). The resulting extracts were concentrated under reduced pressure at 40-60°C to a dark brown syrupy mass in a rotary evaporator. The syrupy mass was further dried using water bath and kept in a desiccator. The final yields were 9.6 % (EAE), 0.9 % (PEE), and 0.7 % (EthE).

**Phytochemical analysis**

Phytochemical analysis of the extracts was performed according to standard methods (Kokate, 2005; Tiwari *et al.*, 2011; Trease *et al.*, 1989).

**Test for Tannins and Phenolic compounds**

About 0.5 g of each of the plant extract was boiled with 25 ml of water for 5 minutes. It was then cooled, filtered and the volume adjusted to 25 ml.

**Lead acetate test**: To 1 ml aliquot of each of the extracts, 10 ml of water and 5 drops of 1% lead acetate solution was added. The formation of white precipitate indicated the presence of tannins (Kokate, 2005).

**Ferric chloride test**: To 1 ml aliquot of each of the extracts 3-4 drops of neutral 5% ferric chloride solution was added. Formation of dark green color indicated the presence of phenols (Kokate, 2005).

**Gelatin test**: To about 1 g of each of the extracts, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins (Tiwari *et al.*, 2011).

**Test for Alkaloids**

Five (5) grams of each of the extracts was stirred with 5 ml of 1% aqueous hydrochloric acid (HCl) on water bath and then filtered. Of the filtrates, 1 ml of each extract filtrates were taken into test tubes to be tested for the presence of alkaloids.

**Dragendorff’s test**: To 1 ml of each of the extracts, 1 ml of Dragendorff’s reagent (potassium bismuth
iodide solution) was added. An orange-red precipitate indicated the presence of alkaloids.

**Wagner’s test:** To 1 ml of each of the extracts, 2 ml of Wagner’s reagent (iodine in potassium iodide) was added. A reddish brown coloured precipitate indicated the presence of alkaloids.

**Test for carbohydrates**

One gram of each of the extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates (Tiwari et al., 2011).

**Benedict’s test:** 1 ml of each of the filtrates were added to 5 ml Benedict’s reagent and heated gently for 2 minutes and cooled. Orange red precipitate indicated the presence of reducing sugars.

**Fehling’s Test:** 1 ml of each of the filtrates was hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling’s A & B solutions. Formation of red precipitate indicated the presence of reducing sugars.

**Test for Phytosterols**

**Salkowski’s test:** One gram of each of the extracts were dissolved in 10 ml of chloroform and filtered. The filtrates were treated with few (3-4) drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes (Tiwari et al., 2011).

**Libermann-Burchard’s test:** One gram of each of the extracts was dissolved in few drops of chloroform, 3 ml of glacial acetic acid and 3 ml of acetic anhydride were added. This solution was warmed and cooled under running tap water. Few drops of concentrated sulphuric acid were added along the side of the test tubes. The appearance of a reddish violet colour at the junction of the two layers and a bluish green colour in the acetic acid layer indicates the presence of unsaturated sterols and/or triterpenes (Wall et al., 1954).

**Test for Flavonoids**

**Shinoda’s test:** About 1 g of each of the extracts was further dissolved with 5 ml of ethanol (98 %). To this was added a small piece of magnesium foil metal, this was followed by drop wise addition of concentrated hydrochloric acid. Intense cherry red colour indicated the presence of flavonones. Orange red colour indicated the presence of flavonols (Brain et al., 1975).

**Lead acetate test:** Few drops of lead acetate solution were added to each of the extracts in test tubes. Formation of yellow coloured precipitate indicated the presence of flavonoids (Tiwari et al., 2011).

**Test for Coumarins**

In a test tube, 1 g of each of the extracts were placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated on water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indicated the presence of coumarins (El-Tawil, 1983).

**Test for Glycosides**

Extracts were hydrolyzed with dilute HCl, and then subjected to test for glycosides.

**Keller-Killiani’s test:** One ml of each of the extracts was mixed with 5 ml of 70% alcohol for 2 minutes. This was filtered and to the filtrates was added 10 ml of water and 0.5 ml of lead acetate. This was filtered and the filtrate was shaken with 5 ml of chloroform. The chloroform layers were separated in a porcelain dish and the solvent removed by evaporation. This was cooled and dissolved in 3 ml glacial acid containing 2 drops of 5 % ferric chloride solution. The solution was carefully transferred to the surface of 2 ml concentrated sulphuric acid. A reddish brown layer formed at the junction of the two liquids and the upper layer which slowly became bluish green and darkening with standing indicated the presence of cardiac glycosides (Harborne, 1998).

**Borntrager's test:** Few drops of dilute sulphuric acid were added to 1 ml of each of the extracts. This was boiled and filtered. The filtrate was extracted with chloroform. The chloroform layer was treated with 1 ml of ammonia. The formation of red colour on the ammoniacal layer showed the presence of anthraquinone glycosides (Harbourne, 1984; Sofowora, 1993).

**Test for Saponins**

**Froth Test:** Extracts (1 g) were diluted with distilled water to 20 ml and this was shaken in a gradu-
Phytochemical & antioxidant properties of *T. monadelpha*

Ben et al.,

The extract (0.5 g portions) was shaken with 2 ml of water. Foam produced which persisted for ten minutes indicated the presence of saponins (Tiwari *et al*., 2011).

**Foam Test:** The extract (0.5 g portions) was shaken with 2 ml of water. Foam produced which persisted for 10 minutes indicated the presence of saponins (Trease *et al*., 1983).

**In vitro anti-oxidant assay**

**Total Phenolic Content**

The total soluble phenolic content of the three extracts (0.3-1 mg ml⁻¹) were quantified using the Folin-Ciocalteu’s phenol reagent (Singleton and Rossi, 1965) with tannic acid (0.01-0.1 mg ml⁻¹) as standard. The extracts (1 ml) were added to 1 ml Folin-Ciocalteu’s reagent (diluted tenfold in distilled water) in separate test tubes. The content of each test tube was mixed and allowed to stand for five minutes at 25°C in an incubator. One millilitre (1 ml) of 2% sodium carbonate solution (Na₂CO₃) was added to the mixture. This was allowed to stand for 2 hours at 25°C in an incubator and centrifuged at 1000 × g for 10 minutes to get a clear solution. The absorbance of the supernatant was then determined at 760 nm using UV mini-1240 single beam spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). Distilled water (1 ml) was added to 1 ml Folin-Ciocalteu’s reagent (diluted tenfold in distilled water) processed in the same way as done for the test samples and used as blank. All measurements were done in triplicates. The total phenolics were expressed as milligrams per milliliter of tannic acid equivalents (TAEs) through the calibration curve with tannic acid.

**Reducing power**

The reducing power of the three extracts (0.1-3 mg ml⁻¹) was determined according to the method of Oyaizu (1986), with tannic acid (0.1-3 mg ml⁻¹) as a reference antioxidant. The reference antioxidant/extract (1 ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution (K₃Fe(CN)₆) in a test tube. The mixture was incubated at 50°C for 20 minutes. Following this, 1.5 ml of 10% trichloroacetic acid solution (TCA) was added to the incubated mixture, and centrifuged at 865 × g for 10 min. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution in a test tube. The absorbance was measured at 700 nm using UV mini-1240 single beam spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). The blank was prepared by adding distilled water (1 ml) to 2.5 ml sodium phosphate buffer and 2.5 ml 1% potassium ferricyanide (K₃Fe(CN)₆) in a test tube. Three replicates were used. Results were then expressed as percentages of blank and presented as concentration-absorbance curves.

**DPPH Scavenging Activity**

The scavenging of the stable 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical is a widely used method to evaluate the free radical scavenging ability of various samples, including plant extracts (Chang *et al*., 2002). The experiment was carried out as described in literature (Blois, 1958) with a few modifications. The extracts (0.1-3 mg ml⁻¹ in methanol) were compared to n-propyl gallate (0.01-0.3 mg ml⁻¹ in methanol) as standard free radical scavenger. The extracts (1 ml) were added to 3 ml methanolic solution of DPPH (20 mg ml⁻¹) in a test tube. The reaction mixture was kept at 25°C for 1 h in an orbital shaker (BoroLabs, Aldermaston Berkshire, UK). The absorbance of the residual DPPH was determined at 517 nm in UV mini-1240 Single beam Spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). Methanol (99.8%, 1 ml) was added to 3 ml DPPH solution, incubated at 25°C for 1 h and used as control. Methanol (99.8%) was used as blank. Each experiment was carried out in triplicates. The percentage radical scavenging capacity was determined using the following formula:

\[
% \text{DPPH Scavenging} = \left( \frac{A_0 - A_e}{A_0} \right) \times 100
\]

where *A₀* is the absorbance of control (DPPH in methanol), and *Aₑ* is the absorbance of tested samples.

A graph was plotted with concentration along X-axis and % DPPH scavenging along Y-axis, and IC₅₀ value was calculated. IC₅₀ value signifies the concentration of tested samples that scavenges 50% of the DPPH radical.
Data Analysis
All experiments were conducted in triplicates, and the data are expressed as Mean ± S.E.M. Concentration responsible for 50% of the maximal effect (EC_{50}/IC_{50}) was determined by using an iterative computer least squares method, with the following non-linear regression (three-parameter logistic) equation

\[ Y = \frac{a + (b - a)}{\left(1 + 10^{(\log ED_{50} - X)}\right)} \]

Where, \(X\) is the logarithm of dose and \(Y\) is the response. \(Y\) starts at \(a\) (the bottom) and goes to \(b\) (the top) with a sigmoid shape. The fitted midpoints (ED_{50}s) of the curves were compared statistically using \(F\) test (Motulsky & Christopulos, 2003). GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA) was used for data analysis and EC_{50}/IC_{50} determinations. P<0.05 was considered statistically significant.

RESULTS
Phytochemical Analysis
Table 1 shows the phytochemical constituents of the various extracts of \(T.\) monadelpha. Alkaloids, terpenoids, phytosterols, reducing sugars and coumarins were present in PEE. EAE had tannins, alkaloids, terpenoids, phytosterols, reducing sugars, flavonoids, cardiac glycosides, anthraquinones and saponins while EthE contained tannins, alkaloids, reducing sugars, cardiac glycosides, anthraquinones, terpenoids and phytosterols.

Total Phenolic Content
Results of the assay of total phenolics in the extracts are shown in Table 2. There was a concentration-dependent increase in the total phenolics in all the extracts when expressed as tannic acid equivalents. Total phenolics were estimated to be 7.51 ± 0.87 mg tannic acid equivalent/g of petroleum ether extract, 34.14 ± 0.78 mg tannic acid equivalent/g of ethyl acetate extract and 119.30 ± 3.20 mg tannic acid equivalent/g of hydroethanol extract. EAE had the highest phenolic content and PEE the least (Figure 1).

Phytochemical & antioxidant properties of \(T.\) monadelpha
Ben et al.,

<table>
<thead>
<tr>
<th>TESTS</th>
<th>PEE</th>
<th>EthE</th>
<th>EAE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tannins and Phenolic compounds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>FeCl_{3} test</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Gelatin test</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dragendroff’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wagner’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Phytosterols/ triterpenoids</strong></td>
<td></td>
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<tr>
<td>Salkowski’s test</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Libermann-Burchard’s test</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Benedict’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Fehling’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Flavonoids</strong></td>
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</tr>
<tr>
<td>Shinoda’s test</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td><strong>Coumarins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for coumarins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cardiac glycosides</strong></td>
<td></td>
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<tr>
<td>Keller-Killiani’s test</td>
<td>-</td>
<td>+</td>
<td>++</td>
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<tr>
<td><strong>Anthraquinones</strong></td>
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<tr>
<td>Borntrager’s test</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td><strong>Saponins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frost test</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Foam test</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

\(-: Not detected, +: Present in low concentration, ++: Present in moderate concentration.)\}

Reducing power
EAE, EthE and tannic acid exhibited significant concentration-dependent reducing activity with EC_{50} values (in mg ml^{-1}) of 0.87±0.11, 13.63±0.38 and 1.04±0.26 respectively (Figure 2; Table 3). PEE, however, showed very weak reducing power (EC_{50}: 81.06±4.35; Figure 2, Table 3).

DPPH scavenging activity
The DPPH scavenging ability of the extracts are shown in figure 3. All extracts exhibited concentra-
Table 2: Total phenol content of the extracts of *T. monadelpha*, expressed as milligram tannic acid equivalent per gram of extract.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total Phenol Content (mg TAE / g of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEE</td>
<td>7.51 ± 0.87</td>
</tr>
<tr>
<td>EthE</td>
<td>34.14 ± 0.78</td>
</tr>
<tr>
<td>EAE</td>
<td>119.30 ± 3.20</td>
</tr>
</tbody>
</table>

Figure 1: Total phenols (expressed as tannic acid equivalents) present in various concentrations of PEE (0.1-1 mg ml$^{-1}$), EAE (0.1-1 mg ml$^{-1}$) and EthE (0.1-1 mg ml$^{-1}$). Each column represents mean ± S.E.M. (n= 3).

<table>
<thead>
<tr>
<th>Extract/standard</th>
<th>Reducing Power</th>
<th>F$_{1,46}$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEE</td>
<td>81.06 ± 4.35***</td>
<td>317.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EthE</td>
<td>13.63 ± 0.38***</td>
<td>300.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EAE</td>
<td>0.87 ± 0.11***</td>
<td>81.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>1.04 ± 0.26</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Values are EC$_{50}$± S.E.M ***P<0.001 compared to EC$_{50}$ of tannic acid.

Table 3: IC$_{50}$ values for extracts of *T. monadelpha* and n-propyl gallate in the DPPH assay.

<table>
<thead>
<tr>
<th>Extract/standard</th>
<th>DPPH scavenging</th>
<th>F$_{1,28}$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEE</td>
<td>0.24 ± 0.04***</td>
<td>50.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EthE</td>
<td>0.08 ± 0.01***</td>
<td>12.07</td>
<td>0.0017</td>
</tr>
<tr>
<td>EAE</td>
<td>0.04 ± 0.04ns</td>
<td>1.69</td>
<td>0.2047</td>
</tr>
<tr>
<td>n-propyl gallate</td>
<td>0.02 ± 0.01</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Values are IC$_{50}$± S.E.M ***P<0.0001; nsP>0.05 compared to IC$_{50}$ of propyl gallate.

**DISCUSSION**

Preliminary phytochemical analysis of the various extracts of *T. monadelpha* demonstrated the presence of saponins, tannins, alkaloids, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, coumarins, triterpenoids and steroidal compounds. Of these, PEE indicated the presence of alkaloids, sterols, triterpenoids, reducing sugars and coumarins. EthE contained reducing sugars, sterols, triterpenoids, tannins, alkaloids, cardiac glycosides and anthraquinones while EAE indicated the presence of all the compounds listed except coumarins. The results obtained confirm earlier reports of some of the phytochemical constituents found in extracts of *T. Monadelpha* (Ainooson et al., 2012; Woode et al., 2012). This study, however, reports for the first time, the presence of coumarins in PEE; cardiac glycosides and anthraquinones in EAE and EthE.
Figure 2: Change in (a) Absorbance and (b) maximal reducing power of the three extracts (0.1-3 mg ml$^{-1}$) of *Trichilia monadelpha* compared to tannic acid (0.1-3 mg ml$^{-1}$). Each point represents mean ± S.E.M. (n= 3).

Figure 3: Free radical scavenging ability of the extracts, PEE, EthE, EAE (0.1-3 mg ml$^{-1}$) compared to *n*-propyl gallate (0.01-0.3 mg ml$^{-1}$) in the DPPH radical assay. Each point represents the mean ± S.E.M (n = 3).

The phytochemical constituents detected in the three extracts of *T. monadelpha* could contribute to the traditional therapeutic use of the whole stem bark. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites and possess diverse biological properties including anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, inhibition of angiogenesis and cell proliferation as well as the improvement of endothelial function (Han *et al.*, 2007). Tannins have also received immense attention in many fields especially in the fields of nutrition, health and medicine due to their physiological activities (e.g. antioxidant, antimicrobial and anti-inflammatory activity)(Mota *et al.*, 1985; Lin *et al.*, 2001; Buzzini *et al.*, 2008; Koleckar *et al.*, 2008). Flavonoids, a group of polyphenolics, are free radical scavengers, super antioxidants which have anti-inflammatory activity, prevent oxidative cell damage through their water soluble property and also possess strong anti-cancer activity (Gurib-Fakim, 2006; Salah *et al.*, 1995). Coumarins are potential antioxidants, according to stud-
ies (Tseng, 1991; Kostova, 2006; Kostova et al., 2011), with the ability of scavenging free radicals and chelating metal ions. Triterpenoids possess analgesic and anti-inflammatory properties (Savithramma et al., 2012). More research is required to determine the specific roles of these phytochemical constituents present in Trichilia monadelpha.

The reducing power and DPPH scavenging tests conducted in this study sought to establish the \textit{in vitro} antioxidant properties of the plant extracts. The detection of phenolic compounds, particularly in EAE and EthE, strongly suggests possible antioxidant activity of the extracts. Phenolic antioxidants are potent free radical terminators (Shahidi et al., 1992). The high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups (Sawa et al., 1999).

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential (Meir et al., 1995) of a substance. From the results, there was significant, concentration-dependent Fe$^{3+}$ reducing activity by EAE and EthE compared with tannic acid. The findings further affirm the antioxidant activity of the extracts.

The DPPH test is widely used as measure for the electron donation capacity of the antioxidant under the assay conditions. DPPH (2, 2-diphenyl-1-picryl hydrazyl) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH radical scavenging ability of EAE was significantly as potent as that of the standard, $n$-propyl gallate, but PEE and EthE were less effective in scavenging the DPPH radical. The DPPH scavenging ability of EAE shows the extract could serve as a free radical inhibitor or scavenger.

From the activities shown by the extracts, particularly EAE, in the antioxidant tests conducted, it is clear that extracts have antioxidant activity. The mechanism of antioxidant activity may be due to the reduction of free radicals as well as scavenging of reactive oxygen species and other free radicals. EAE had the highest amounts of phenolics and hence it is not surprising that it also exhibited most reducing power and free radical scavenging ability. The antioxidant activities observed in this study could account, partly, for the anti-inflammatory effect observed in an earlier study on \textit{T. monadelpha} (Ainooson et al., 2012) since a large pool of evidence implicates free radicals in the process of inflammation (Closa et al., 2004; Conner et al., 1996; Reuter et al., 2010). Further studies are required to clarify the \textit{in vivo} potential of \textit{T. monadelpha} in the management of human diseases resulting from oxidative stress.

**CONCLUSION**

The current study has shown that the petroleum ether, ethyl acetate and ethanol extracts of the stem bark of \textit{Trichilia monadelpha} contain saponins, tannins, alkaloids, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, coumarins, triterpenoids and steroidal compounds. The extracts also possess antioxidant and radical scavenging properties \textit{in vitro}.

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**COMPETING INTERESTS**

The authors declare that they have no competing interests.

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