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

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ORIGINAL ARTICLE

Preliminary Phytochemical Screening and *In vitro* Antioxidant Properties of *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 Trichilia monadelpha (Thonn) JJ De Wilde (Family: Meliaceae). Petroleum ether (PEE), ethyl acetate (EthE) and ethanol extracts (EAE) of the stem bark of T. monadelpha were screened for the presence of phytochemicals. The 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 suggests that the stem bark of Trichilia monadelpha may be a good source of natural antioxidants and might be useful in treating the diseases associated with oxidative stress.

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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 *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 *et al.*, 2007). Plants are en-

Correspondence: Prof. E. Woode, Department of Pharmacology, CHS, KNUST, Kumasi. Ghana. E-mail: <u>ewoode.pharm @knust.edu.gh</u>, <u>ericwoode@yahoo.com</u>, Tel. No.: +233 244 589793 dowed 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.

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

(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) while2, 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 (\geq 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 colour 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.

Dragendroff's test: To 1 ml of each of the extracts, 1 ml of Dragendroff'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 carbo-hydrates (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

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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-

ated cylinder for 15 minutes. Formation of 1 cm layer of foam 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 ten 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 $\times g$ 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 ten-fold 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 mgl-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:

% DPPH Scavenging = $[(A_0-A_s)/A_0] \ge 100$ where A_0 is the absorbance of control (DPPH in methanol), and A_s is the absorbance of tested samples.

A graph was plotted with concentration along Xaxis 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 \pm S.E.M. Concentration responsible for 50% of the maximal effect (EC₅₀/IC₅₀) was determined by using an iterative computer least squares method, with the following non-linear regression (three-parameter logistic) equation

$$Y = \frac{a + (b - a)}{(1 + 10^{(LogED_{50} - X)})}$$

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 (ED₅₀s) 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 EC₅₀/IC₅₀determinations. 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 whiles 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 concentrationdependent 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).

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Table 1: Phytochemical constituents of stem b	ark
extract of T. monadelpha	

TESTS	PEE	EthE	EAE
Tannins and Phenolic			
compounds			
Lead acetate test	-	+	++
FeCl ₃ test	-	-	++
Gelatin test	-	+	++
Alkaloids			
Dragendroff's test	+	+	+
Wagner's test	+	+	+
Phytosterols/			
triterpenoids			
Salkowski's test	++	++	+
Libermann-	++	++	+
Burchard's test			
Carbohydrates			
Benedict's test	+	+	+
Fehling's test	+	+	+
Flavonoids			
Shinoda's test	-	-	++
Lead acetate test	-	-	++
Coumarins			
Test for coumarins	+	-	-
Cardiac glycosides			
Keller-Killiani's test	-	+	++
Anthraquinones			
Borntrager's test	-	+	++
Saponins			
Frost test	-	-	++
Foam test	-	-	++

-: 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-

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Table 2: Total phenol content of the extracts of *T. monadelpha*, expressed as milligram tannic acid equivalent per gram of extract.

Extract	Total Phenol Content (mg TAE / g of extract)	
PEE	7.51 ± 0.87	
EthE	34.14 ± 0.78	
EAE	119.30 ± 3.20	



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 \pm S.E.M. (n= 3).

tion-dependent scavenging activity in a similar manner to the reference antioxidant, n-propyl gallate (figure 3). The IC₅₀ correlates directly with the effectiveness of the substrate/extract to scavenge the DPPH radical. The IC₅₀ values (in mg ml⁻¹) obtained (table 4) suggests PEE has the least ability to scavenge free radicals compared to *n*-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, couma-

Table 3: EC_{50} values for extracts of *T. monadel-pha* and tannic acid in the reducing power assay.

Extract/ standard	Reducing Power	F 1,46	P value
PEE	81.06 ± 4.35***	317.10	< 0.0001
EthE	13.63 ± 0.38***	300.20	< 0.0001
EAE	$0.87 \pm 0.11^{***}$	81.21	< 0.0001
Tannic acid	1.04 ± 0.26	-	

Values are $EC_{50} \pm S.E.M ***P < 0.001$ compared to EC_{50} of tannic acid.

Table 4: IC_{50} values for extracts of *T. monadel-pha* and *n*-propyl gallate in the DPPH assay.

Extract/ standard	DPPH scaveng- ing	F1,28	P value
PEE	$0.24 \pm 0.04^{***}$	50.21	< 0.0001
EthE	$0.08 \pm 0.01^{***}$	12.07	0.0017
EAE	$0.04 \pm 0.04^{\text{ns}}$	1.69	0.2047
n-propyl gallate	0.02 ± 0.01	-	

Values are $IC_{50} \pm S.E.M ***P < 0.0001$; nsP>0.05 compared to IC_{50} of propyl gallate.

rins, 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 \pm 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 \pm 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-apotosis, anti-aging, anti-carcinogen, antiinflammation, 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 *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 Fe³⁺ 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 *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 *in vivo* potential of *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 *Trichlia monadelpha* containsaponins, tannins, alkaloids, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, coumarins, triterpenoids and steroidal compounds. The extracts also possess antioxidant and radical scavenging properties *in vitro*.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

- Abbiw DK (1990). Useful Plants of Ghana. edn. Intermediate Technology Publications and Royal Botanic Gardens Kew.
- Ainooson GK, Owusu G, Woode E, Ansah C, Annan K (2012). Trichilia monadelpha Bark Extracts Inhibit Carrageenan-Induced Foot-Oedema in the 7-Day Old Chick and the Oedema Associated with Adjuvant-Induced Arthritis in Rats. *Afr. J. Tradit. Complement. Altern. Med.***9**(1): 8-16.
- Blois MS (1958). Antioxidant determinations by the use of a stable free radical. *Nature***181:** 1199-1200.
- Brain KR, Turner TD (1975). The practical evaluation

of phytochemicals. . Wright Science Technical: Bristol.

- Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, Ieri F., Romani A. (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini Rev. Med. Chem.*8(12): 1179-1187.
- Chang C-C, Yang M-H, Wen H-M, Chern J-C (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analysis***10**(3): 178-182.
- Closa D, Folch-Puy E (2004). Oxygen free radicals and the systemic inflammatory response. *IUBMB Life***56**(4): 185-191.
- Conner EM, Grisham MB (1996). Inflammation, free radicals, and antioxidants. *Nutrition***12**(4): 274-277.
- Dasgupta N, De B (2007). Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chemistry***101**(2): 471-474.
- Dokosi OB (1998). *Herbs Of Ghana*. 1 edn. Ghana Universities Press: Accra.
- El-Tawil BDH (1983). Chemical constituents of indigenous plants used in Native Medicines of Saudi Arabia, II. *Arab Gulf J Sci Res*A1(12): 395 -410.
- Gurib-Fakim A (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.***27**(1): 1-93.
- Halliwell B (2011). Free radicals and antioxidants quo vadis? *Trends Pharmacol. Sci.***32**(3): 125-130.
- Halliwell B (2012). Free radicals and antioxidants: updating a personal view. *Nutr. Rev.***70**(5): 257 -265.
- Halliwell B, Gutteridge JM, Cross CE (1992). Free radicals, antioxidants, and human disease: where are we now? J. Lab. Clin. Med.119(6): 598-620.
- Han X, Shen T, Lou H (2007). Dietary Polyphenols and Their Biological Significance. *Int. J. Mol. Sci***8**(9): 950-988.
- Harborne JB (1998). *Phytochemical Methods*—A Guide to Modern Techniques of Plant Analysis. 1stedn: Chapman and Hall; London, UK.
- Harbourne JB (1984). Phytochemical Methods: A

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> Guide to Modern Technique of Plant Analysis, 2nd ed. Chapman & Hall, London. 282.

- Kamanzi Atindehou K, Schmid C, Brun R, Kone MW, Traore D (2004). Antitrypanosomal and antiplasmodial activity of medicinal plants from Cote d'Ivoire. J. Ethnopharmacol.90(2-3): 221-227.
- Kokate CK (2005). A Textbook for Practical Pharmacognosy. 5th Ed edn.
- Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, Opletal, L. (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini Rev. Med. Chem.*8(5): 436-447.
- Kostova I, Bhatia S, Grigorov P, Balkansky S, Parmar VS, Prasad AK, Saso L. (2011). Coumarins as antioxidants. *Curr Med Chem*,18(25), 3929-3951.
- Kostova I. (2006). Synthetic and natural coumarins as antioxidants. *Mini Rev Med Chem*, 6(4), 365 -374.
- Lin CC, Hsu YF, Lin TC (2001). Antioxidant and free radical scavenging effects of the tannins of Terminalia catappa L. *Anticancer Res.***21** (1A): 237-243.
- Mota ML, Thomas G, Barbosa Filho JM (1985). Anti-inflammatory actions of tannins isolated from the bark of Anacardium occidentale L. J. Ethnopharmacol.13(3): 289-300.
- Meir S, Kanner J, Akiri B, Hadas SP (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric.Food Chem.43: 1813.
- Oyaizu M (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **44:** 307–315
- Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010). Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic. Biol. Med.***49**(11): 1603-1616.
- Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch.Biochem. Biophys.***322**(2): 339-346.

- Savithramma N, M. LR, Ankanna S (2012). Preliminary Phytochemical Analysis of Traditionally used Medicinal Plants. *Res. J. Pharm. Bol. Chem. Sci.***3**(1): 308-314.
- Sawa T, Nakao M, Akaike T, Ono K, Maeda H (1999). Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumorpromoter effect of vegetables. J. Agric. Food Chem.47(2): 397-402.
- Shahidi F, Wanasundara PK (1992). Phenolic antioxidants. Crit. Rev. Food Sci. Nutr.32(1): 67-103.
- Singleton V.L., Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *Am. J. Enol. Vitic.***16:** 144-158.

Sofowora A (1993). Phytochemical screening of me-

dicinal plants and traditional medicine in Africa 2nd Edition Spectrum Books Limited, Nigeria; 150-156.

- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia***1**(1): 98-106.
- Trease GE, Evans WC (1989). Textbook of Pharmacognosy. 12th ed. edn: Balliere; Tindall, London, UK
- Tseng A (1991). Proc. Am. Assoc. Cancer Res32: 2257.
- Woode E, Amoh-Barimah AK, Abotsi WK, Ainooson GK, Owusu G (2012). Analgesic effects of stem bark extracts of Trichilia monadelpha (Thonn.) JJ De Wilde. *Indian J.Pharmacol.*44(6): 765-773.



