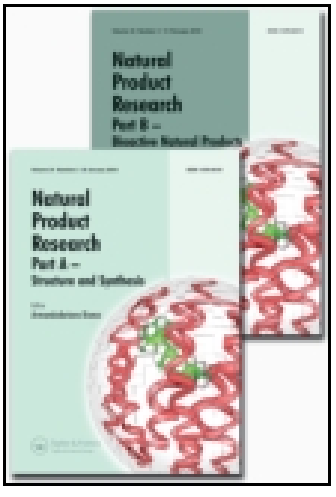

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Phenolic contents, antioxidant and antibacterial activities of Hymenocardia acida

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Phenolic contents, antioxidant and antibacterial activities of *Hymenocardia acida*

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This study investigates the antioxidant and antibacterial activities of aqueous and methanolic extracts from *Hymenocardia acida* Tul. (Hymenocardiaceae). The inhibition values of the extracts and quercetin were found to be very close, with no significant differences at a concentration of $0.05\,mg\,mL^{-1}$ in their ability to inhibit 1,1-diphenyl-2-picrylhydrazyl (DPPH). Total proanthocyanidins for both water and methanol extracts were 20.2 ± 0.01 and $30.6 \pm 0.51\,mg\,g^{-1}$ (catechin equivalent) while the total phenol contents were 20.0 ± 0.52 and $35.6 \pm 1.42\,mg\,mL^{-1}$ (tannic acid equivalent), respectively. Positive correlations $R^2 = 0.85$, $R^2 = 0.94$, $R^2 = 0.97$ for DPPH, reducing power and 2′-azino-bis(3-ethylbenzo thiazoline)6-sulphonic acid (ABTS). Linear regression analysis also produced a high correlation coefficient with total proanthocyanidins (DPPH, $R^2 = 0.69$; ABTS, $R^2 = 0.94$). *H. acida* extracts showed low antibacterial activity (minimum inhibitory concentration (MIC) value $\geq 5.0\,mg\,mL^{-1}$) against gram negative bacteria but significantly (MIC value $\leq 2.5\,mg\,mL^{-1}$) inhibited the growth of the gram positive strains tested. Qualitative TLC of the extract was positive for flavonoids, phenols, steroids and triterpenoids.

The results of this study support the use of *H. acida* in traditional Nigerian medicine and show that the alcoholic extract of the leaves can be used as an easily accessible source of natural antioxidant and can be of assistance in some dermatological problems.

Keywords: *Hymenocardia acida*; antioxidant activity; antibacterial activity; polyphenols

1. Introduction

Medicinal plants contain a variety of antioxidants; prominent among these are phenolic compounds (Kang, Yu, & Lee, 2003). Various phenolic antioxidants, such as flavonoids, tannins, coumarins, xanthones and more recently, procyanidins, scavenge radicals dose-dependently; thus they are viewed as promising therapeutic drugs for free radical pathologies (Lee, Kim, Hong, Kang, & Kim, 2000). Studies have also shown that many

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dietary polyphenolic constituents, derived from plants, are more effective antioxidants *in vitro* than vitamin E or C, and thus might contribute significantly to the protective effects *in vivo* (Catherine, Rice-Evans, & Georg, 1997; Rene, Yolanda, & Zeno, 2001). Attention has also been paid to the antimicrobial activity of phenolics, but no dramatic evidence of their effectiveness has been reported (Rauha et al., 2000).

Hymenocardia acida Tul. (Hymenocardiaceae) is a small tree of about 6 m high, gnarled and twisted with characteristic rough, rusty-red bark. It is widespread in tropical Africa (Burkhill, 1994). Ethnopharmacological studies of *H. acida* revealed an extensive array of medicinal uses, particularly from tropical African countries. In Senegal and Ivory Coast, an infusion or decoction of its leaves is used for the treatment of chest complaints, small pox, in baths and draughts as a febrifuge, and is taken as snuff for headaches or applied topically for rheumatic pains and toothaches. The bark and leaves are prescribed together in various ways in Nigeria for abdominal and menstrual pains and as poultices on abscesses and tumours. The powdered leaves of this tree are also used for the treatment of arthritis (Burkhill, 1994). Pharmacological activities reported on the plant include antiulcer (Ukwe, 1997), antitrypanosomal (Hoet, Opperdoes, Brun, Adjakidje, & Quetin-Leclercq, 2004), antitumour and anti-HIV (Muanza, Eule, Williams, & Newman, 1995) and antiplasmodial and cytotoxic (Vonthron-Senecheau et al., 2003). Silva et al. (1996) also reported the antimicrobial property of the root extract of this plant. Despite the widespread ethnomedical data reported on this plant, no information is available on the antioxidant and antibacterial properties of its leaves. Yet these are necessary for the proper and scientific evaluation of the medicinal potential of *H. acida*. This study was aimed at investigating the phenolic contents, antioxidant and antibacterial properties of the plant, with a view to relating its phenolic content to its medicinal values.

2. Materials and methods

2.1. *Plant material*

The leaves of *H. acida* were collected in November 2004 at Olokemeji, Oyo State, Nigeria (0.722N, 03.58E). The plant was authenticated at the Forestry Research Institute of Nigeria, Ibadan. Voucher specimens were prepared and deposited at the herbaria of both the Institute and the Pharmacognosy Department, University of Lagos, Nigeria.

2.2. *Preparation of extracts*

The leaves were air dried at room temperature for 21 days, pulverised, and a portion (250 g) of the dried material was extracted with 2.5 L of water on a shaker overnight at room temperature. The extract was filtered through Whatman (No. 1) filter paper and the filtrate concentrated in a lyophiliser. The residue was extracted with methanol (1.0 L) and filtered through Whatman filter paper. The filtrate was evaporated to dryness at 40°C *in vacuo*. The yields of the aqueous and methanol extracts were 28.6 and 18.3 g, respectively.

2.3. *Antioxidant assays*

2.3.1. *Chemicals*

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzo thiazoline)6-sulphonic acid (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulphonic acid, potassium

ferricyanide, catechin, ascorbic acid, catechin, tannic acid, quercetin and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA), vanillin from BDH (Poole, UK), Folin–Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All the other chemicals used, including the solvents, were of analytical grade.

2.3.2. *TLC analysis and rapid screening for antioxidants*

The chemical constituents of the extracts were identified qualitatively using TLC. The extract showed a positive Shibata test for flavonoids, positive ferric chloride test for phenols, Liebermann-Burchard reaction for steroids and a positive Noller test for triterpenoids (Harborne, 1992). These results were confirmed by silica gel TLC using ethylacetate : methanol : water (8 : 1 : 1) as the solvent system. Visualisation was done under UV 254 and 366. Spots on the developed plate were also observed for antioxidant activity using 0.2% DPPH in methanol. Antioxidant compounds showed clear zones against a purple background (Cuendent, Hostettman, Potterat, & Dyatmiko, 1997).

2.3.3. *Determination of DPPH radical scavenging ability*

The effect of the extracts on DPPH radical was estimated adopting the method of Liyana-Pathirana and Shahidi (Liyana-Pathiranan & Chide, 2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of the extract in methanol, containing 0.005–0.05 mg mL^{-1} of extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercetin was used as the standard. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity } (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/(\text{Abs}_{\text{control}})] \times 100$$

where $\text{Abs}_{\text{control}}$ was the absorbance of DPPH radical + methanol, $\text{Abs}_{\text{sample}}$ was the absorbance of DPPH radical + sample extract/standard.

2.3.4. *Determination of the reducing power of the extract*

The reducing capacity of the extract was determined by the method of Oyaizu (1986). Varying concentrations of the extract in methanol (0.005–0.05 mg mL^{-1}) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%), and incubated at 50°C for 20 min. Aliquots (2.5 mL) of 10% trichloroacetic acid were added to the reaction mixture, which was then centrifuged at 1000 g for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). The absorbance was measured at 700 nm.

2.3.5. *ABTS radical cation scavenging activity*

The free radical scavenging activity of the extracts was determined by ABTS radical cation decolourisation assay (Re et al., 1999). It involved the generation of $ABTS^+$ chromophore by the oxidation of ABTS with potassium persulphate. The $ABTS^+$ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulphate after incubation at room temperature in the dark for 16 h. The solution was then diluted by mixing 1 mL $ABTS^+$ solution with 60 mL methanol to obtain an absorbance of 0.7074 ± 0.001 at 734 nm using the spectrophotometer. The reactive mixture (1 mL of extract and 1 mL of $ABTS^+$)

was allowed to stand at room temperature for 6 min and the absorbance was immediately recorded at 734 nm. Quercetin standard solution was prepared and assayed using the same conditions.

2.3.6. *Determination of total flavonols*

The total flavonols were determined according to the method of Kumaran and Karunakaran (2007). The rutin calibration curve was prepared by mixing 2 mL of 0.4, 0.2, 0.1 0.05 and 0.25 mg mL^{-1} ethanolic solutions of rutin with 2 mL (20 g L^{-1}) aluminum trichloride and 6 mL (50 g L^{-1}) sodium acetate. The absorption at 440 nm was read after 2.5 h at 20°C. The same procedure was carried out with 2 mL of plant extract (10 g L^{-1}) instead of rutin solution. All determinations were carried out in triplicate. The content of flavonols, in rutin equivalents, was calculated by the following equation: $X = CV/m$, where X is the flavonol content, in milligrams per gram of plant extract; C is the concentration of rutin solution, established from the calibration curve, in milligrams per millilitre; and V and m are the volume and the weight of the plant extract, in millilitres and grams, respectively.

2.3.7. *Determination of total flavonoids*

Total flavonoids were estimated according to Ordonez, Gomez, Vattuone and lsla (2006). To 0.5 mL of sample, 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After 60 min at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

2.3.8. *Determination of total proanthocyanidins*

The determination of proanthocyanidin was done by adopting the procedure of Sun et al. (1998). An extract of 0.5 mL of 50 mg mL^{-1} was mixed in 3 mL of 4% vanillin–methanol solution and 1.5 mL hydrochloric acid, and the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm and the result expressed as catechin equivalent.

2.3.9. *Determination of total phenolic contents*

Total phenol contents in the extract were determined by the modified Folin–Ciocalteu method of Wolfe, Wu and Liu (2003). An aliquot of the extract was mixed with 5 mL of Folin–Ciocalteu reagent (previously diluted with water 1 : 10 v/v) and 4 mL (75 g L^{-1}) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for colour development. Absorbance was measured at 765 nm using a Hewlett Packard UV–VS spectrophotometer. The amount of total phenolics was calculated as tannic acid equivalents (TAE (milligram)) from the calibration curve. The experiment was replicated thrice.

2.4. *Antibacterial testing*

Laboratory isolates of 10 bacterial species, which included five gram positive and five gram negative strains, were obtained from the Department of Microbiology, Rhodes University. They were *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*,

Micrococcus kristinae, *Streptococcus pyrogens*, *Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. They were maintained on nutrient agar slants and recovered by culturing in nutrient broth (Biolab No. 2) for 24 h. Before use, each bacterial culture was diluted 1 : 100 with fresh sterile nutrient broth (Grierson & Afolayan, 1999). The bacteria were streaked in radial patterns on the agar plates, incubated at 37°C and examined after 24 and 48 h; complete suppression of growth by a specific concentration of the extract was required to be declared active. The extract was tested at 2.0, 1.0, 0.5 and 0.1 mg mL^{-1}. Blank plates containing only nutrient agar and another set containing nutrient and 2% methanol served as controls (Zheng & Wang, 2001).

2.5. *Statistical analysis*

Where applicable, the data were subjected to a one-way analysis of variance and the significance of the difference between the means was determined by Duncan's multiple range test ($p < 0.05$).

3. Results and discussion

3.1. *TLC analysis and rapid screening for antioxidant*

The extract showed a positive Shibata test for flavonoids, ferric chloride test for phenols, Liebermann-Burchard reaction for steroids and a positive Noller test for triterpenoids. Five spots with the same R_f were detected in both water and methanol extracts after spraying with 1% vanillin HCl and 5% $FeCl_3$. This was an indication that some compounds were extracted by both water and methanol. DPPH spray detected four potential radical scavenging compounds. The aqueous fraction was, however, devoid of some compounds present in the methanol extract. Hence, methanol appeared to be a better solvent of extraction, especially for antioxidant assay.

3.2. *Total flavonol, flavonoid, phenol and proanthocyanidins*

Total flavanol, flavonoid, phenol and proanthocyanidin contents in the extracts are summarised in Table 1. The percentage composition of proanthocyanidin in the extracts was much higher than the flavonoid and flavonol (Table 1). This might make a major contribution to the observed antioxidant activity of the extracts. A similar contribution of tannins to the antioxidant property of Rosaceae species was earlier reported by

Table 1. Total phenolics, flavonoids, proanthocyanidins and flavanol of *Hymenocardia acida* extracts.

Components	Water extract	Methanol extract
Total phenol [mg (tannic acid g^{-1})]	20.0 ± 0.52	35.6 ± 1.42
Total flavonoids [mg (quercetin g^{-1})]	1.6 ± 0.10	5.4 ± 0.21
Total proanthocyanidins [mg (catechin g^{-1})]	20.2 ± 0.01	30.9 ± 0.51
Total flavanol [mg (quercetin g^{-1})]	1.1 ± 0.01	3.7 ± 0.13

Note: Values are means of three replicates ± SD.

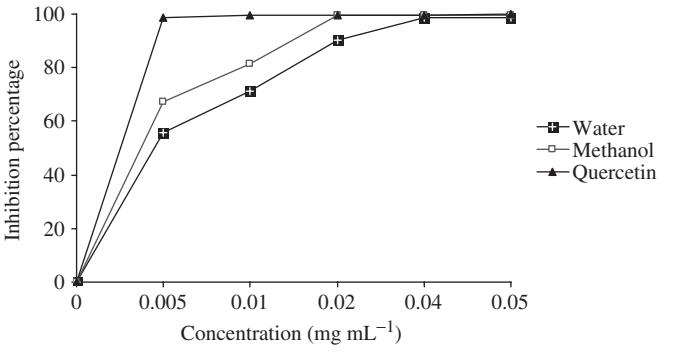

Figure 1. $ABTS^{+}$ scavenging effects of methanol and water extracts of *Hymenocardia acida*.

Santos-Beulga and Scalbert (2000) and Sun, Chu, Wu and Liu (2002). Proanthocyanidins are of great interest in nutrition and medicine because of their antioxidant activity and possible protective effects on human health (Bagchi et al., 2000). It has been hypothesised that the free radical scavenging properties of proanthocyanidins may reduce the risk of cardiovascular diseases and cancer (Wang & Lin, 2002). The total phenolic compounds in methanol extract from *H. acida* leaves were higher than in the water extract. These values are higher when compared with other total phenol contents reported in some vegetables (Amarowicz, Pegg, Rahini-Moghaddam, Barl, & Weil, 2004). However, the total flavonoid and flavonol contents of the extracts were relatively low when compared to some medicinal plants (Ligangli, Scott, Jonathan, John, & Minq, 2002).

3.3. *Antioxidant activities*

A steady increase in the ABTS radical scavenging capacity of the extracts was observed up to a concentration of $0.02\,mg\,mL^{-1}$, followed by a relatively low increase, with further increase in concentration (Figure 1). The scavenging effect of the extracts and the standard on ABTS radical decreased in the order of quercetin > methanol > water and were 99, 69 and 56% at the concentration of $0.005\,mg\,mL^{-1}$, respectively. Higher concentrations of the extracts were more effective in quenching free radicals in the system (Yen & Chen, 1995).

In DPPH assay, the radical scavenging activities of the extracts were determined from the reduction in the optical density of DPPH free radical at 517 nm. There was a significant decrease in the concentration of DPPH, with increases in the concentration of the extracts (Figure 2). The antioxidant activity of the samples was in the decreasing order of quercetin > methanol > water extract. At $0.02\,mg\,mL^{-1}$, quercetin, methanol and water extracts of *H. acida* exhibited 93.4, 85.5 and 55.4% scavenging activity, respectively. There was no significant difference in scavenging activity between the extracts and quercetin at $0.05\,mg\,mL^{-1}$. Figure 3 shows the reductive capabilities of the extracts compared with quercetin and ascorbic acid. Like the free radical scavenging activity, the reductive capacity of both extracts increased with the concentration. There was no significant difference ($p < 0.01$) between the reducing power of the methanol extract and ascorbic acid. However, the activity of quercetin was found to be significantly more pronounced than

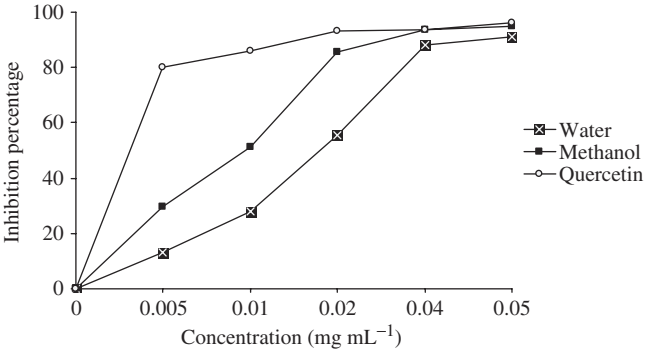

Figure 2. DPPH radical scavenging capacity of methanol and water extracts of *Hymenocardia acida*.

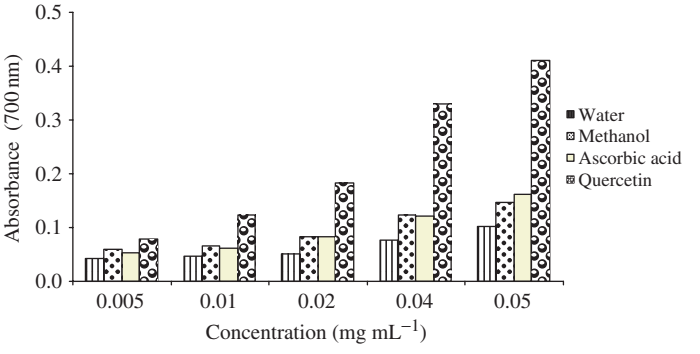

Figure 3. Reducing power of methanol and water extracts of *Hymenocardia acida*.

ascorbic acid, methanol and water extracts. These results revealed that the extracts of *H. acida* were electron donors and could react with free radicals, converting them to more stable products and terminating the radical chain reaction (Oktay, Gulcin, & Kufrevioglu, 2003). The antioxidant activity trend obtained in this result corresponds directly with the content of total phenolics in the extracts, with a correlation coefficient of $r = 0.85$, $r = 0.94$, $r = 0.97$ for DPPH, reducing power and ABTS, respectively. Linear regression analysis also produced a high correlation coefficient with content in total proanthocyanidins (DPPH, $r = 0.69$; ABTS, $r = 0.94$). Thus, it is apparent that there is a strong relation between total phenolic content and antioxidants (Duffy & Power, 2001; Sofidiya, Odukoya, Familoni, & Inya-Agha, 2006).

3.4. *Antibacterial activity*

The antibacterial activity of *H. acida* against some selected gram negative and gram positive bacteria has revealed that the methanol extract exhibited stronger and broader activity than the water extract against 8 out of the 10 bacteria strains used in the assay (Table 2). Greater and more remarkable activity was recorded though, for the two extracts, against *E. coli*, *Streptococcus pyogenes* and *B. cereus*. The activity of the

Table 2. Antibacterial activity of methanol and water extracts of *Hymenocardia acida*.

Bacterial species	Gram positive/ negative	MIC ($mg\,mL^{-1}$)			
		Methanol	Water	Chloramphenicol ($\mu g\,mL^{-1}$)	Streptomycin sulphate ($\mu g\,mL^{-1}$)
B. cereus	+	0.5	1.0	<2	<2
S. epidermidis	+	2.5	na	<2	<2
S. aureus	+	2.5	2.5	<2	<2
M. kristinae	+	2.5	1.0	<0.2	<2
S. pyrogens	+	0.5	1.0	<2	<2
E. coli	−	0.5	1.0	<2	<2
S. pooni	−	5.0	na	<2	<2
S. marcescens	−	5.0	na	<2	<2
P. aeruginosa	−	na	na	<2	<5
K. pneumoniae	−	na	na	<2	<2

Note: na represents not active.

extracts was found to be higher on gram positive bacteria than the gram negative strains. Two of the test organisms, *P. aeruginosa* and *K. pneumoniae*, were found to be resistant to the extracts. The generally low activity of the extracts against the gram negative organisms may be due to the fact that gram negative bacteria possess an outer membrane and a periplasmic space, both of which are absent in gram positive bacteria (Cowan, 1999). The outer membrane of gram negative bacteria is known to present a barrier to the penetration of numerous antibiotic molecules. In addition, the periplasmic space contains enzymes which are capable of breaking down foreign molecules introduced from outside.

In general, the difference of activity demonstrated by *H. acida* leaf extracts appears to be directly related to the qualitative and/or quantitative diversity of the compounds that are present in the extracts. It is believed that plants which are rich in a wide variety of secondary metabolites belong to chemical classes such as tannins and terpenoids. This suggests that the strength of the biological activities of a natural product is dependent on the diversity and quantity of such constituents. Since a significant correlation was obtained between the total phenolics in the extracts, we believe that the antioxidant activity shown by the extracts is probably due to the presence of phenolic compounds. Our results are in agreement with those that reported the ability of phenolic compounds to scavenge free radicals and active oxygen species (Duh, Tu, & Ye, 1999; Hatano, Edamatsu, Mori, Fujita, & Yasuhara, 1989) and to show antimicrobial activity (Behera, Verma, Sonon, & Makhija, 2005; Rauha et al., 2000). Hence, the bioactive molecules responsible for these activities are being given attention in our laboratory. The results of this study support, therefore, the use of *H. acida* in traditional Nigerian medicine and show that methanol extract of the leaves of this plant can be used as an easily accessible source of natural antioxidants and in addressing some dermatological problems.

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References

Amarowicz, R., Pegg, R.B., Rahini-Moghaddam, P., Barl, B., & Weil, J.A. (2004). Free radical scavenging capacity and antioxidant activity of selected plant species from Canadian prairies. *Food Chemistry, 84*, 551–562.

Bagchi, D., Bagchi, M., Stohs, S.J., Das, D.K., Ray, S.D., & Kuszynski, C.A. (2000). Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. *Toxicology, 148*, 187–197.

Behera, B.C., Verma, N., Sonon, A., & Makhija, U. (2005). Antioxidant and antibacterial activities of lichen *Usnea ghattensis in vitro*. *Biotechnology Letters, 27*, 991–995.

Burkhill, H.M. (1994). *Useful plants of West Tropical Africa* (Vol. 2, p. 85). Kew, England: Families E-I. Royal Botanical Gardens.

Catherine, A., Rice-Evans, N.J.M., & Georg, P. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*, 564–582.

Cuendent, M., Hostettman, K., Potterat, O., & Dyatmiko, W. (1997). Iridioid glucosides with free radical scavenging properties from *Fragraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1151.

Duffy, C.F., & Power, R.F. (2001). Antioxidant and antimicrobial properties of some Chinese plant extracts. *International Journal of Antimicrobial Agents, 17*, 527–529.

Duh, P.D., Tu, Y.Y., & Ye, G.C. (1999). Antioxidant activity of aqueous extract of harn jyur (*Chyrsanthemum morifolium* Ramat). *Lebensmittel-Wissenschaft Technologie, 32*, 269–277.

Grierson, D.S., & Afolayan, A.J. (1999). Antibacterial activity of some indigenous plants used for the treatment of wound in the Eastern Cape, South Africa. *Journal of Ethnopharmacology, 66*, 103–106.

Harborne, J.B. (1992). *Phytochemical methods*. London: Chapman and Hall publications.

Hatano, T., Edamatsu, R., Mori, A., Fujita, Y., & Yasuhara, E. (1989). Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chemical and Pharmaceutical Bulletin, 37*, 2016–2021.

Hoet, S., Opperdoes, F., Brun, R., Adjakidje, V., & Quetin-Leclercq, J. (2004). *In vitro* antitrypanosomal activity of ethnopharmacologically selected Beninese plants. *Journal of Ethnopharmacology*, *91*, 37–42.

Kang, D.G., Yu, C.K., & Lee, H.S. (2003). Screening and comparison of antioxidant activity of solvent extracts of herbal medicines used in Korea. *Journal of Ethnopharmacology, 87*, 231–236.

Kumaran, A., & Karunakaran, R.J. (2007). Screening and comparison of antioxidant activity of solvent extracts of herbal medicines used in Korea. *Lebensmittel-Wissenschaft Technologie, 40*, 344–352.

Lee, Y.M., Kim, H., Hong, E.K., Kang, B.H., & Kim, S.J. (2000). Water extract of 1:1 mixture of *Phellodendron cortex* and *Aralia cortex* as inhibitory effects on oxidative stress in kidney of diabetic rats. *Journal of Ethnopharmacology, 73*, 429–436.

Ligangli, Y., Scott, H., Jonathan, M., John, W., & Minq, Q. (2002). Free radical scavenging properties of wheat extracts. *Journal of Agricultural Food Chemistry, 50*, 1619–1624.

Liyana-Pathiranan, C.M., & Chide, F. (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L) as affected by gastric pH conditions. *Journal of Agricultural and Food Chemistry, 53*, 24–33.

Muanza, D.N., Eule, K.L., Williams, L., & Newman, D.J. (1995). Screening for antitumor and HIV activities of nine medicinal plants from Zaire. *International Journal of Pharmacognosy, 33*, 98–106.

Oktay, M., Gulcin, I., & Kufrevioglu, O.I. (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Lebensmittel-Wissenschaft Technologie, 36*, 263–271.

Ordonez, A.A.L., Gomez, V., Vattuone, M.A., & lsla, M.I. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chemistry, 97*, 452–458.

Oyaizu, M. (1986). Studies on products of browning reaction prepared from glucoseamine. *Japanese Journal of Nutrition, 44*, 307–315.

Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., et al. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology, 56*, 3–12.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C.A. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*, 1231–1237.

Rene, D.T., Yolanda, V., & Zeno, A. (2001). Comparison of the antioxidant content of fruits, vegetables and tea measured as vitamin C equivalents. *Toxicology, 166*, 63–69.

Santos-Beulga, C., & Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds-nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture, 80*, 1094–1117.

Silva, O., Duarte, A., Cabrita, J., Pimentel, M., Diniz, A., & Gomes, E. (1996). Antimicrobial activity of Guinea-Bissau traditional remedies. *Journal of Ethnopharmacology, 50*, 55–59.

Sofidiya, M.O., Odukoya, O.A., Familoni, O.B., & Inya-Agha, S.I. (2006). Radical scavenging activity of some Nigerian medicinal plant extracts. *Pakistan Journal of Biological Sciences, 9*, 1438–1456.

Sun, J.S., Tsuang, Y.W., Chen, I.J., Huang, W.C., Hang, Y.S., & Lu, F.J. (1998). An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. *Burns: Journal of the International Society for Burn Injuries, 24*, 225–256.

Sun, J., Chu, Y.F., Wu, X.Z., & Liu, R.H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry, 50*, 7449–7454.

Ukwe, C.W. (1997). Antiulcer activity of aqueous stembark extract of *Hymenocardia acida* Tul (Euphorbiaceae). *International Journal of Pharmacognosy, 35*, 354–357.

Vonthron-Senecheau, C., Weniger, B., Quattara, F., Kamenan, A., Lobstein, A., Brun, R., et al. (2003). *In vitro* antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. *Journal of Ethnopharmacology, 87*, 221–225.

Wang, S.Y., & Lin, H.S. (2002). Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry, 48*, 140–146.

Wolfe, K., Wu, X., & Liu, R.H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry, 51*, 609–614.

Yen, G.C., & Chen, H.Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural Food Chemistry, 42*, 27–32.

Zheng, W., & Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*, 5165–5170.