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Free Radical Scavenging Activity of Some Nigerian Medicinal Plant Extracts

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Abstract: The present research evaluates the DPPH radical scavenging, total antioxidant activities, reducing power and total contents of phenolic compounds in methanolic leaf extracts of five Nigerian medicinal plants (*Dalbergia saxatilis* Hook.f. (Papilionacae), *Ekebergia senegalensis* A.Juss.(Meliaceae), *Hymenocardia acida* Tul. (Hymenocardiaceae), *Icacina tricantha* Oliv. (Icacinaceae) and *Salacia pallescens* Oliv.(Celastraceae). Total phenols were analysed according to the Folin-Ciocalteu method. Each sample under assay condition, showed a dose-dependent effect both on free radical scavenging 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and also on Fe^{3+} reducing power. The antioxidant activity of the plant extracts with the DPPH radical scavenging and reducing power method, were in the order *Hymenocardia*> *Ekebergia*> *Salacia*> *Icacina*> *Dalbergia*. *H. acida* and *E. senegalensis* possess very high radical scavenging activity in both assays. Potency of *H. acida* extract was of the same magnitude as that of reference α-tocopherol. Total phenols in all the samples expressed as GAE (Gallic Acid Equivalent) varied from 1.83 to 15.47mg g^{-1} of dry plant material. Total antioxidant activities correlated with total phenols (R^2 = 0.6640) an indication that 66% of the antioxidant capacity of these extracts results from contribution of phenolic compounds. A linear positive relationship existed between the reducing power and total phenolics of the tested plant extracts (R^2 = 0.9564).

Key words: Free radical scavenging, antioxidant activity, reducing power, total phenolic content, Nigerian medicinal plants

INTRODUCTION

Free radicals are compounds generated from normal body processes and also from environmental pollutants. They tend to attack the cells of our body causing them to deteriorate. Antioxidants quench free radicals so that they will not attack living cells. Antioxidant properties have been extensively studied and are among the first links between chemical reactions and biological activity (Trouillas *et al*., 2003). It has been shown that antioxidants and free radical scavengers are relevant in the prevention of pathologies such as arteriosclerosis, heart diseases, cancer and arthritis, in which reactive oxygen species or free radicals are, implicated (Middleton, 2000).

Several types of plant materials such as vegetables, fruits leaves, oilseeds, cereals crops, barks and roots, spices and herbs and crude plant drugs are potential sources of antioxidant compounds. Most of the isolated constituents with antioxidant activity are phenolic compounds (Ramarathnam *et al*., 1997; Odukoya *et al*., 2005; Olukemi *et al*., 2005).

Phenolic natural products such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals. The antioxidant activity of these phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quencher. In addition, they have metal chelating potentials (Rice-Evans *et al*., 1995).

A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. There is a worldwide trend toward the use of natural antioxidants. For this reason, an extensive search for different types of antioxidants in various kinds of plants has been undertaken. According to a conservative estimation, 300,000-400,000 plants species grow on earth; however,

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only a small percentage have had their phytochemistry and biological function investigated (Kitani *et al*., 2001).

In this regard, while some individual plant species such as *Ginkgo biloba* and *Panax ginseng* have been investigated in some detail, relatively little information is available concerning the antioxidant potential of plant species in general and Nigerian plant species in particular. The objective of the present research is to carry out a systematic survey of the relative levels of antioxidant activity in selected Nigerian medicinal plant species used in traditional medical practice. *D. saxatilis* is used in the treatment of sores, sharp stomach pains and gonorrheoa; *E. senegalensis* and *I. tricantha* for dysentery; *H. acida* as a febrifuge and also in the treatment of gonorheoa and *S. pallescens* as an antidiabetic and anti obesity.

MATERIALS AND METHODS

Plant collection and extraction: The plants were collected between November 2004 and January 2005, from various locations in Oyo State, Nigeria and were identified at the herbarium of the Forestry Research Institute of Nigeria [FRIN], Ibadan. Voucher specimens were deposited at the Institute and also in the Department of Pharmacognosy, University of Lagos. The plants were air dried at room temperature.

Ten gram of each dried powdered sample was macerated with 100 mL of methanol for 24 h at room temperature. Each extract was filtered through Whatman No.1 filter paper and the residue re-extracted with the same solvent. All extracts were combined together and left on the bench to dry. The extracts were weighed and preserved for further use in the refrigerator.

DPPH Radical scavenging assay: Radical scavenging activity of plant extracts was measured by slightly modified method of Miliauskas *et al*. (2004). One mg mL^{-1} methanolic stock solution of extract was prepared and various concentrations of the extracts were obtained by serial dilution. To these was added 0.5 mL methanol solution of DPPH (1 mM). The mixture was shaken and then left to stand at room temperature for 30 min. The absorbance of the resulting solution was read spectrophotometrically at 517 nm. α-Tocopherol (Sigma) was used as standard antioxidant while a blank of methanol (Analytical BDH) was ran with each assay. All determinations were carried out in triplicate. The same procedure was repeated using control sample (DPPH without the extracts). The inhibition of DPPH was calculated as a percentage according to the following equation:

$$\%\,\text{Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Reducing power: The reducing power of the prepared extracts was determined using Ferricyanide Trichloroacetic acid method according to Yen and Chen (1995). Mean values from three independent samples were calculated for each extract.

Analysis of phenolic compounds: Plant extracts were screened for phenolics by the addition of few drops of 5% $FeCl_3$ solution. Total phenol contents in plant methanol extract was determined with Folin-Ciocalteu reagent (1927) as recorded by Slinkard and Singleton (1977) using gallic acid as a standard. All determinations were performed in triplicate. The concentrations of total phenolic compounds in the extracts were determined as milligrams of Gallic Acid Equivalents (GAE) using the following linear equation based on the calibration curve:

$$A = 10.564X + 0.1036,\ R^2 = 0.9998$$

A is the absorbance and X is gallic acid equivalents (mg).

Statistical analysis: Experimental results were the mean±SD of 3 measurements. Analysis of variance and regression coefficients were from Excel programme.

RESULTS AND DISCUSSION

The DPPH assay measured hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. All extracts showed dose- dependent increase in activity (Table 2).

The reducing power of the crude methanol extracts of the samples was examined as a function of their concentration. In this assay, the yellow colour of the test solution changed to various shades of green and blue depending upon the reducing power of each extract. Reducing power was similar to antioxidant activity, *H. acida* showed a higher reducing power than the other plant extract and α-tocopherol (Table 3). However the

Table 1: Plant yield to solvent

Plants	Voucher No.	Yield (%)
D. saxatilis	62771	0.968
E. senegalensis	89500	1.149
H. acida	38672	2.217
I. tricantha	43676	1.548
S. pallescens	67183	1.607

Table 2: Free radical scavenging effect % inhibition on DPPH at different concentrations of plant extract

	Inhibition at mg mL^{-1} concentration (%)			
Samples	0.025	0.050	0.10	0.20
α-Tocopherol	96.9±0.002	97.0±0.005	97.2±0.002	97.9±0.001
D. saxatilis	31.2±0.053	46.4±0.051	54.4±0.053	71.1±0.019
E. senegalensis	81.0±0.002	95.8±0.003	96.2±0.003	96.5±0.006
H. acida	96.9±0.001	97.0±0.002	97.1±0.001	97.4±0.002
I. tricantha	30.9±0.004	50.5±0.029	77.1±0.047	93.2±0.002
S. pallescens	69.2±0.108	93.1±0.030	94.3±0.005	93.0±0.003

Data are represented as means±SE (n = 3)

Table 3: Reducing power of extracts at different concentrations

	Reducing power at mg mL^{-1} concentration				
Sample	0.025	0.050	0.10	0.20	0.40
α-Tocopherol	0.610±0.0647	0.736±0.0817	0.846±0.0453	1.160±0.0474	1.589±0.0895
D. saxatilis	0.697±0.0195	0.698±0.0441	0.781±0.0448	0.799±0.0800	0.846±0.0560
E. senegalensis	0.638±0.0102	0.682±0.0554	0.808±0.0701	1.029±0.0488	1.305±0.0329
H. acida	0.666±0.0218	0.828±0.0735	0.989±0.0967	1.177±0.0463	1.483± 0.2370
I. tricantha	0.712±0.0200	0.738±0.0441	0.765±0.0395	0.887±0.0330	0.846±0.0560
S. pallescens	0.653±0.0477	0.663±0.0500	0.807±0.0836	0.936±0.0782	1.218±0.0365

are represented as means±SE (n = 3)

Table 4: Total phenol contents of the plant extracts

Extracts	Total phenols (mg g^{-1} of gallic acid equivalents)
D. saxatilis	4.47±0.001
E. senegalensis	9.63±0.001
H. acida	15.47±0.001
I. tricantha	5.00±0.000
S. pallescens	8.60±0.000

Data are represented as means±SE (n= 3)

absorbance value was lower than that of α-tocopherol at 0.4 mg mL^{-1}. Using the DPPH radical scavenging method and reducing power method, the antioxidant activity of the plant extracts were in the order *Hymenocardia> Ekebergia> Salacia> Icacina> Dalbergia.* This result was consistent with the order of total phenolic content of each extract.

Several studies have reported the relationship between phenolic content and antioxidant activity. Velioglu *et al.* (1998) and Odukoya *et al.* (2005) reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products, while Kahkonen *et al.* (1999) found no correlation between antioxidant activity and phenolic content of some plant extracts.

In this study, our findings showed a good linear correlation (R^2 = 0.9564) between reducing power and the total phenol content in each extract. These results indicated that the reducing power of each extract might be mostly related to their concentration of hydroxyl hydrogen. Phenolic compounds could easily donate hydroxyl hydrogen due to resonance stabilization (Meir *et al.*, 1995). Reducing power of a compound is related to electron transfer ability of the compound. Therefore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity as indicated in our results.

However, free radical scavenging activity and total phenolic content of the plant extract had a correlation coefficient of R = 0.6640 (Y = 1.2281×-103.11) (Table 4). This suggests that about 66% of the antioxidant capacity of these extracts result from contribution of phenolic compounds. Thus confirming the radical scavenging and antioxidant activities of these extracts, as determined by reducing power and scavenging effect on the DPPH free radical.

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