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Original article

Evaluation of antioxidant activities of some Nigerian medicinal plants

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Abstract

Objective: The methanol extracts of the leaves of *Kolancheo* (*K.*) *pinnata*, *Aspilia* (*A.*) *africana*, *Mucuna* (*M.*) *pruriens*, *Emilia* (*E.*) *coccinea*, *Triumfetta* (*T.*) *rhomboidea*, *Laportea* (*L.*) *ovalifolis*, *Celosia* (*C.*) *trigynea*, *Cucurbita* (*C.*) *moschata* and *Asystesia* (*A.*) *gangestica* were evaluated for antioxidant activities, the reducing potentials of these plants were also determined and phytochemical screening of some of these plants were carried out. **Methods**: The antioxidant activities of these plants were evaluated by the use of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay and spectrophotomer. The reducing potentials of these plants were determined using Ferric chloride, Potassium Ferricyanide and Trichloroacetic acid proceedures. The phytochemical screenings of the medicinal plants were equally done using standard procedures. **Results**: The percentage of antioxidant activities values for the plants were: (31.00 ± 1.80) %, (58.40 ± 1.26) %, (59.10 ± 1.60)%, (60.00 ± 1.05) %, (60.80 ± 1.28) %, (62.40 ± 1.26) %, (64.80 ± 2.10) %, (75.70 ± 2.60) % and (82.70 ± 2.80)% for *A. gangestica*, *C. moschata*, *C. trigynea*, *L. ovalifolia*, *E. coccinea*, *M. pruriens*, *A. africana*, *K. pinnata* and *T. rhomboidea*, respectively. These values were dose-dependent and statistically significant at $P < 0.05$ (ANOVA). The results showed that *T. rhomboidea* had the highest antioxidant activity value (82.70 ± 2.80 %) while *A. gangestica* had the least value (31.00 ± 1.80 %). The percentage of antioxidant activities of these plants were comparable to the standard used, *Tocopherol* which was found to be (97.20 ± 1.06) %. The reducing potentials of the plants were found to be positively correlated to the antioxidant activities of the plants. Phytochemical screenings revealed the presence of alkaloids, flavonoids, terpenoids, saponins and tannins in the medicinal plants. **Conclusion**: The findings from this study have revealed the potentials of these plants as antioxidants. This could be exploited in drug development in search of powerful antioxidants which are urgently needed to challenge free radicals in biological systems. It will consequently help to prevent the body from free radicals originated ailments. However, further study needs to be done to isolate and characterize the active principles in these plants.

Keywords: Phytochemical screening; Antioxidant activity; Reducing potentials; Nigerian medicinal plants

INTRODUCTION

Chemical compounds with unpaired radicals such as powerful oxidants and free radicals are capable, when present in the body, to damage lipids, proteins, and DNA and consequently may bring about mutation [1]. Free radicals play a prominent role in human health. Free radical reactions have been implicated in the etiology and pathogenesis of chronic

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diseases that are life limiting such as cancer, hypertension, cardiac infarction, arteriosclerosis, diabetes etc.

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions[2]. Antioxidant activity is a very important pharmacological property. Many of the pharmacological functions such as antimutagenicity, anticarcinogenicity, antiaging etc originate from this property [3,4].

The most important free radicals in the body are the reactive oxygen species and reactive nitrogen species, such as super oxide, hydroxyl and nitric oxide radicals. They are generated in the body as a consequence of cellular and metabolic activities. They also arise from exogenous sources (exposure to ionizing radiations, injury, oxidative drugs, pollutants etc). Excessive production and 'Leakages' from their site of generation are damaging to cells and tissues due to their reactivity with other biologically functional compounds. The body maintains a balance in reactive oxygen and nitrogen species by various scavenging mechanisms. These include a number of antioxidant enzymes and antioxidant molecules. Oxidative stress occurs when there is an imbalance in free radicals and antioxidants in the body. This could lead to serious health problems.

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay has been used extensively for screening antioxidants from fruit and vegetable juices or extracts[5]. DPPH is a stable free radical which is reduced to DPPH-H on reaction with antioxidants. As a consequence, the absorbance is decreased and the original purple colour of DPPH is changed to yellow.

The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. The antioxidant activities of several plant materials have been reported [6-11].

Presence of antioxidants has been also confirmed in soybeans [12], garlic, red wine, green tea[13] and in Tridax [14].

In fact, many research works have been done on screening of some medicinal plants and vegetables for antioxidant activities [6,11,15-18]. However, no scientifically proven information is available on the antioxidant activity of *K. pinnata*, *A. africana*, *L. ovalifolia*, *E. coccinea*, *A . gangetica*, *C . trigyna* and *C. moschata*. It is that this study seeks to establish for the first time the antioxidant activities of these plants in the light of this. Natural products still represent an important source of interesting leads for drug development.

MATERIALS AND METHODS

All the chemicals used for the extraction, phytochemical screening, reducing potential and DPPH assay were of analytical grade; DPPH free radical was a product of Sigma-Aldriech USA.

Collection and drying

The fresh leaves of the experimental plants were collected in May, 2008 from local gardens at the University of Port Harcourt and were authenticated by Edwin Wosu of the Department of Botany Herbarium, University of Port Harcourt. Voucher specimens were maintained at the Herbarium. The voucher specimens´numbers are: 558, 559, 560, 561, 562, 563, 564, 565 and 568 for *K. pinnata*, *A. africana*, *E. coccinea*, *C. moschata*, *L. ovalifolia*, *A. gangetica*, *C. trigyna*, *M. prurients* and *Triumfetta rhomboidea*, respectively.

The leaves were cleaned of sand particles, air-dried until they attained a constant weight (10 days). They were pulverized to powder and stored in air-tight containers in the refrigerator for subsequent use.

These samples were brought out and allowed to assume room temperature prior to use for analysis.

Preparation of the extracts

Samples of the powdered leaf of each plant (100 g each) were cold macerated with 100 mL of methanol for 72 hrs at room temperature.

Each extract was filtered (Whatman No. 1 filter paper) and the residue re-extracted with the same solvent. The extracts were combined and concentrated in a rotary evaporator under reduced pressure to give the methanol extract for phytochemical analysis and antioxidant-activity assay.

Phytochemical screening

Chemical tests were carried out on the methanolic extracts and on the powdered specimens using standard procedures to identify the constituents[19-22] by characteristic colour changes as described by Sofowara(1993), Odebedy and Sofowara(1978).

DPPH assay for antioxidant activity

The ability of the extract to scavenge DPPH radical

was determined according to Mensor et al., 2001[15] with little modification. 1.0 mL of 0.3 mM DPPH methanol solution was added to the solution of the extract or standard (250 μg/mL, 2.5 mL) and allowed to react at room temperature for 30 mins. The absorbance of the resulting mixture was measured at 518 nm with spectrophotometer and converted to percentage antioxidant activity (AA%). Methanol (1.0 mL) plus extract solution (2.5 mL) was used as a blank, while 1.0 mL of 0.3 mM DPPH plus methanol (2.5 mL) was used as a negative control. Solution of ascorbic acid served as positive control. Antioxidant activity (AA) was calculated as percentage of inhibition relative to control using the following equation[6].

$$AA\% = (Rcontrol - Rsample)/Rcontrol \times 100$$

Where Rcontrol = absorbance of control
Rsample = absorbance with each sample
AA% = percentage of antioxidant activity.

Determination of reducing potential

Reducting potential was determined according to the method of Afolabi, et al (2007) with little modifications[23]. The extract or standard (100 μg/mL or 250 μmg/mL, respectively) was mixed with phosphate buffer and potassium ferricyanide. The mixture was incubated at 50℃ for 20 mins. Trichloroacetic acid (10%, 2.5 mL) was added to the mixture. A portion of the resulting mixture was mixed with Ferric chloride ($FeCl_3$, 0.1%, 0.5 mL) and the absorbance measured at 700 nm using Spectrophotometer. Higher absorbance of the reaction mixture indicates higher reductive potential.

Statistical analysis

Results were analyzed using one way analysis of variance (ANOVA). Data was expressed as Mean ± SEM and further subjected to Graph Pad prism 5 demo (software) analyses, the differences between mean accepted as significant at $P < 0.05$ (ANOVA).

RESULTS

The phytochemical screening of *K. pinnata* (fam. Crassulaceae), *A. africana* (Fam. Asteraceae), *M. pruriens* (Fam. Fabaceae), *E. coccinea* (Fam. Asteraceae) and *T. rhomboidea* (Fam. Tiliaceae) shows that these plants contain alkaloids, saponins, tannins terpenoids, cardiac glycosides and flavoniods (Table 1).

The antioxidant activity assay shows that *A. gangestica* (Acanthaceae), *C. moschata* (Cucurbitaceae), *C. trigyna* (Amaranthaceae), *L. ovalifolia* (Urticaceae) and *E. coccinea* exhibit percentage of antioxidant activity values as (31.00 ± 1.80) %, (58.40 ± 1.26) %, (59.10 ± 1.60) %, (60.00 ± 1.05) % and (60.80 ± 1.20) % with $P < 0.05$, respectively (ANOVA) (Table 2, Figure 1). While *M. pruriens*, *A. africana*, *K. pinnata* and *T. rhomboidea* show antioxidant activity values as (62.40 ± 1.26) %, (64.80 ± 2.10) %, (75.70 ± 2.60) % and (82.70 ± 2.80) % with $P < 0.001$, respectively (ANOVA, Table 2, Figure1). These values were comparable to the observed antioxidant activity of *tocopherol* which was used as standard (97.20 ± 1.06)% (Table 2 and Figure 1).

The reducing potentials of these plants were found to have a direct positive linear relationship with the percentage antioxidant activity of these plants (Table 2, Figure 2).

Table 1 The phytochemical screening of some of the Nigerian medicinal plants.

Phytochemicals	*E. coccinea*	*M. Pruriens*	*A. africana*	*T. rhombiodea*	*K. pinnata*
Carbohydrate	+	+	+	+	+
Alkaloids	+	+ +	+	+	+
Flavonoids	+	+ +	+ +	+ +	+ +
Anthraquinone	−	+	−	+ +	+
Saponins	+	+	+	+	+
Tannins	+	+	+	+ +	+
Steroids	+	+	+	+	+
Fats & oils	+	+	+	+	+
Resins	−	−	−	+	−
Terpenoids	+	+	+ +	+	+
Cardiac glycoside	+	+	+	−	+

Table 2 The antioxidant activities and reducing potentials of Nigerian medicinal plants.

	AOA	IC_{50} (μg/mL)	% AA	RP
Control/blank (methanol)	0.56	2.50mL	0.00	0.00
A. gangestica	0.30	100.00	31.00 ±1.80 [a]	0.20 ±0.01
C. moschata	0.23	150.00	58.40 ± 1.26 [a]	0.40 ± 0.05
C. trigyna	0.27	120.00	59.10 ± 1.60 [a]	0.60 ± 0.04
L. ovalifolia	0.22	100.00	60.00 ±1.05 [a]	0.80 ± 0.02
E. coccinea	0.22	120.00	60.80 ± 1.20 [a]	1.00 ± 0.07
M. pruriens	0.21	100.00	62.40 ± 1.26 [b]	1.20 ± 0.06
A. africana	0.20	160.00	64.80 ± 2.10 [b]	1.40 ± 0.03
K. pinnata	0.12	180.00	75.70 ± 2.60 [b]	1.60 ± 0.03
Triumfeta rhomboidea	0.10	120.00	82.70 ± 2.80 [b]	1.70 ± 0.02
Tocopherol	0.02	50.00	97.20 ± 1.06 [b]	2.00 ± 0.02

[b] represents significant at $P < 0.001$; a significant at $P < 0.05$ (ANOVA); AOA = Antioxidant activity, AA = Percentage antioxidant activity, RP = reducing potentials, IC_{50} = 50% inhibitory concentration. Results expressed as mean ± standard error of mean (SEM).

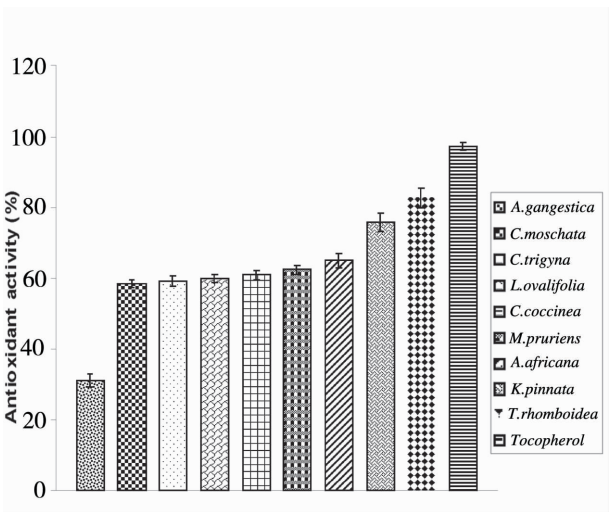

Figure 1 The antioxidant activities of Nigerian medicinal plants.

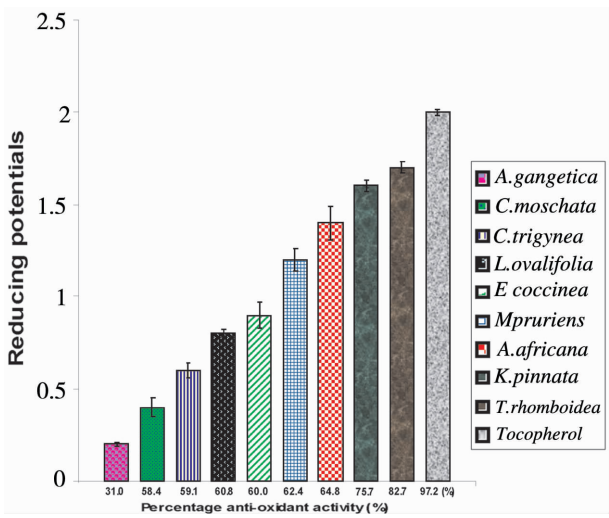

Figure 2 Reducing potentials and anti-oxidant activities of Nigerian plants.

DISCUSSION

The results show the phytochemicals which detected in the methanolic extracts of the leaves of *K. pinnata*, *T. rhomboidea*, *A. africana*, *M. pruriens* and *E. coccinea*. The extracts tested positive for alkaloids, flavonoids, terpeniods, saponins and cardiac glycosides. These compounds detected have been documented to possess medicinal properties and potent therapeutic effects[23-26]. These are consistent with the previous works [27-29].

The percentage antioxidant activity of *K. pinnata* was found to be the highest at (75.70 ± 2.60) %. This is very comparable to the antioxidant activities

of *tocopherol* which was used as standard and got to be (97.20 ± 1.06) %. The percentage antioxidant activity of *K. pinnata* was found to be statistically significant at $P < 0.001$ (ANOVA). The high percentage antioxidant activity value of *K. pinnata* could be attributed to its high content of Favonoids, Phenols and ascorbic acid which have been evaluated to be 1.72, 1.86 and 44.03 mg/100 g in dry weight, respectively [25]. This is equally consistent with the previous works[30-32].

The least percentage of antioxidant activity was obtained with *A. gangestica* at (31.00 ± 1.80)%. This is about three times less than the percentage of antioxidant activities of the tocopherol used as standard (97.20 ±1.06) %. This is statistically significant at $P < 0.05$ (ANOVA).

The percentage antioxidant activity of *A. africana* was found to be (64.80 ± 2.10) %. This value is comparable to the standard used (97.20 ±1.06) %. The value is also statistically significance at $P < 0.001$ (ANOVA). The high percentage antioxidant activity value of *A. africana* can be attributed to its high content of flavonoids, ascorbic acid and phenols which have been evaluated to be 1.48, 26.42 and 1.46 mg/100 g in dry weight, respectively [25] (Okwu and Josiah 2006). This is also consistent with the previous works [27-29].

The percentage of antioxidant activities of *M. pruriens*, and *E. coccinea* were (62.40 ± 1.26) % and (60.80 ± 1.20) %, respectively. These were significant at $P < 0.05$ (ANOVA). The values were comparable to the standard used. These were consistent with the past works[24,33,34]. The percentage of antioxidant activities of *C. moschata*, *C. trigyna* and *L. ovalifolia* were found to be (58.40 ± 1.26) %, (59.10 ± 1.60) % and (60.00 ± 1.05) %, respectively. These were statistically significant at $P < 0.05$ (ANOVA), and are consistent with the works [34].

The reducing potentials of the plants were found to have a direct linear relationship with the percentage antioxidant activity. This is consistent with the past work[18].

These novel plants can be good potential sources for new drug development. This is because the findings from this study have revealed the potentials of these plants as antioxidants. Therefore, this could be exploited in drug development in search of powerful antioxidants which are urgently needed to challenge free radicals in biological systems. It will consequently help to prevent the body from free radicals originated ailments. However, further study needs to be done to isolate and characterize the active principles in these plants.

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