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POLYPHENOL CONTENTS, CYTOTOXICITY AND ANTIOXIDANT ACTIVITIES OF SOME SELECTED NIGERIAN VEGETABLE FOODS

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ABSTRACT

The present investigation was carried out to evaluate the relative antioxidant activity, cytotoxicity and some polyphenol contents in selected Nigerian vegetable foods. The flavouoid contents varied from 1.55± 4.23 to 17.300 ± 0.200% in the extracts. Total phenol contents were between 0.263 ± 0.00 to 1.328 ± 0.318% in the plant extracts. 2,2- Diphenyl-1- picrylhydrazyl (DPPH) radical scavenging effects of the extracts was determined spectrophotometrically. Rapid radical scavenging screening using thin layer chromatographic plate (TLC) was also performed. The highest radical scavenging effect was observed in *C. esculenta* leaf extract with IC_{50} = 180.20 µg/mL. This is comparable to the standards quercetin and ascorbic acid used.

The higher amount of the polyphenolic compounds is attributable to more potent radical scavenging effects as demonstrated by *C. esculenta* leaf extract.

The extracts possessed very low cytotoxicity to brine-shrimp, when compared with the reference standard (Potassium dichromate, LC_{50} = 180.142 µg/mL). The results obtained in the present study indicate that these vegetables can be potential sources of natural antioxidant agents and are relatively safe for the purposes utilized.

Key words: Cytotoxicity, 2,2-Diphenyl-1-picryl hydrazyl, Antioxidants, Polyphenols, Vegetable foods.

INTRODUCTION

Polyphenols are common constituents of human diet, present in most foods and beverages of plant origin. They are considered to contribute to the prevention of various

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degenerative diseases, including cardiovascular diseases[1]. Polyphenols have a variety of anti-inflammatory and immune-modulating effects that may be of relevance to arteriosclerosis.

They are antioxidants containing a polyphenolic structure and are a class of antioxidant agents which act as free radical terminators[2]. Polyphenols are widely distributed in most legumes, fruits vegetables such as broccoli, cabbage, onion etc and include such compounds as phenols, anthocyanins, flavonoids, and tannins. They are effective hydrogen donor, which makes them good antioxidants[3]. Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals ($O_2^{\bullet}$), hydroxyl radicals ($^{\bullet}OH$) and non-free radical species such as H_2O_2 and and singlet oxygen ($^1O_2$), are various forms of activated oxygen[4, 5].

The importance of free radicals and ROS has attracted increasing attention over the past decade. These molecules are exacerbating factors in cellular injury and in the ageing process[6]. ROS have aroused significant interest among scientists. Their broad range of effects in biological and medicinal systems have been studied in many experimental investigations[7].

In living organisms, various ROS can form in different ways. Normal aerobic respiration stimulate polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents, and pesticides[8,9].

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies.

The use of plant compounds for pharmaceutical purposes has gradually increased in Nigeria and the world over. About 80% of individuals from developed countries use plant food as medicine. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Beside well known and traditionally used natural antioxidants from tea, wine fruits, vegetables and spices, some natural antioxidant (e. g. Rosemary and Sage) are already exploited

commercially either as antioxidant additives or nutritional supplements[10]. Also many other plant species have been investigated in the search for novel antioxidants[11-14], but generally there is still a demand to find more information concerning the antioxidant potential of plant species.

The purpose of the present study was to evaluate the *in vitro* antioxidant activities, cytotoxicity and polyphenol contents of the methanolic extract of selected Nigerian leafy vegetables.

EXPERIMENTAL

Materials and methods

Plant materials

The leaves and stems of the selected vegetables, *pterocarpus mildbraedii*, *Gnetum africanum Adansonia digitata* and *Colocasia esculenta* were obtained from Oboloafor market, Enugu State and Kogi State University Staff Quarters, Anyigba, Nigeria. The plant materials were washed with water to remove dirts and were air dried in the laboratory for two weeks. The dried plant materials were pulverized using pestle and mortar.

Preparation of crude methanolic extracts

Cold extraction method was employed. 20 g of the powdered samples were weighed into conical flasks. 150 mL of pure methanol was added and left for 72 hours. The mixtures were filtered and the filtrate were concentrated using rotary evaporator.

Chemicals

DPPH (2,2-Diphenyl-1-picrylhydrazyl) and quercetin were purchased from Sigma chemical company (Sigma, Germany). Vitamin C used was a product of Glaxo Smithkline, methanol, Folin-Ciocalteu reagent, potassium dichromate, ferric chloride, and amyl alcohol were products of BDH. Tannic acid used was M & B product. All other chemicals and reagents used, but not mentioned, are of analytical grade.

Total phenol and polyphenol determination

The total phenol composition was determined using the Folin-Ciocalteu reagent as described by McDonald et al.[15] The method of Harbone[16] was followed in the determination of the total flavonoids content. The colorimetric method of van-Burden and Robinson[17] was employed in the determination of tannin content.

Rapid radical scavenging screening

The methods of Mensor et al[18], Burits and Bucar[19] and Adebayo et al.[20] were followed in screening the antioxidant property of the extracts. With the aid of a capillary tube, stock solutions (1 mg/mL) of extracts were spotted on silica gel thin layer chromatographic (TLC) plate and developed with a solvent system of ethanol : methanol (90 : 10). After development, the chromatograms were dried and sprayed with a 0.3 mM solution of the stable radical DPPH. Yellow spot formed against purple background were taken as positive results. The duration for the development of yellow colour indicated, whether the antioxidant activity was strong or not.

Antioxidant assay-scavenging of DPPH radicals

The free radical scavenging activity of the plant extracts were determined using the modified method of Blois[21]. 1 mL of different concentration (500, 250, 62.5, 31.25 µg/mL) of extracts or standard (Vitamin C and quercetin) in a test tube was added to 1 mL of 0.3 mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was measured at 517 nm against a DPPH control containing only 1 mL of methanol in place of the extract. Percentage scavenging activity was calculated using the expression.

$$\% \text{ Scavanging activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control

IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radical and this was computed using Jandel scientific sigma plot for windows version 1.2.

Cytotoxicity to brine-shrimps

Modified methods of Solis et al[22] and Potduang et al.[23] were used to determine the inhibitory activity on *Artemia Salina*. 50 µL of different concentrations of crude methanolic extracts (1000, 500, 250 and 125 µg/mL) and control (methanol) was added into graduated vial bottles containing 10 newly hatched brine-shrimps in 5 mL of artificial sea water, and then incubated at room temperature for 24 hours. All samples were repeated in two wells to make the overall tested organism of 20 for each. The living brine-shrimps were counted under a hand-magnifying lens. Same procedure was followed using potassium dichromate as the reference standard or positive control and data analysed based

on US; EPA probit analysis programme version 1.5 (Finney, 1971) to determine the LC_{50} at 95% confidence limit.

Statistical analysis

Data are reported as the mean ± S.E of three determinations. Statistical analysis was performed by student t-test. IC_{50} values for all the experiments were computed using Jandel scientific sigma plot for windows version 1.2, LC_{50} was determined using united state Environmental protection Agency Probity analysis programme version 1.5 (Finney, 1971).

RESULTS AND DISCUSSION

Phenol and polyphenol contents of the plant extracts

Phenols and flavonoids posses hydroxyl groups, which are responsible for free radical scavenging effect[2]. It has been known that flavonoids posses antioxidant activity and thereby being considered as having positive effect on human health and nutrition. Flavonoids are anti-inflammatory, anti-tumor, antiviral and antiplatelets[2, 24].

Table 1: Phenol and some polyphenol contents in the studied plant extracts

Plant species	Total phenol (%)	Tannins (%)	Flavonoids (%)
Pterocarpus mildbraedii (stem)	0.386 ± 0.007	0.297 ± 0.047	3.133 ± 0.100
Pterocarpus mildbraedii (leaf)	0.372 ± 0.453	0.283 ± 0.010	1.600 ± 0.200
Gnetum africanum (leaf)	0.286 ± 0.010	0.138 ± 0.000	17.300 ± 0.200
G. africanum (stem)	0.263 ± 0.010	0.098 ± 0.010	5.200 ± 0.200
Adansonia digitata (stem)	1.175 ± 0.159	1.328 ± 0.318	2.440 ± 0.662
A. digitata (leaf)	3.753 ± 0.340	1.276 ± 0.300	1.550 ± 4.226
Colocasia esculenta (stalk)	1.578 ± 0.371	0.347 ± 0.082	1.750 ± 0.326
C. esculenta (leaf)	1.385 ± 0.327	0.540 ± 0.127	13.700 ± 3.239

Each value in the table was obtained by calculating the average of three determinations ± standard error of mean (SEM).

Phenol acts as free radical chain reaction terminators; thereby acting as antioxidant[25]. Phenols also have a potential of combating oxidative stress, a syndrome

causative of some neurodegenerative diseases and cardiovascular diseases. The mechanisms of action of flavonoids are through scavenging or chelating process[26, 27].

The result of the determination of the flavonoid content of the selected vegetables is presented in Table 1. The flavonoid composition of the extracts of *G. africanum* leaf (17.300 ± 0.200%), *C. esculenta* leaf (13.700 ± 3.239%) and *G. africanum* stem (5.200 ± 0.200%) were higher than that in the extracts of other vegetables studied (Table 1); thus, the flavonoid contents varied between 17.300 ± 0.200 to 1.550 ± 4.226% in the extracts. *G. africanum* leaf possesses the highest amount of flavonoids among the plant in this investigation.

According to the present study, the high amount of these polyphenols in *C. esculenta* leaf *and G. africanum* leaf extracts can account for their high free radical scavenging capacity. The studied vegetables are richer in flavonoids than any other phytochemicals and tannin composition being the lowest.

Antioxidant capacity

The result of the rapid radical scavenging screening of the plant extracts are as presented in Table 2. The observation of weak, moderate and strong antioxidant abilities of these vegetables is in consonance with those reported for other plants[28-30]. The screening reveal that only the leaf extracts posses strong antioxidants and stems of plant studied indicated weak intensity of yellow coloration affirming weak antioxidant capacity and no activity (Table 2).

Table 2: Radical scavenging abilities of the methanolic extracts from vegetable plants using rapid DPPH, TLC screening

Plant species	Reaction speed	Intensity of spots
Gnetum africanum (leaf)	Fast	+ + +
G. africanum (stem)	Very slow	+
Pterocarpus mildbraedii (leaf)	Fast	+ + +
P. mildbraedii (Stem)	-	-
Adansonia digitata (leaf)	Fast	+ + +
A. digitata (Stem)	-	-

Cont…

Plant species	Reaction speed	Intensity of spots
*Colocasia esculent*a (leaf)	Fast	+ + +
C. esculenta (stalk)	Very slow	+

Keys:– No yellow coloration

+ + + Strong intensity (immediate reaction)

+ Weak intensity of yellow colouration (15-30 minute before colour developments)

The intensity of the spots and reaction speed of the DPPH radical scavenging activities of the methanolic extracts using the TLC method indicated that *G. africanum* leaf, P. *mildbraedii* leaf, *A. digitata* leaf, and *C. esculenta* leaf showed the strongest antioxidant activities (Table 2). The IC_{50} values of the extracts using the DPPH spectrophotometric assay are as presented in Table 3. *C. esculenta* showed the highest antioxidant capacity (IC_{50} = 180.20 (µg/mL).

Table 3. Average scavenging activity and IC_{50} values of plant species (stem, stalk and leaf)

Plant species	Average scavenging activity (%)	IC_{50} (µg/mL)
G. africanum (leaf)	51.27	201.92
G. africanum (stem)	10.48	-
P. mildbraedii (leaf)	48.32	218.4
P. mildbraedii (stem)	11.2	-
C. esculenta (leaf)	55.68	180.2
C. esculenta (stalk)	50.2	207.53
A. digitata (leaf)	33.94	329.47
A. digitata (stem)	24.64	-
Quercetin -	66.44	166.51
Vitamin C -	58.72	178.24

This value is comparable with the standards used (quercetin and ascorbic acid,

166.51 and 178.24 µg/mL, respectively.

The generation of the reactive oxygen species (ROS) beyond what the ability of the body can cope with, leads to oxidative stress[7, 31]. Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases like: arteriosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS[32]. The use of DPPH scavenging assays in assessing the cell membrane integrity/cell membrane stabilizing capacities of plant constituents has given explanations as to the possible ways by which phytomedicines could help to reduce diseases caused by infections, inflammation and oxygen radicals generation affecting the cell membrane[33]. The models of scavenging DPPH free radicals used are the rapid screening and the photometric assay methods commonly employed for evaluating antioxidants activities based on their abilities to donate hydrogen ion[34].

The DPPH is a free radical, stable at room temperature. The methanolic solution gives a purple colouration which when reduced by an antioxidant molecule gives rise to a yellow solution. In the present study, quercetin (Q) and ascorbic acid (A) were used as reference standards. On comparison with these standards, the antioxidant capacities of the extracts in decreasing order were :Q > A > *C. esculenta* leaf > *G. africanum* leaf > *P.* mildbraedii leaf > *C. esculenta* stalk > *A. digitata* leaf. The low polyphenol content of the stem extracts may be attributable to the in activity or weak antioxidant capacity exhibited.

Cytotoxicity to brine-shrimps

The result of the cytotoxicity studies is presented in the Table 4.

Table 4. Cytotoxicity of plant species

Extracts	% Lethality	LC_{50} (µg/mL)
G. africanum (leaf)	30	3.63.721
G. africanum (stem)	37.5	1039.552
P. mildbraedii (leaf)	36.3	1266.256
P. mildbraedii (stem)	33.8	1330.958
C. esculenta (leaf)	51.3	1067.946
C. esculenta (stalk)	40.8	7582.855

Cont…

Extracts	% Lethality	LC_{50} (µg/mL)
A. digitata (leaf)	42.0	5631.467
A. digitata (stem)	31.3	32623.871
Potassium dichromate -	80.0	180.142

All the extracts showed mild brine-shrimp inhibition, when compared with the reference standard used with LC_{50} = 180.142 µg/mL. *C. esculenta* leaf showed the highest percentage of inhibition. This could be due to the nature of polyphenol present in this plant part, which need further investigation .On the whole, extracts are relatively non-toxic to the cell. This inference is drawn from the mild inhibition shown on the brine shrimps.

CONCLUSION

On the basis of the results obtained in this present study, we conclude that the methanolic extracts of these selected Nigerian vegetable foods have significant amount of antioxidant and this is attributable to the polyphenol contents. These vegetables are relatively non-toxic and could be well integrated into Nigerian food stuff as they posses beneficent attributes.

The antioxidant activities were found in the leaf extracts, the parts that are highly consumed. There is need to further investigate these vegetables especially to isolate the active component, which are responsible for the antioxidant activities.

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