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A Comparative Study of the Antioxidant Properties of some Edible and Medicinal Seeds and Leaves Consumed in Calabar

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Abstract: Herbs are plants with leaves, seeds or flowers used for food and/or medicine. The herbs and seeds are beneficial in the management of various degenerative diseases because of their inherent phytochemicals, which function mostly as antioxidants. In this study, the antioxidant activity of eight leaves: *Vernonia amygdalina (VA), Baphia nitida (BN), Acalypha torta (AT), Alchornea cordifoliia (AC), Lansianthera africana (LS), Gongronema latifolium (GL), Hensia crinata (HC), Telfairia occidentalis leaf (TOL)* and two edible seeds: *Treculia africana seed (TAS) and Telfairia occidentalis seed (TOS)* commonly eaten in Calabar, a Metropolitan city in Nigeria, were studied using the ferric reducing antioxidant power (FRAP) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay methods. Compared to the standard antioxidant, vitamin C, *VA* and *HC* demonstrated significantly high antioxidant activity at 500ug/ml. This was closely followed by *GL, TOL* and *TOS* at 400ug/ml and 500ug/ml respectively, when compared to vitamin C. However, *TAS* and *AT* showed the least antioxidant activity even at the highest concentration (500ug/ml). The antioxidant activity increased with increase in the concentration of the leaf/seed extracts used and the trend in increasing order of antioxidant activity was as follows: *TOS→TOL→GL→HC→VA. TAS, AC, AT, BN* and *LA* showed negligible antioxidant activity. Therefore, the incorporation of these herbs: *VA, HC, GL, TOS* and *TOL* with high antioxidant activity singly or in combination in meals would enhance the nutritional and health value especially for the aged and people suffering from oxidative-related diseases including cardiovascular diseases.

Keywords: Antioxidants, cardiovascular diseases, seeds, leaves, herbs and Calabar.

Antioxidant Properties of some Medicinal Plants in Calabar

1.1. INTRODUCTION

Plant-based foods are rich in antioxidants, though they are diverse in their antioxidant profiles and content. Due to the enormous number and different types of individual antioxidant in foods, their measure is difficult, thus requiring a commonly used approach to measure the 'total antioxidant content (or activity, capacity or power) of foods. FRAP and DPPH assays are among the widely adopted methods (Wachtel-Galor *et al.,* 2014). Free radicals are molecules produced by normal cellular metabolism usually derived from oxygen (reactive oxygen species, ROS) and nitrogen (reactive nitrogen species, RNS). Free radicals are very reactive because of the unpaired electron in their valance shell; as a result, they can attack the nearest stable molecule and elicit a chain reaction (Wachtel-Galor *et al.,* 2014; Badarinath *et al.,* 2010) which stimulate apoptosis, and can provoke various cardiovascular, neurological and physiological disorders (San *et al.,* 2014; Shad *et al.,* 2013). The characteristics common to free radicals is the ability to cause oxidative damage to most important macromolecules such as carbohydrates, proteins, lipids and nucleic acids. The damage causes adenosine triphosphate (ATP) depletion and eventually cell death (Wachtel-Galor *et al.,* 2014; Shad *et al.,* 2013). Oxidative stress causes serious cell damage which gives rise to degenerative diseases such as cancer, cardiovascular diseases, Alzheimer's disease, Parkinson's disease, neurodegenerative disorders and immunological incompetence. ROS and RNS include radicals such as: the superoxide ($O_2^{-\bullet}$), the hydroxyl ($^{\bullet}OH$), the hydroperoxyl (HO_2), the peroxyl ($ROO^{\bullet}$), the alkoxyl

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($RO^{\bullet}$), the lipid peroxyl ($LOO^{\bullet}$), the nitrogen dioxide ($NO_2^{\bullet}$) and nitric oxide ($NO^{\bullet}$); and the non-radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxynitrate ($ONOO^-$), nitrous acid (HNO), dinitrogen trioxide (N_2O_3) and lipid peroxide (LOOH) (Mekha *et al.*,2014; Badarinath, et al., 2010; Sen *et al.*, 2010). Non-radicals are also capable of free radical reaction in living system (Sen *et al.*, 2010). There is a balance between the free radicals and antioxidant ratio in a normal cell. However, overproduction or low concentration of either the free radicals or the antioxidants leads to oxidative stress.

Oxidative stress describes a disturbance in the pro-oxidant-antioxidant balance leading to oxidative damage (Wachtel-Galor *et al.*, 2014). It has been implicated in the etiology of chronic degenerative diseases such as cardiovascular diseases, cancer and metabolic diseases. Studies have shown that frequent intake of antioxidant rich foods such as fruits and vegetables reduce the risk incidence of chronic diseases. Antioxidants are molecules that scavenge free radicals by delaying or inhibiting their activities. Antioxidants can be synthesized in the body or supplied through diet like phytochemicals (Garrido *et al.*, 2014; Wachtel-Galor *et al.*, 2014; Sen *et al.*, 2010). The protection that fruits and vegetables provide against diseases has been attributed to the various phytochemicals contained in them. Increased dietary intake of some phytochemicals may retard age-related decrements in immune functions and prolong life span. Vegetarians have lower oxidative stress, provided their diet contains iron, vitamin B_{12} or sulfur. Hence, they live longer and healthier than non-vegetarians do, because of the frequent intake of antioxidant rich diet (Wachtel-Galor *et al.*, 2014). Lack of knowledge on the importance of good diet and prevalence of poverty has influenced the composition of diets taken by some Nigerians coupled with the exposure to different kinds of toxic substances such as smoke from generators, vehicles and pesticides from farm which are capable of inducing oxidative stress through production of free radicals. Thus, this study was set to determine the antioxidant content of commonly eaten vegetables (eight) and seeds (two) in south-south Nigeria in other to extrapolate their importance in health and disease. Nigeria is blessed with enormous biodiversity resources. In view of the diversity of the habitat and the climate of the country, the biota exhibits considerable diversity. Plants are rich sources of antioxidants and their phyto-constituents confer fewer side effects, and are compatible with the human physiology (Sen *et al.*, 2010).

2.1. MATERIALS AND METHODS

2.2. Chemicals

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was purchased from Sigma chemical company (Sigma, Germany) and Vitamin C used was a product of Glaxo Smithkline. $FeCl_3 \bullet 6H_2O$, methanol and tripyridyltriazine (TPTZ) solution were products of BDH while sodium citrate buffer was prepared using the pH meter in the laboratory. All other chemicals and reagents were of analytical grade or purer.

2.3. Plant Material

The leaves of eight vegetable plants: *Vernonia amygdalina, Baphia nitida, Acalypha torta, Alchornea cordifoliia, Lansianthera africana, Gongronema latifolium, Hensia crinata, Telfairia occidentalis and* two seeds of *Treculia africana and Telfairia occidentalis* were obtained from Akamkpa forest in Akamkpa Local Government Area of Cross River State. The Plant was identified and authenticated in the Department of Botany, Faculty of Sciences, University of Calabar. The plant materials were washed and dried for two weeks under room temperature varying between 25 ± 3^0C. The dried plant materials were homogenized using manual blender.

2.4. Preparation of Crude Methanol Extracts

Cold extraction method was employed. 100g of the powdered samples were weighed into conical flask. 200ml of pure methanol was added and left for 72 hours. The mixtures were filtered and the filtrates were concentrated using water bath at 37^0C.

2.5. Determination of Antioxidant Scavenging Activity of DPPH Radicals

The free radical scavenging activity of the plant extracts were determined, 1ml of different concentration (500, 400, 200, 100, 50, 10ug/ml) of extracts and standard (Vitamin C) respectively in a test tube was added 1ml of 0.3mM 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 1ml of methanol in place of the extract. Percentage scavenging activity was calculated using the expression;

$$\% \text{ scavenging activity} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} x\ 100$$

2.6. Determination of antioxidant activity by the FRAP method

Antioxidant activity was measured using Ferric reducing/antioxidant power (FRAP) assay 1mL of freshly prepared FRAP reagent (Acetate buffer:

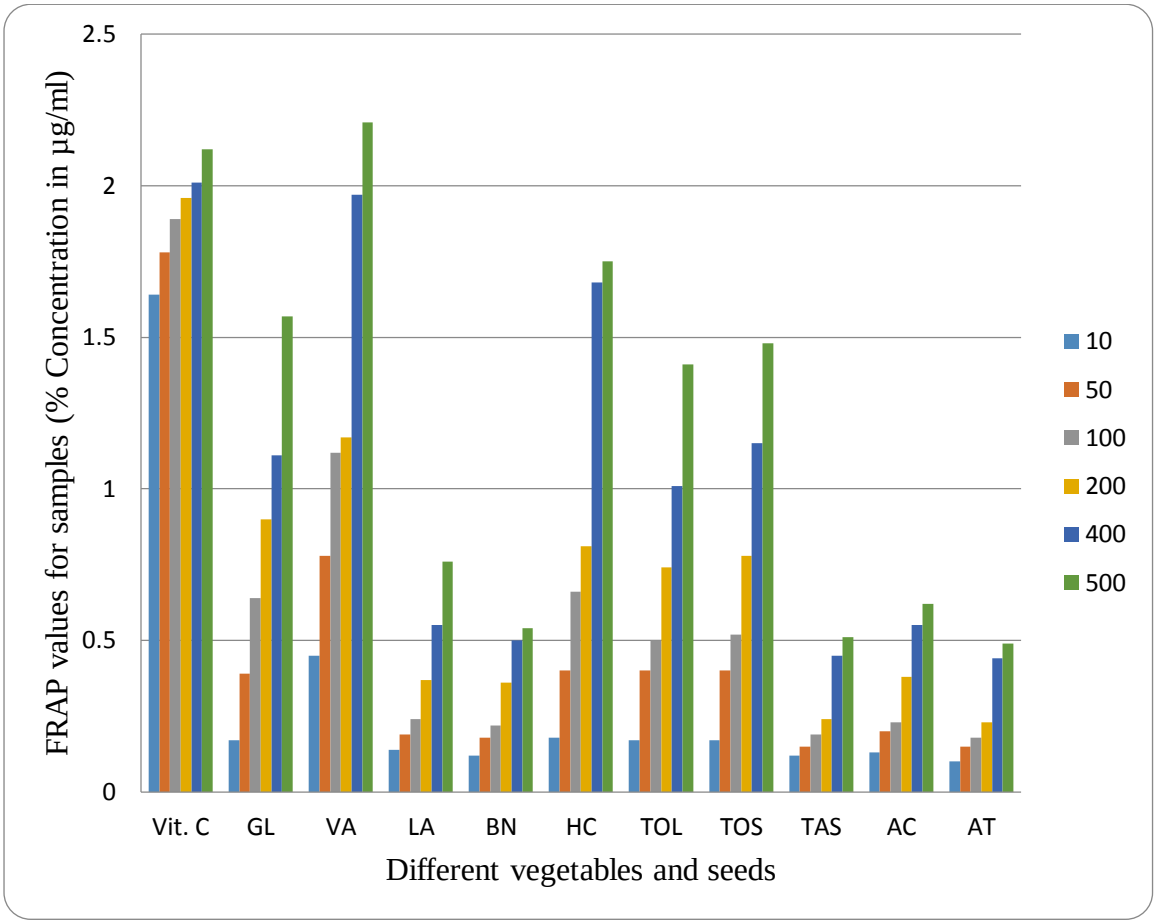
TPTZ solution: $FeCl_3.6H_2O$ solution in the ratio of 10:1:1) was mixed with the extract and absorbance measured exactly after 4 minutes at 593nm. All analysis was carried out in triplicates.

TAA was calculated using 1mm $FeSO_4$ standard and expressed as µmol/g(w/w).

3.1. STATISTICAL ANALYSIS

The data obtained was analyzed by two ways analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) version 17.0. All data was expressed as mean ±SEM (n=3), and hypothesis was tested at 99.9% level of significance.

4.1. RESULTS



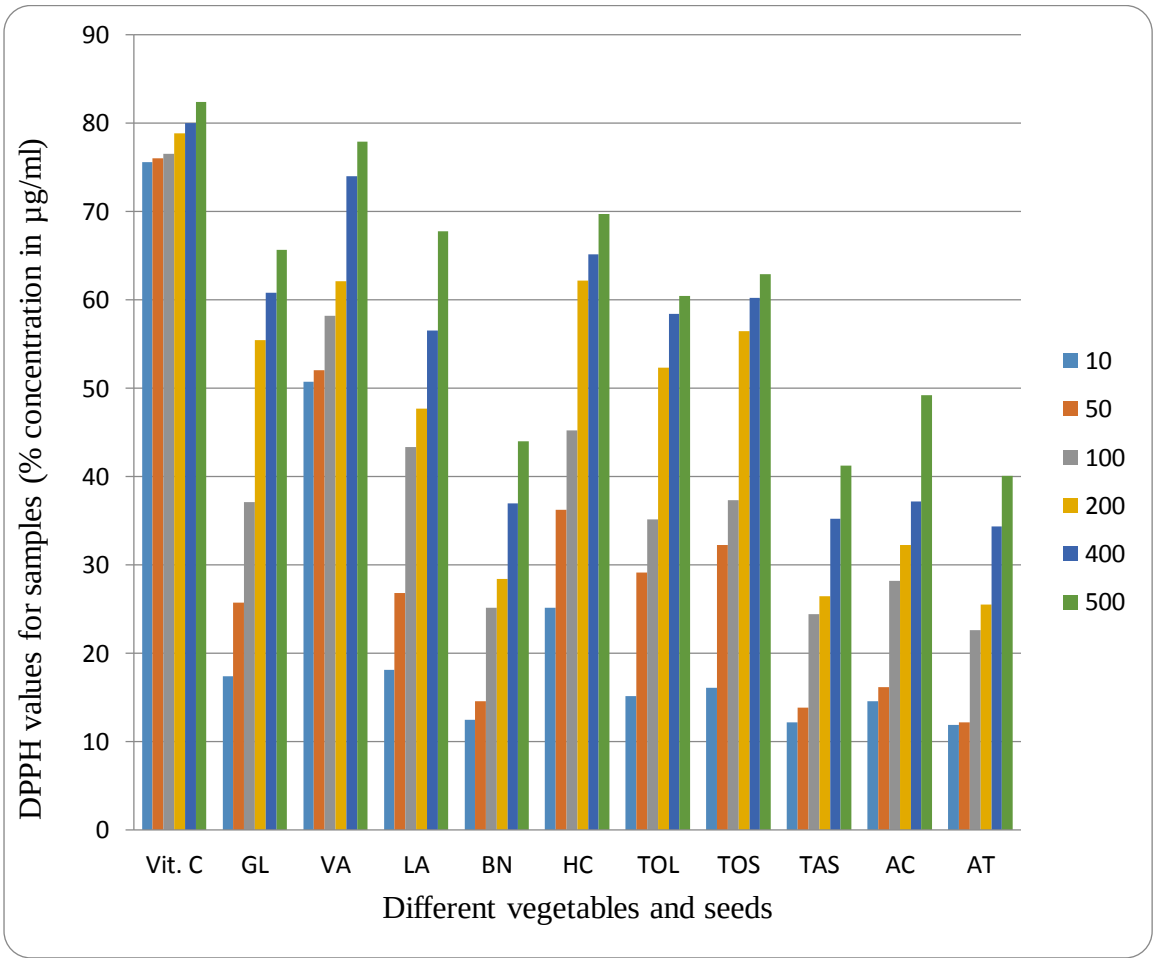
Values are expressed as mean ±SEM, n = 3.

i) The FRAP values of the different extracts varies significantly with their concentrations ($F=1x10^7$; df = 5; $p<0.001$)

ii) There are significant differences in FRAP values of the different extracts ($F = 9x10^6$; df = 10; $p<0.001$)

iii) There is also a significant interaction between the FRAP of the extracts and concentrations.

Figure 1 Antioxidant activity values of the different experimental sample using FRAP assay method.



Values are expressed as mean ±SEM, n = 3.

i) The DPPH values of the different extracts varies significantly according to their concentrations ($F= 3x10^7$; df = 5; $p<0.001$)

ii) There are significant differences in DPPH values of the different extracts ($F = 2x10^7$; df = 10; $p<0.001$).

iii) There is also a significant interaction between the DPPH of the extracts and concentrations.

Figure 2 Antioxidant activity values of the different experimental sample using DPPH assay method.

4.2. DISCUSSION

Changes were observed in the antioxidant activities of various herbal samples on the basis of their concentrations (Fig. 1 and Fig. 2 above) using the FRAP and DPPH methods respectively. It is evident from the results (Fig. 1) that the methanol extracts of *VA* and *HC* have the highest antioxidant potential at 500ug/ml while *TAS* and *AT* have the lowest; *LA* and *AC* have only vestigial amounts at that same concentrations using the FRAP assay methods when compared to vitamin C. The highest absorbance of FRAP was observed in *VA* and there is no significant difference in the antioxidant activity values of *HC* at 400ug/ml and 500ug/ml respectively. The results of the FRAP assay show that the antioxidant activity values in each herbal sample increased with increase in concentration with *VA* having the highest activity followed by *HC*, *GL* through *AT* which had the lowest activity when compared to vitamin C. The compound 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical and the bleaching of DPPH indicates the antioxidant potential of the test sample. The higher the bleaching effects the more potent the test sample is in scavenging free radicals. Antioxidant values of the herbal samples using DPPH assay method (Fig. 2) showed similarity to the values obtained using the FRAP assay method except for *LA* which had very remarkable values at 400ug/ml and 500ug/ml respectively as observed in DPPH assay method. Vitamin C antioxidant activity was virtually high at all the concentrations while *GL*, *VA*, *HC*, *TOS* and *TOL* were observed to possess high activity at concentrations 200ug/ml, 400ug/ml and 500ug/ml respectively. In both assay methods, *BN* had

relatively low antioxidant activity even at high concentrations when compared to vitamin C as well as *VA* and *HC*. There was no significant difference between *TOL* and *TOS* at 400ug/ml and 500ug/ml respectively. Moreover, no significant difference was observed in antioxidant activity values of vitamin C in virtually all concentrations.

The electron donating ability of fruits, seeds and vegetables is a suitable parameter to establish the possession of oxidative stress quenching ability. Their health promoting studies have shown that *Vernonia amygdalin, Gongronema latifolium and Moringa oleifera* are good sources of antioxidants in rats as these herbs have been reported to prevent lipid peroxidation and reverse tissue toxicity in the studied animals (Imaga & Bamigbetan, 2013; Omodamiro & Ekeleme, 2013). Issues of bioavailability means that caution is needed when interpreting results of *in vitro* measures, as have been performed here, in an *in vivo* context. Nonetheless, knowing the antioxidant content of individual foods, vegetables and seeds is useful in planning dietary strategies to meet the new reference daily intake when compared to ascorbic acid. In addition, the total antioxidant activity and the relative contribution when combined may be useful indices of the potential health benefits of individual dietary agents.

Furthermore, measurement of total antioxidant activity could be a valuable tool in food technology as the effect of growing conditions, seasonality, storage, processing, preservation techniques, cooking and genetic modification of plant-based foods could be determined. The results presented here show that the total antioxidant capacity of some vegetables and fruits consumed in Calabar, Nigeria, the most populous country in Africa varies widely, indicating clearly that all servings are not equal in terms of antioxidant intake.

5.1. CONCLUSION

Vernonia amygdalina (VA) had almost twice the total antioxidant activity compared to the other vegetables used in this study. Ferric reducing antioxidant power and DPPH free radical scavenging ability of *VA* was also much higher than those of the selected vegetables followed by *HC, GL, TOS* and *TOL*. However, based on our knowledge on synergistic action of therapeutic substances, the combined effect of any of the four herbs (*HC, GL, TOS*, and/or *TOL*) can result in a significant effect which may be close to or more than that of VA. Hence, these findings suggest that those (*VA, HC, GL, TOS* and *TOL)* herbs can serve more as a potent source of antioxidants than are common vegetables. We therefore recommend the combination of these herbs: *VA, HC, GL, TOS* and *TOL* to our fruits/food meals to increase the nutritional value especially for the aged, sick and young ones to help reduce oxidative-related diseases such as stroke and cardiovascular diseases, boost immune system, enhance proper growth and tissue maintenance. Furthermore, these data can be used to aid in dietary planning in order to promote higher antioxidant intake.

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