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

Emmanuel Effiong Edet¹, Desmond Nwabuobi Anaka¹, Justin Item Atangwho¹, Daniel Ejim Uti¹

¹Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P.M.B.1115, Calabar, Nigeria

Abstract: Herbs are plants with leaves, seeds or flowers used for food and/or medicine. The herbs and seeds are beneficial mostly as antioxidants. In this study, the antioxidant activity of eight leaves: Vernonia amygdalina (VA), Baphia nitida (BN), Acalypha torta (AT), Alchornea cordifolia (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 valence 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 (O₂•−), the hydroxyl (·OH), the hydroperoxyl (HO₂•), the peroxyl (ROO•), the alkoxyl...
(RO\(^2\)), the lipid peroxyl (LOO\(^•\)), the nitrogen dioxide (NO\(^2\)) and nitric oxide (NO\(^•\)); and the non-radicals such as hydrogen peroxide (H\(_2\)O\(_2\)), hypochlorous acid (HOCl), peroxynitrate (ONOO\(^−\)), nitrous acid (HNO), dinitrogen trioxide (N\(_2\)O\(_3\)) 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 (Sen et al., 2014) (Galor 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 order 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 was used as a product of Glaxo Smithkline. FeCl\(_3\cdot6\)H\(_2\)O, 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 cordifolia, 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\(^\circ\)C. 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\(^\circ\)C.

#### 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:

\[
\% \text{ scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

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

TPTZ solution: FeCl$_3$.6H$_2$O 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 vary significantly according to their concentrations ($F= 3 \times 10^7$; df = 5; p<0.001)

ii) There are significant differences in DPPH values of the different extracts ($F = 2 \times 10^7$; 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 500µg/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 400µg/ml and 500µg/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 400µg/ml and 500µg/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 200µg/ml, 400µg/ml and 500µg/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 amygdalina, 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.

REFERENCES

1) Badarinath, A. V., Mallikarjuna, K. R., Madhu, S. C., Ramanth, S., Rajan, T. V., & Gnanaprakash, K. (2010). A review on in vitro antioxidant methods: comparisons, correlations and considerations. International Journal of PharmaTech Research, 2(2), 1276-85.
2) Garrido, M., Rodriguez, A. B., & Terron, M. P. (2014). Tryptophan and Melatonin-Enriched Foodstuff to Improve Antioxidants Status in Aging. In R. V. Preedy (Ed.), Aging: Oxidative Stress and Dietary Antioxidants (1st ed., pp. 129-136). London, UK: Academic Press.
3) Imaga, N. O., & Bamigbetan, D. O. (2013). In vivo biochemical assessment of aqueous extract of Vernonia amygdalina (Bitter leaf). International Journal of Nutrition and Metabolism, 5(2), 22-7.
4) Mekha, M. S., Shobha, R. I., Rajeshwari, C. U., & Andallu, B. (2014). Aging and Arthritis: Oxidative Stress and Antioxidant Effects of Herbs and Spices. In R. V. Preedy (Ed.), Aging: Oxidative Stress and Dietary Antioxidants (1st ed., pp. 233-245). London, UK: Academic Press.
5) Omodamiro, O. D., & Ekeleme, C. M. (2013). Comparative study of in vitro antioxidant and antimicrobial activities of Piper guineense, Curcumina longa, Gongronema latifolium, Allium sativum Ocinum gratissimum. World Journal of Medicine and Medical Science, 1(4), 51-69.
6) San, S., Chakraborty, R., Verma, A., De, B., Devanna, N., & Dey, K. B. (2014). Evaluation of antioxidants activities of Saraca indica leaves extracts using in vitro and ex vivo models. Journal of Scientific and Industrial Research, 73, 157-62.
7) Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. S., & De, B. (2010). Free radicals, antioxidants, disease and phytomedicines: current status and future prospects. International Journal of Pharmaceutical Sciences Review and Research, 3(1), 91-100.
8) Shad, M. A., Nawaz, H., Rehman, T., & Ikram, N. (2013). Determination of some biochemicals, phytoc hemicals and antioxidant properties of different parts of Cichorium intybus L.: A comparative study. The Journal of Animal and Plant Sciences, 23(4), 1060-6.
9) Wachtel-Galor, S., Siu, P. M., & Benzie, I. F. (2014). Antioxidants, Vegetarian Diets and Aging. In R. V. Preedy (Ed.), Aging: Oxidative Stress and Dietary Antioxidants (1st ed., pp. 81-91). London, UK: Academic Press.