CORRELATION OF TOTAL POLYPHENOLIC CONTENTS WITH ANTIOXIDANT POTENTIALS OF AFRAMOMUM MELEGUETA AND PIPER GUINEENSE

Owoade, A.O.[1], Adetutu, A.[2], Olorunnisola, O.S.[3]

[1- 3] Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso. NIGERIA.

[1]aoowoade@lautech.edu.ng

ABSTRACT

This study compared the in-vitro antioxidant potential of Aframomum melegueta and Piper guineense. The results indicate that methanolic extracts of Aframomum melegueta and Piper guineense effectively scavenge DPPH radical cation in a dose and time-dependent manner with IC_{50} of 316.91µg/mL and 241.54 µg/mL respectively. Also the two extracts scavenge $ABTS^{\bullet+}$ radical cations in dose-dependent manner with TEAC value of Aframomum melegueta found to be 0.13 mmol/L while TEAC value of Piper guineense was found to be 0.27 mmol/L. In both cell free assays, Piper guineense was found to be more effective as an antioxidant when compared with Aframomum melegueta under the same experimental conditions. Piper guineense was found to contain higher amount of phenolic compounds (37.15 mg/g) than Aframomum melegueta (21.29 mg/g) in gallic acid equivalent. Therefore, the effectiveness of antioxidant activities of these two extracts may be related to their phenolic content.

Keywords: Aframomum melegueta, Piper guineense, oxidative stress, antioxidant

INTRODUCTION

Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko *et al.,* 2001; Maritim *et al.,* 2003). This occurs in biological systems when there is overproduction of ROS/RNS on one side and deficiency of enzymatic and non-enzymatic antioxidants on the other (Valko *et al.,* 2007). The excess ROS are harmful because they can damage cellular lipids, proteins, and DNA which are the most important biomolecules in the human body (Orhan *et al.,* 2006). Oxidative stress plays an important role in the pathogenesis of several degenerative or chronic diseases, such as atherosclerosis, cardiovascular diseases (CVD), stroke, diabetes, Parkinson's disease, renal disease, AIDS, and cancer (Dalle-Donne *et al.,* 2006).

Antioxidant defenses are extremely important as they represent the direct removal of free radicals, thus providing protection for biological sites (Cevalls-Casals and Cisneros-Zevallos, 2003). However, as this protection may not be sufficient to entirely prevent the damage by ROS/RNS, consumption of food rich in dietary antioxidants which offers a supportive role in antioxidant defense system in removing excessive ROS/RNS becomes even more important in protecting cell biomolecules against oxidative damage. Such antioxidant compounds present in human diet include vitamin E, vitamin C, carotenoids and phenolics (flavonoids and phenolic acids). These compounds are reported to play preventing role in the development of various pathological diseases (Schlesier *et al.,* 2002).

Aframomum melegueta and *Piper guineense* are two medicinal plants which their seeds are regularly added to food as seasoning and spice (Ilic *et al.,* 2010; Van Andel *et al.,* 2012; Ekanem *et al.,* 2010). *Aframomum melegueta* (*A. melegueta*) (family Zingiberaceae) is commonly known as Alligator pepper. In traditional medicine, the extract of the seed is used to relieve stomachache, diarrhoea, and inflammatory disorders (Ilic *et al.,* 2010; Gbolade, 2012). Several experimental evidences have shown that *A. melegueta* may exert antioxidant and antibacterial effects (Gabriel *et al.,* 2003; Ogbonna *et al.,* 1998) neuroprotective potentials (Adefegha and Oboh, 2012) and manage erectile dysfunction (Mbongue *et al.,* 2012). On its part, *Piper guineense* (*P. guineense*), commonly named African black pepper is a plant of the family of Piperaceae. It has culinary, medicinal, cosmetic and insecticidal applications (Arong *et al.,* 2011). In folk medicine, it is used in the treatment of rheumatism and bronchitis (Sofowora, 2008), cough, stomach disorder, intestinal diseases and gonorrhoea (Mensah *et al.,* 2008), obstetrics and fertility enhancement in women (Mbongue *et al.,* 2005), control of weight/obesity (Mba, 1994), the seeds as an aphrodisiac (Mbongue *et al.,* 2005) and in the treatment of mental illness (Odugbemi, 2008).

Although *A. melegueta* seeds have been shown to contain alkaloids (piperine) and essential oils (gingerol, shagaoland paradol) (Ajaiyeoba and Ekundayo, 1999; Ilic *et al.,* 2010), while *P. guineense* extracts has also been shown to contain numerous compounds such as alkaloids, sterols, lignans and amides (Noumi *et al.,* 1998). Till date, work that examine the phenolic content as well as determine contributory role played by phenolic compounds to the antioxidant effects of *A. melegueta* and *P. guineense* are scanty

Therefore in the present study, attempt was made to compare the total phenolic content of *A. melegueta* and *P. guineense* methanolic extracts as a basis for the *in vitro* antioxidant potential of the two extracts.

MATERIALS AND METHODS

Reagents

6-Hydroxy- 2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sufonic acid) ABTS, Gallic acid, thiobarbituric acid (TBA), were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). All other chemicals used were analytical grade.

Plant material Piper guineense and Aframomum melegueta

The seeds of *P. guineense* and *A. melegueta* were bought from Sabo market, in Ogbomoso. Identification and authentication of the seeds were carried out by Prof. A.J. Ogunkunle of Department of Pure and Applied Biology of Ladoke Akintola University of Technology, Ogbomoso. The seeds were dried at room temperature and blended to a coarse powder.

Preparation of Piper guineense and Aframomum melegueta extracts

Dried seeds of *P. guineense* and *A. melegueta* were milled into powdered. The powdered seed was extracted with methanol for 72 hours. The extract was filtered and the solvent was removed from the extract with a vacuum rotary evaporator at 45°C. The concentrated dried methanolic extract was then stored at -20°C before use.

Determination of total phenolic compounds in Piper guineense and Aframomum melegueta

The content of total phenolic compounds in *P. guineense* and *A. melegueta* were determined by Folin–Ciocalteu method as described by Miliauskas *et al.,* (2004). Briefly, 1 ml aliquots of 0.024, 0.075, 0.0105 and 0.3 mg/ml ethanolic gallic acid solutions were mixed with 5ml

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

Folin-ciocalteu reagent (diluted ten-fold) and 4ml (75g/L) sodium carbonate. The absorption was read after 30 min at 20^0C at 765 nm and the calibration curve was drawn. One ml of either *P. guineense* or *A. melegueta* (1mg/ml) were mixed with the same reagents as described above, and after l hour the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$C = c \cdot V/m'$

Where: C-total content of phenolic compounds, mg/g plant extract, in GAE; c-the concentration of gallic acid established from the calibration curve, mg/ml; V- the volume of extract, ml; m´- the weight of pure plant methanolic extract, g.

Trolox equivalent antioxidant capacity (TEAC) assay

The assay was performed essentially as described by Re *et al.,* (1999). ABTS radical cation was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–24 h before use. The $ABTS^{\bullet+}$ solution was diluted with water and adjusted to an absorbance of 0.700 ± 0.020 at 734nm. For the photometric assay, 1ml of the $ABTS^{\bullet+}$ solution and various concentrations of the extracts were mixed for 45 seconds and measured immediately after 1 minute at 734nm. The antioxidant activity of the extracts was calculated by determining the decrease in absorbance at different concentrations by using the following equation.

% antioxidant activity = $((A_{(ABTS^{\bullet+})} - A_{(Extracts)}) / (A_{(ABTS^{\bullet+})}) \times 100$.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

The assay was performed as previously described by Schelesier *et al.,* (2002). The radical solution is prepared by dissolving 2.4 mg $DPPH^{\bullet}$ in 100 ml methanol. For the photometric assay 1.95 ml $DPPH^{\bullet}$ solution and 50 µl antioxidant solution were mixed. At first, the absorbance of the disposable cuvette with 1.95 ml $DPPH^{\bullet}$ was measured as blank, then the antioxidant solution was added and mixed. The reaction was measured at 5 min interval at 515 nm until $\Delta A=0.003$ min^{-1}. The anti-oxidative activity was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

% Inhibition activity = $((A_{(DPPH^{\bullet})} - A_{(Extracts)}) / (A_{(DPPH^{\bullet})}) \times 100$

Statistical analysis

Results are expressed as means ± SEM. Statistical analyses were performed using one way analysis of variance followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and $p < 0.05$ was considered statistically significant.

RESULTS

Trolox equivalent antioxidant capacity (TEAC) assays [mmo]I^{-1} of trolox, gallic acid, *Piper guineense* and *Aframomum melegueta*

In TEAC assay, the inhibition of $ABTS^{\bullet+}$ radical cation was directly related to the concentration of the extracts and the TEAC value of Trolox is 1.00 (Table 1 & Figures 1). Gallic acid responded as the strongest antioxidant in the assay while *P. guineense* showed greater antioxidant activity than *A. melegueta* (Table 1 & Figure 1).

Table 1. Trolox equivalent antioxidant capacities (TEAC) (mmol/L) of trolox, gallic, *Piper guineense* and *Aframomum melegueta*

Sample	*Trolox equivalent antioxidant capacities (TEAC)*
Trolox	1.00
Gallic	4.25 ± 0.12
Piper guineense	0.27 ± 0.03
Aframomum melegueta	0.13 ± 0.01

Values are the means of three experiments ± SEM.

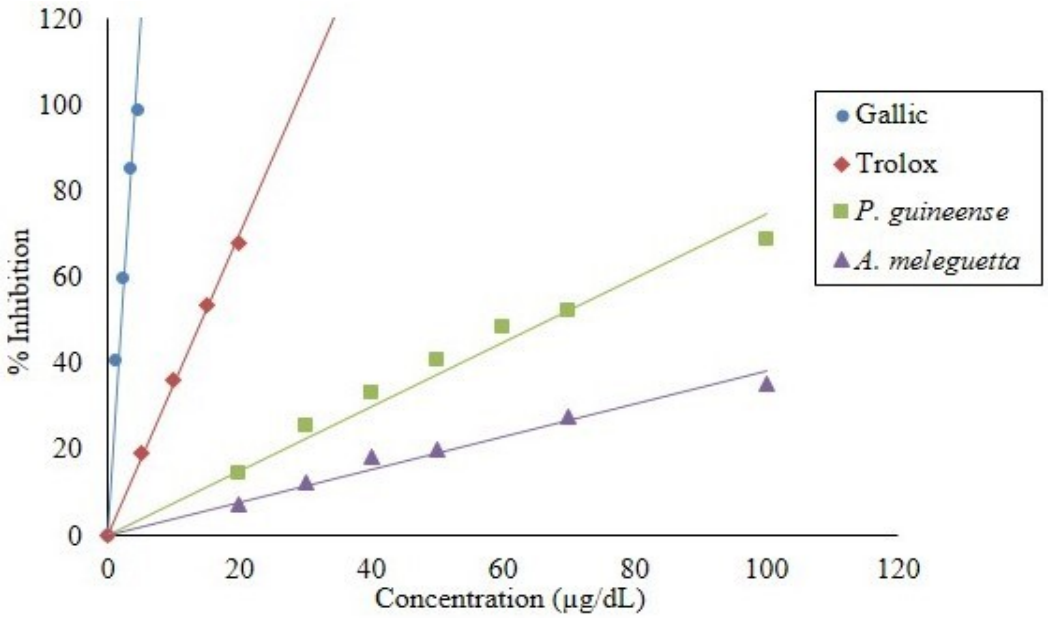

Figure 1. The effects of different concentrations of Gallic, Trolox, *Piper guineense* and *Aframomum melegueta* on the inhibition of the ABTS radical. Values are the means of three experiments ± SEM.

Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of *Piper guineense* and *Aframomum melegueta*

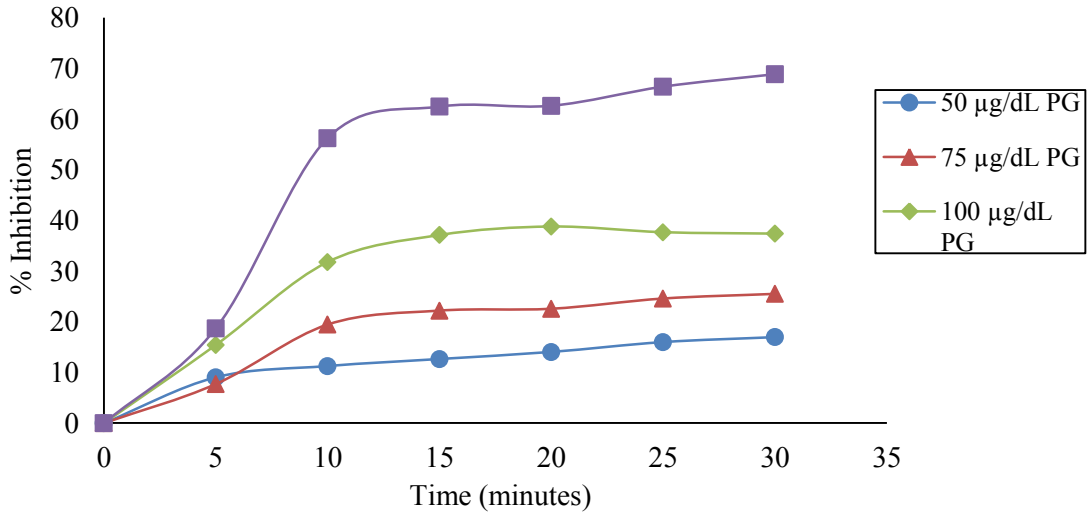

Figure 2. The effects of time on different concentration of methanolic extract of *Piper guineense* on inhibition of DPPH radical

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

P. guineense and *A. melegueta* demonstrated a concentration and time dependent scavenging activity by quenching DPPH radicals (Figure 2 & 3) and was compared with gallic acid, as a positive control. The IC_{50} values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by *P. guineense*, *A. melegueta* and gallic acid were 241.54 ± 5.28 µg/dL, 316.91 ± 6.71 µg/dL and 16.32 ± 1.50 respectively (Table 2).

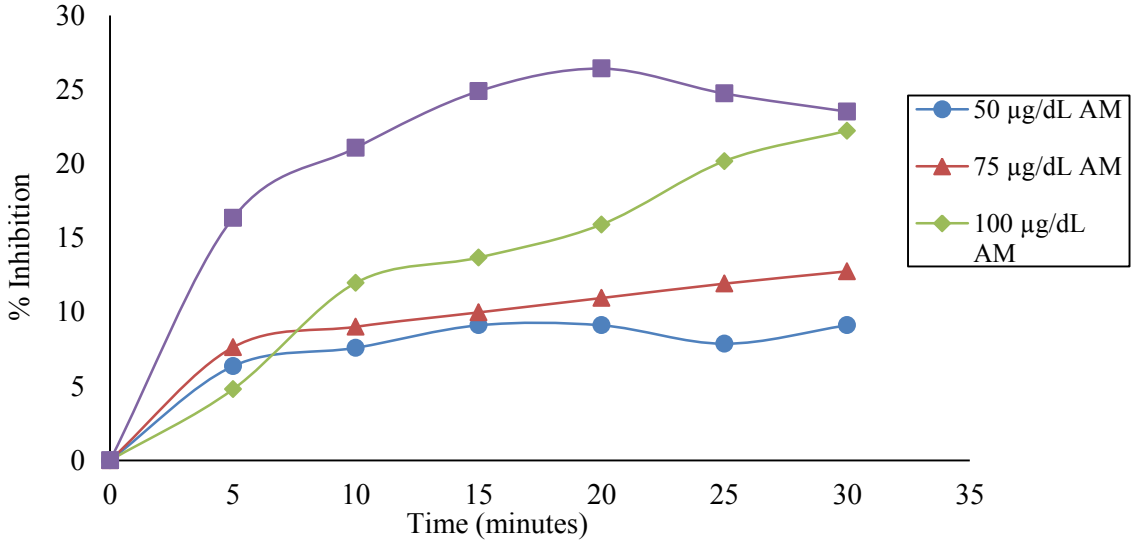

Figure 3. The effects of time on different concentration of methanolic extract of *Aframomum melegueta* on inhibition of DPPH radical

Table 2. DPPH radical scavenging value of Gallic acid, *Piper guineense* and *Aframomum melegueta* (µg/mL)

Sample	*DPPH scavenging activity (IC_{50})*
Gallic	16.32 ± 1.50
P. guineense	241.54 ± 5.28
Aframomum melegueta	316.91 ± 6.71

Values are the means of three experiments ± SEM

The Total Phenolic Content of *Piper guineense* and *Aframomum melegueta*

The phenolic contents of *P. guineense* and *A. melegueta* were determined using Folin-Ciocalteu assay and by constructing a standard curve using gallic acid. The total amount of phenolic compounds present in *P. guineense* and *A. melegueta* were found to be 37.15 ± 2.62 and 21.29 ± 1.12 mg/g respectively in Gallic acid equivalent (Table 3)

Table 3. The total phenolic content of *Piper guineense* and *Aframomum melegueta* in mg gallic acid equivalent/g dry weight.

Sample	*Total phenol*
P. guineense	37.15 ± 2 .62
Aframomum melegueta	21.29 ± 1.12

Values are the means of three experiments ± SEM

DISCUSSION

The present study investigated the antioxidant potential of *A. melegueta* and *P. guineense.* Among the various methods used to evaluate the total antioxidant activity of vegetables or other plants, the $ABTS^{\bullet+}$ and DPPH radical scavenging assay are among the common applied methods. The abilities of antioxidants to scavenge the pre-formed $ABTS^{\bullet+}$ radical cation measured in TEAC value have been reported to be influenced by the presence of functional groups and number of conjugated double bonds in carotenoids (Miller *et al.,* 1996), as well as polyphenols and ascorbic acid content in the beverages (Schlesier *et al.,* 2002). The ABTS radical-scavenging study revealed that *P. guineense* has stronger antioxidants potential than *A. melegueta*. The result also shows that inhibition of the $ABTS^{\bullet+}$ radical by the two antioxidants is concentration dependent.

DPPH is a dark-coloured crystalline powder composed of stable free radical molecules. DPPH assay is based on its change of colour from Violet to pale yellow when mixed with a substance that can donate hydrogen atom. The colour change is based on the transformation from the oxidized to its reduced form and this makes it a useful tool in assessing antioxidant activities of phytochemicals in vitro (Molyneux, 2004). The quantitative DPPH assay results provided here revealed the concentration and time-dependent antioxidant activity of methanolic extract of *P. guineense* with IC_{50} of 241.54 ± 5.28 g/ml and that of *A. melegueta* with IC_{50} of 316.91 ± 6.71 g/ml, both comparable to that of the standard compound Gallic acid (IC_{50} of 16.32 ± 1.50 g/ml). The results also indicates that *P. guineense* has higher DPPH radical scavenging ability than *A. melegueta*. Although, IC_{50} of the extracts are low when compared to that of Gallic acid, but the result showed the hydrogen donating ability of the active component of the two extracts.

Antioxidant activities of plant extracts were usually linked to their phenolic content, though it is well accepted that non phenolic antioxidants might also contribute to the antioxidant activity of plant extract (Hassimotto *et al.,* 2005; Harish and Shivanandappa, 2006). Phenolic compounds are commonly found in the plant kingdom, and they have been reported to have multiple biological effects, including antioxidant activity (Schroeter *et al.,* 2002; Fraga, 2007). In the present study, the amount of phenolic compounds in *A. melegueta* and *P. guineense* were determined. The content of phenolic compounds in methanolic extract of *P. guineense* was found to be 37.15 ± 2 .62 (mg GAE/g of extract in dried weight) which is higher than total phenolic content in *A. melegueta* found to be 21.29 ± 1.12 (mg GAE/g of extract in dried weight).

A detailed look at the two cell-free antioxidant assays used in this study showed *P. guineense* to respond as strongest antioxidants in the two assays. *P. guineense* also contained higher amount of phenolics than *A. melegueta* suggesting that the effectiveness of the antioxidant activity of the extracts may be associated with phenolic content. It is proposed that the phenolic compounds of *A. melegueta* and *P. guineense* may play an important role in the observed antioxidant activities of the extracts. Although antioxidant potential of *A. melegueta* and *P. guineense* have been ascribed to polyphenols content of the two extracts in this study, but other antioxidant compounds such as ascorbic acid that are not subject of this study may also contribute.

CONCLUSION

The result of this study indicates that extracts of *A. melegueta* and *P. guineense* scavenge both $ABTS^{\bullet+}$ and DPPH radical cations and these abilities are found to be dose-dependent. In the two antioxidant assays, *P. guineense* is found to be more effective as an antioxidant when compared with antioxidant ability displayed by *A. melegueta*. The same extract also has

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

higher amount of phenolic compounds than *A. melegueta* suggesting that antioxidant activities of these two extracts may be related to their phenolic content. Therefore, the strong antioxidant properties and the presence of phenolic compounds in *A. melegueta* and *P. guineense* may justify their popular consumption and usage in herbal medicine.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.

85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Faculty of Basic Medical Sciences,

LAUTECH, Ogbomoso ethics committee.

REFERENCES

[1] Adefegha, S. A., & Oboh, G. (2012). Acetylcholinesterase (AChE) inhibitory activity: Antioxidant properties and phenolic composition of two Aframomum species. *J. Basic Clin. Physiol. Pharmacol.*, *23*, 153–161.

[2] Ajaiyeoba, E. O., & Ekundayo, O. (1999). Essential oil constituents of Aframomummelegueta (Roscoe) K. schum seeds (Alligator pepper) from Nigeria, *FlavourFragrance J.*, *14*, 109–111.

[3] Arong, G. A., Oku, E. E., Obhiokhenan, A. A., Adetunji, B. A., & Mowang, D. A. (2011). Protectantability of Xylopiaaethiopica and *P. guineense* leavesagainstthe cowpea bruchid Callosobruchusmaculatus (Fab.)(Coleoptera: Bruchidae). *World J. Sci.Technol.*, *1*(7), 14–19.

[4] Cevalls-Casals, B. A., & Cisneros-Zevallos, L. (2003). Stoichimetric and kinetic studies of antioxidants from Audean purple corn and red—fleshed. *Chemistry*, *51*, 3313-3319.

[5] Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D., & Milzani, A. (2006). Biomarkers of oxidative damage in human disease. *Clin Chem.*, *52*, 601-623.

[6] Ekanem, Q., Udoh, V., & Oku, E. (2010). Effects of ethanol extract of Piperguineense seeds (Schum. and Thonn) on the conception of mice (MusMusculus). *Afr. J. Pharm. Pharmacol.*, *4*, 362–367.

[7] Fraga, C.G. (2007). Plant polyphenols: How to translate their *in vitro* antioxidant actions to *in vivo* conditions. *International Union of Biochemistry and Molecular Biology Life* (*IUBMB LIFE*), *59*, 308-315.

[8] Gabriel, A. O., Makinde, O., Falade, O. K., Phyllis, I., & Peters, U. (2003). Extraction andcharacterization of antioxidants from *A. melegueta*and Xylopiaaethiopica, *Eur. Food Res. Technol.*, *216*, 526–528.

[9] Gbolade, A. (2012). Ethnobotanical study of plants used in treating hypertension in Edo State of Nigeria. *Journal of Ethnopharmacology*, *144*, 1–10.

[10] Harish, R., & Shivanandappa, T. (2006). Antioxidant activity andhepatoprotective potential of *Phyllanthusniruri*. *Food Chem.*, *95*(2), 180-185.

[11] Hassimotto, N., Genovese, M., & Lajolo, F. (2005). Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J. Agric. Food Chem., 53*(8), 2928-2935.

[12] Ilic, N., Schmidt, B.M., Poulev, A., & Raskin, I. (2010). Toxicological evaluation of Grains of Paradise (Aframomummelegueta) [Roscoe] K. Schum. *Journal of Ethnopharmacology, 127*, 352–356.

[13] Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: *Journal Biochemistry and Molecular Toxicology, 17*, 24–38.

[14] Mba, M.A. (1994). Effect of dietary intake of *P. guineense* on growth and indices of fitness in Rattusrattus. *Isc.Innoa*, *4*, 383–388.

[15] Mbongue, F.G.Y., Kamtchouing, P., Essame, O.J.L., Yewah, P.M., Dimo, T., & Lontsi, D. (2005). Effect of the aqueous extract of dry fruits of *P. guineense* on the reproductive function of adult male rats. *Indian J. Pharmacol*., *7*, 30–32.

[16] Mbongue, G. Y. F., Kamtchouing, P., & Dimo, T. (2012). Effects of the aqueous extract of dryseeds of *A. melegueta*on some parameters of the reproductivefunction of mature male rats, *Andrologia*, *44*, 53–58.

[17] Mensah, J.K., Okoli, R.I., Ohaju-Obodo, J.O., & Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *Afr. J. Biotechnol.*, *7* (14), 2304–2309.

[18] Miliauskas, G., Venskutonis, P., & Beek, T. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem*., *85*, 231-237.

[19] Miller, N.J., Sampson, J., Vandeias, L.P., Bramley, P.M., & Rice-Evans, C.A. (1996). Antioxidant activities of carotenes and xanthophylls. *FEBS Letters*, *384*, 240-242.

[20] Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity Songklanakarin. *J. Sci. Technol*., *26* (2), 211-219.

[21] Noumi, E., Amvam, W., & Lontsi, D. (1998). Aphrodisiac plants used in Cameroon. *Fitoterapia*, *69*, 5–34.

[22] Odugbemi, T. (2008). *A textbook of medicinal plants from Nigeria*. Lagos, Nigeria: University of Lagos Press.

[23] Ogbonna, C. N., Nozaki, K., & Yajima, H. (1998). Antimicrobial activity of Xylopiaaethiopica: *A. melegueta*and *P. guineense*ethanolic extracts and the potential of using Xylopiaaethiopica to preserve fresh orange juice. *Afr. J.Biotechnol*., *12*, 1993–1998.

[24] Orhan, H., Orhan, G. H., Vriese, E., Vermeulen, N. P. E., & Meerman, J. N. N. (2006). Application of lipid peroxidation and protein oxidation biomarkers for oxidative damage in mammalian cells: A comparison with two fluorescent probes. *Toxicology in Vitro*, *20*, 1005-1013.

[25] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M.,& Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cationdecolorization assay. *Free Radical Biology and Medicine*, *26* (9–10), 1231–1237.

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

[26] Schlesier, K., Harwat, M., Böhm, V., & Bitsch, R. (2002). Assessment of Antioxidant Activity by Using Different *in Vitro* Methods. *Free Radical Research*, *36*, 177-187.

[27] Schroeter, H., Boyd, C., Spencer, J.P.E., Williams, R. J., Cadenas, E., & Rice-Evans, C. (2002). MAPK signaling in neurodegeneration: Influences of flavonoids and of nitric oxide. *Neurobiology of Aging*, *23*, 861-880.

[28] Sofowora, A. (2008). *Medicinal plants and traditional medicine in Africa*. Ibadan: Spectrum Books Limited.

[29] Turko, I. V., Marcondes, S., & Murad, F. (2001). Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. *American Journal of Physiology, Heart and Circulatory Physiology*, *281* (6), H2289-94.

[30] Valko, M., Leibfritz, D., Moncola, J., Mark T.D., Cronin, M. T. D., Mazura, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology, 39*, 44–84.

[31] Van Andel, T., Myren, B., & Onselen, S. (2012). Ghana's herbal market. *Journal of Ethnopharmacology, 140*, 368–378.