Journal of Scientific Research & Reports
10(6): 1-9, 2016; Article no.JSRR.25930
ISSN: 2320-0227

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Some Antinutritive and Antioxidative Properties of Pulverized *Citrus sinensis* (Sweet Orange) Peels and Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Author ACCE designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors RIU and CEO managed the literature searches and analyses of the study author ODO cross-checked the methods and reported results. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2016/25930
Editor(s):
(1) Tzasna Hernandez Delgado, Laboratory of Pharmacognosie, Biology Unit and Prototypes (UBIPRO). National Autonomous University of Mexico, Mexico.
Reviewers:
(1) Myrene Roselyn D'souza, Mount Carmel College, India.
(2) Maria Luisa Kennedy, Universidad Nacional De Asuncion, Paraguay.
Complete Peer review History: http://sciencedomain.org/review-history/14336

Original Research Article

Received 26th March 2016
Accepted 12th April 2016
Published 26th April 2016

ABSTRACT

Introduction: *Citrus sinensis* (sweet orange) fruit peels and seeds may have antinutriitve and antioxidative properties but are essentially discarded with abundant waste generation.
Aim: This study evaluated some antinutrients and antioxidative property of pulverized *Citrus sinensis* (sweet orange) peels and seeds, using standard methods.
Place and Duration of Study: The study was conducted at the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria between May and August, 2015.
Methodology: The antinutrients were determined by standard methods. The antioxidative property was measured as the 2,2-Diphenyl-1-picryhydrazyl (DPPH) scavenging activity of the respective sample at 100 mg/ml. The potency was measured by estimating the half maximal effective concentration (EC_{50}).
Results: The hydrogen cyanide content in the peels (18.16±0.06 µg/g) was higher ($p<0.05$) than that in the seeds (11.67±0.07 µg/g) or any other determined antinutrient followed by phenol in the

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peels (1.06±0.05 mg/100 g) and flavonoid in the seeds (0.69±0.03%). The DPPH radical scavenging activity (percentage inhibition, %) was higher ($p<0.05$) in the peels (68.58±0.02) than in the seeds (43.73±0.02). Similarly, the EC_{50} (µg/ml) was higher ($p<0.05$) in the peels (937.40±0.10) than in the seeds (658.60±0.21).

Conclusion: The antinutrient mix in the peels may confer it with higher cyanide toxicity, higher antioxidant activity but lowered antioxidant potency than the seeds. The study seemingly underscores the importance of hydrogen cyanide in the antioxidative activity and potency of the samples. This could be central to other food wastes, warranting further studies aimed at determining and possibly eliminating hydrogen cyanide content of these and other food wastes.

Keywords: Potency; antioxidant; hydrogen cyanide; EC_{50}; DPPH; antinutrients.

1. INTRODUCTION

The practice of consuming the juice of most fruits while discarding the peel and seed could contribute to increasing solid food wastes with potential adverse environmental and public health implications [1,2]. Reactive Oxygen Species (ROS) were implicated in unhealthy states, including cancer, coronary heart disease and aging [3]. The effects of ROS could be mitigated by natural antioxidants present in plants and plant parts [4]. This could explain the use of various plants with antioxidant properties in ethnomedication [5] and for reducing the risk of immune and infectious diseases [6]. In particular, antioxidant potential of plants and plant parts was associated with the presence of phenols and flavonoids [7] which are antinutrients. The antioxidant activity of natural products could be measured by DPPH procedure [8] among other methods [9]. High contents of antioxidant compounds such as polyphenols and flavonoids have high potential to diminish the negative effects of free radicals as a result of their electronic and molecular structures [10]. It is interesting to find out if the peels and seeds contained antinutrients and had antioxidant property. In particular, antioxidant property is of interest in mitigating adverse effects of reactive oxygen species that underlie serious health problems, including cancer [10].

The seeds and peels of grapes and pomegranates are rich in natural antioxidant [11] while the seeds and peels of watermelon have nutritive and antimicrobial properties [12-15,2]. Possible roles of bioactive compounds in improving and managing even metabolic diseases have been suggested [16,17]. Sweet orange fruits contain vitamin C, fiber, as well as other bioactive components, including carotenoids and phenolic compounds [18]. The edible orange fruits juice has antioxidant property [19] attributable to the rich vitamin C, flavonoids and phenolic compounds contents. *Citrus limonum* (lemon) contain esculetin, a bioactive compound that improved markers of health functions in rats [20] while the sweet orange peel essences had antiseptic, analgesic and anti inflammatory values [21,22]. Human health challenges are seemingly ever growing and could result from adverse effects of foods and food condiments [23,24] warranting constant search for scientific basis to utilize plants/plant parts, fruits and fruit wastes as antioxidants. Studies on orange fruits were reported [25,1], but not essentially on the antinutrient composition or the potency and antioxidative properties of the peels and seeds. The food and pharma-food potentials of a food source could be further assessed through the antinutrient compositions of the food source while the possible pharmacologic properties could be assessed through the potency and antioxidative activities, warranting the present study of the pulverized/milled peels and seeds from *Linnaeus osbeck* variety of sweet orange (*Citrus sinensis*).

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The chemicals used, including those used in the preparation of reagents, were of analytical grade and products of reputable companies, including Sigma Chemical Company, St. Louis, U.S.A. May and Baker, Dagenham, England and British Drug House (BDH) Ltd., Poole, England. This study was conducted between May and August, 2015 at the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria.

2.2 Collection, Identification and Preparation of Samples

The sweet orange fruits were purchased from a particular supplier at Eke-Okigwe market, a weekly market in Okigwe Imo state, Nigeria. The

fruits were identified as *Linnaeus osbeck* variety of the *Citrus sinensis* (sweet orange) by a taxonomist in the Central Laboratory Unit of National Root Crop Research Institute Umudike, Nigeria. As described in a similar study [1], the orange fruits were thoroughly washed to remove sand and unwanted particles and sliced longitudinally into four equal parts, using a home choice European knife. The juicy flesh or pulp containing the seeds was carefully removed from the peels. The seeds were carefully picked from the pulp and washed off the orange juice using clean water while the peels were chopped into bits. The samples were separately placed on a foil and weighed with a Satorious Digital Weighing Balance, Model BP210S, Germany before and after sun drying for seven days to obtain the respective wet weight (seeds = 86.05 g, peels =169.74 g), dry weight (seeds =46.89 g, peels = 153.12 g) and percentage yield (seeds = 54.49%, peels = 90.21%). The respective dry weight sample was separately pulverized into powder using Arthur Thomas Laboratory Mill Crypto model, USA, covered separately in a labeled white nylon and kept in the desiccator until used.

2.3 Determination of Antinutrients

The alkaloid content was determined by the alkaline precipitation gravimetric method [26] while the flavonoid content was determined by gravimetric method [26]. The tannin content was determined by the Folin- Dennis spectrophotometric method [27] whereas the saponin content was determined by the double solvent extraction gravimetric method [26]. The hydrogen cyanide content was determined by the alkaline picrate colorimetric method [28] while the phytate content was determined by the colorimetric method described by Oberleas [29]. The phenol content was determined by the method in Association of Official Analytical Chemists, AOAC [30].

2.4 Determination of Antioxidant Activity (2,2-Diphenyl-1-picryhydrazyl (DPPH) Radical Scavenging Activity) and Potency or Half Maximal Effective Concentration (EC_{50})

2,2-Diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity was determined by the method of Bougatef et al. [31] as modified by Ladda et al. [32]. The respective sample, respectively dispersed in distilled water at concentration of 2% protein (w/v) DPPH radical scavenging activity, was incubated for 30 minutes at 37ºC. Distilled water was used as a blank. The radical scavenging capacity of the respective sample extract was determined by using Jenway Digital Spectrophotometer (Model 6320D, UK) set at 550 nm to measure the disappearance of DPPH. A decreasing DPPH solution absorbance indicates an increase in the DPPH radical-scavenging activity. This activity is given as percentage DPPH radical-scavenging as calculated from the equation:

$$\%\ DPPH\ radical\text{-}scavenging = (AC - AS)/AC \times 100,$$

Where AC is the absorbance of the control solution (containing only DPPH), AS is the absorbance of the sample in DPPH solution.

The percentage of DPPH radical-scavenging was plotted against the sample concentrations (µg/ml) to determine the concentration of the sample required to scavenge DPPH by 50% which is the antioxidant potency or half the maximal effective concentration (EC_{50}).

2.5 Data Analysis

The data obtained by triplicate determinations were subjected to analysis of variance (ANOVA) using SPSS 16.0 for Windows. Comparison of difference in means was based on Students t-test. Difference in mean at a p value < 0.05 was regarded as statistically significant. Results were expressed as mean± standard deviation (SD).

3. RESULTS AND DISCUSSION

As shown in Table 1 and Figs. 1 and 2, the hydrogen cyanide content in the peels (18.16±0.06 µg/g) was higher ($p<0.05$) than that in the seeds (11.67±0.07 µg/g) or any other determined antinutrients followed by phenol in the peels (1.06±0.05 mg/100 g) and flavonoid in the seeds (0.69±0.03%).

The DPPH radical scavenging activity/percentage inhibition (%) was higher ($p<0.05$) in the peels (68.58±0.02) than in the seeds (43.73±0.02). Similarly, the EC_{50} (µg/ml) was higher ($p<0.05$) in the peels (937.40±0.10) than in the seeds (658.60±0.21) (Table 2).

Table 1. Some antinutritive composition of pulverized *Citrus sinensis* (sweet orange) peels and seeds

Antinutrients	Peels	Seeds	Difference
Phenols (Mg/100 g)	1.06±0.05[b]	0.36±0.10[a]	±0.7*
Flavonoids (%)	0.37±0.01[b]	0.69±0.03[a]	±0.32*
Phytates (%)	0.12±0.10[a]	0.37±0.02[b]	±0.25*
Saponins (%)	0.24±0.04[b]	0.19±0.02[b]	±0.05[ns]
Hydrogen cyanide (µg/g)	18.16±0.06[a]	11.67±0.07[b]	±6.49*

*Result = Value ± SD of triplicate determinations. ns = difference is not significant (p > 0.05). * = difference is significant (p < 0.05)*

Table 2. DPPH radical scavenging activity (%) and the concentration of pulverized *Citrus sinensis* (sweet orange) peels and seeds at 50% DPPH radical scavenging activity 50% (EC_{50}) (ug/ml)

Sample	DPPH scavenging activity or percentage inhibition (%)	Concentration of samples at DPPH radical scavenging activity 50% (DPPH EC_{50} (ug/mL))
Orange peels	68.58±0.02*	937.40±0.10*
Orange seeds	43.73±0.02*	658.60±0.21*
Difference	±24.85*	±278.08*

*Result = Value ± SD of triplicate determinations. ns = difference is not significant (p > 0.05). * = difference is significant (p < 0.05)*

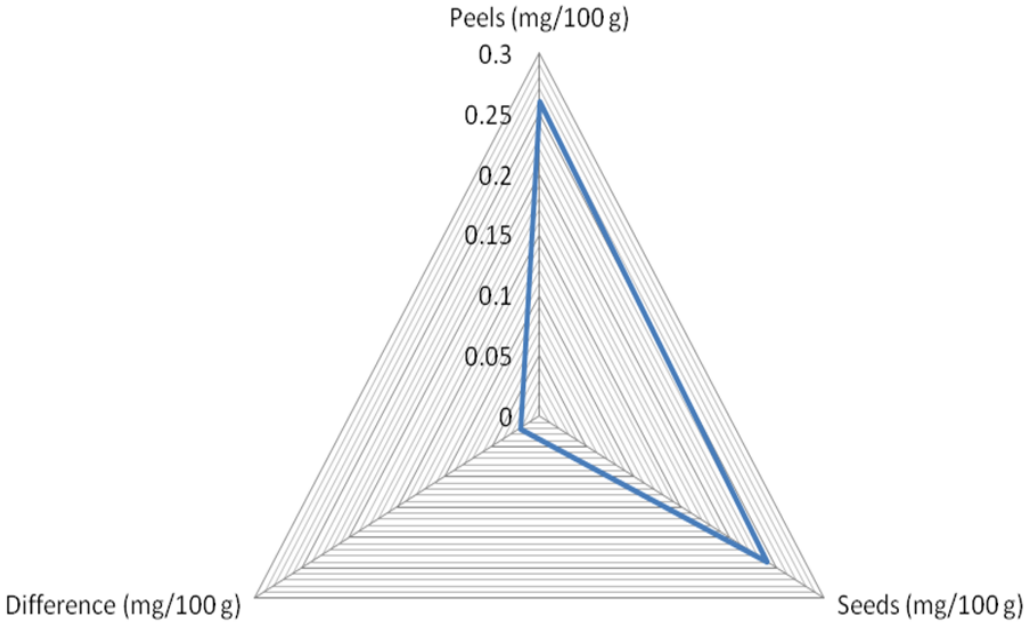

Fig. 1. Tannins composition (mg/100 g) of pulverized *Citrus sinensis* (sweet orange) peels and seeds

[ns] indicates non significant difference p>0.05)

The sweet orange (*Citrus sinensis)* peels and seeds are usually discarded as food wastes. This could adversely affect the environment and public health. Studies that could provide scientific basis are needed for enhanced beneficial use of these wastes to reduce the burden on the environment. Thus, the present study investigated some antinutritive and anitoxidative properties of the *Citrus sinensis* (sweet orange) peels and seeds. The result could provide basis for the possible antioxidant property of these fruit wastes.

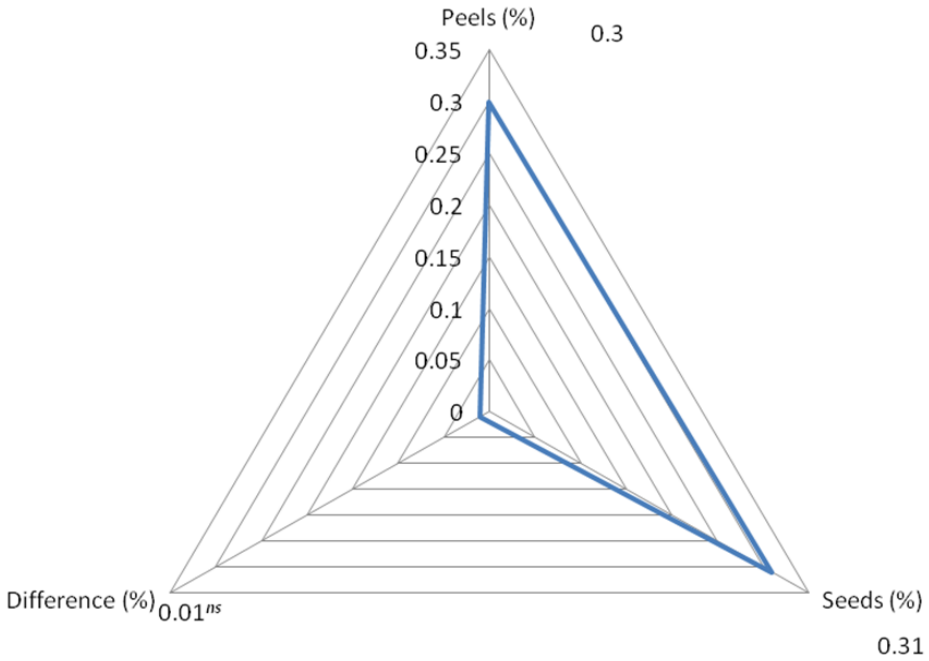

Fig. 2. Alkaloids composition (%) of pulverized *Citrus sinensis* (sweet orange) peels and seeds
ns indicates non significant difference p>0.05)

Hydrogen cyanide content in the respective sample (peels and seeds) was highest among the other determined antinutrients followed by phenols in the peels (1.06±0.05 mg/100 g) and flavonoids in the seeds (0.69±0.03%). This suggests that the peels more than the seeds could be susceptible to deterioration. It also suggests apparent higher cyanide-related toxicity of the peels than the seeds when consumed by animals. The significantly ($p<0.05$) higher hydrogen cyanide content in the peels than in the seeds was lower than the range (40.80 to 42.82 mg/100 g) reported in varieties of groundnut seeds [33]. Hydrogen cyanide (HCN) is a toxic compound, and the toxicity was associated with its dissociation product, cyaninde ion which could be eliminated or reduced by processing. Processing altered food compositions [34,35], hence adequate processing methods for these samples are advocated. Saponins, the glycosidic compounds found in most of the plants, exerted both beneficial and adverse effects on human health [36]. The saponins content in either the peels or the seeds was quite low compared to its median lethal dose, LD_{50} [37], hence could be expected to elicit health benefits when consumed by humans.

Flavonoids, tannins, and phenols are the main phenolic compounds [38], known to protect plants against micro-organisms with possible potential to improve animal health and elicit antioxidant activity. The antioxidant activity of the phenolic compounds was linked to the presence of hydroxyl groups in their structures [39], but the resultant multiple hydrogen bonds with the carboxylic group of the dietary proteins and enzymes could ultimately, reduce the digestibility of proteins with accompanying adverse health effects related to altered protein metabolism. Beside the antioxidant activity, these phenolic compounds contribute to the nutritional and commercial uses *via* their sensory properties [40,41]. Tannins content in the seeds was lower ($p>0.05$) than in the peels. The tannins content in these samples was much lower than those reported for groundnut seeds [33] and for leafy vegetables [42]. The higher phenols in the peels (1.06±0.05 mg/100 g) compared to that in the seeds suggests apparent higher antioxidant property in the peels than in the seed. Higher phenolic compounds caused higher antioxidant activity [43]. Antioxidant potential of plants and plant parts was associated with the presence of phenolic compounds [7] which due to the presence of one or more hydroxyl groups have the potential to quench free radicals by forming stabilized phenoxyl radicals [44,45].

Free radicals-mediated oxidative damage to proteins, lipids and nucleic acids in plant foods results to food deterioration due to resultant toxic

compound products [46] which explains why antioxidants could maintain the nutritional value of foods [36]. The DPPH assay is a rapid and sensitive method for the scavenging activity of antioxidant as it could form a stable product after accepting an electron or hydrogen from an antioxidant [47]. The reducing power, hence antioxidant activity of a compound is related to its electrons donating ability [48]. The DPPH· scavenging capacity for the peels (68.58±0.02%) was higher ($p<0.05$) than for the seeds (43.73±0.02%) suggesting that the peels antinutrient mix had good antioxidant properties or a good electron donating capability to scavenge free radicals. Flavonoids and phenolic compounds might contribute to higher antioxidant activity as phenolic hydroxyl groups act as a reducing agent by donating electron. Furthermore, higher DPPH radical scavenging activity was consistent with higher total phenolic content and *vice versa* implying a positive relationship between the polyphenolic content and DPPH radical scavenging activity [8]. Generally, EC_{50} which is the half maximal effective concentration or the concentration of drug that induces a response half way between the baseline and maximum after a given time is a measure of drug potency. Thus, potency (EC_{50}), could be determined by measuring the concentration of drug sample required to produce 50% of the maximum possible effect (EC_{50}). However, the EC_{50} with the least concentration had the highest radical scavenging effect, hence the highest potency [44]. This implies that the smaller the value of EC_{50} is, the more potent the drug sample. The EC_{50} (ug/ml) was higher ($p<0.05$) in the peels (937.40±0.13) than in the seeds (658.60±0.42), suggesting that the seeds had higher antioxidant potency than the peels [44] and that the seeds could be better source of potent natural antioxidants [40].

Interestingly, the DPPH radical scavenging activity/percentage inhibition (%) was higher ($p<0.05$) in the peels than in the seeds (Table 2) in apparent contradiction of the result and suggestion on the phenol content in this study. The reason for these contradictions, notably the apparent higher antioxidant activity but lower antioxidant potency of the peels compared to the seeds, was not explored. The reasons, however, could be linked to the higher hydrogen cyanide content of the peels recorded in this study. The reducing power, hence antioxidant activity of a compound relates to its electrons donating ability [48], seemingly implicating the high hydrogen cyanide content in the peels that could readily dissociate into hydrogen and cyanide ion. Furthermore, hydrogen cyanide-induced transient accumulation of reactive oxygen species, though during seed germination, has been reported [49]. Such ROS accumulation could overwhelm the antioxidant capacity of the peels resulting to the lowered antioxidant potency. Further studies are therefore warranted to verify these speculations as the observation may underscore the importance of hydrogen cyanide in the antioxidative activity and potency of other food wastes and natural products.

4. CONCLUSION

The antinutrient mix in the peels may confer it with higher cyanide toxicity, higher antioxidant activity but lowered antioxidant potency than the seeds. The study seemingly underscores the importance of hydrogen cyanide in the antioxidative activity and potency of the samples. This could be central to other food wastes, warranting further studies aimed at determining and possibly eliminating hydrogen cyanide contents of these and other food wastes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Egbuonu ACC, Osuji CA. Proximate compositions and antibacterial activity of *Citrus sinensis* (sweet orange) peel and seed extracts. European Journal of Medicinal Plants. 2016;12(3):1-7. DOI: 10.9734/EJMP/2016/24122
2. Egbuonu ACC, Aguguesi RG, Samuel R, Ojunkwu O, Onyenmeri F, Uzuegbu U. Some physicochemical properties of the petroleum ether-extracted watermelon (*Citrullus lanatus*) seed oil. Asian Journal of Scientific Research. 2015;8(4):519-525. DOI: 10.3923/ajsr.2015.519-525
3. Gulcin I. Antioxidant activity of food constituents: An overview. Arch. Toxicol. 2012;86:345-391.

4. Fazlina M, Zurina H, Mohd ZA. Antioxidant activity of *Cinnamomum iners* leaves standardized extract. Asian Journal of Biochemistry. 2016;11:90-96. DOI: 10.3923/ajb.2016.90.96
5. Kumaran A, Karunakaran RJ. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus.* Food Chem. 2007;100:356-361.
6. Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and antiproliferative activities of red pitaya. Food Chem. 2006; 95:319-327.
7. Chua MT, Tung YT, Chang ST. Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. Bioresour. Technol. 2008;99:1918-1925.
8. Ajibola CF, Oyerinde VO, Adeniyan OS. Physicochemical and antioxidant properties of whole-wheat biscuits incorporated with *Moringa oleifera* leaves and cocoa powder. Journal of Scientific Research & Reports. 2015;7(3):195-206.
9. Moure A, Dominguez H, Parajo JC. Antioxidant properties of ultrafiltration recovered soy protein fractions from industrial effluents and their hydrolysates. Process Biochemistry. 2006;41(2):447–456.
10. Romeilah RM. Chemical compositions, antioxidant, anticancer activities and biological effects of *Myrtus communis L*. and *Origanum vulgare* essential oils. Asian Journal of Biochemistry. 2016;11:104-117. DOI: 10.3923/ajb.2016.104.117
11. Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed *Vitis vinifera* extracts on peroxidation models *in-vitro:* Fd. Chem. 2001;73:285-290.
12. Egbuonu ACC. Comparative investigation of the proximate and functional properties of watermelon (*Citrullus lanatus*) rind and seed. Res. J. of Environ. Toxicol. 2015a;9(3):160-167.
13. Egbuonu ACC. Assessment of some antinutrient properties of watermelon (*Citrullus lanatus*) rind and seed. Res. J. Environ. Sci. 2015b;9(5):225-232.
14. Egbuonu ACC. Comparative assessment of some mineral, amino acid and vitamin compositions of watermelon (*Citrullus lanatus*) rind and seed. Asian Journal of Biochemistry. 2015c;10(5):230-236. DOI: 10.3923/ajb.2015.230.236.
15. Egbuonu ACC. Comparative investigation of the antibacterial and antifungal potentials of the extracts of watermelon (*Citrullus lanatus*) rind and seed. European Journal of Medicinal Plants. 2015d;9(4):1-7. DOI: 10.9734/EJMP/2015
16. Ezeanyika LUS, Egbuonu ACC. Impaact of nitric oxide and insulin resistance on the pathophysiology of the metabolic syndrome: Possible role of L-arginine and glutamate. J. Med. and Med. Sci. 2011;2(20):657-662.
17. Egbuonu ACC, Ezeanyika LUS. L-arginine exposure improves renal function markers of metabolic syndrome in female rats. Am. J. Biochem and Mol. Biol. 2013;3(1):50-60.
18. Atolani O, Omere J, Otuechere CA, Adewuyi A. Antioxidant and cytotoxicity effects of seed oils from edible fruits. Journal of Acute Disease. 2012;130-134. DOI: 10.1016/S22216189(13)60073-6
19. Tripoli E, La Guardia M, Giammanco S, Di Majo D, Giammanco M. *Citrus* flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chemistry. 2007;104:466-479.
20. Egbuonu ACC, Ogbu AE, Ezeanyika LUS. Dose-related influence of esculetin (6,7-dihydroxy-coumarin) on some liver and prostate function markers of male Wistar rats. Journal of Biological Sciences. 2012;12(4):253-257.
21. Muhammad G, Sajid M, Nasir M, Nyla J, Rehana K, Kokab J, Gulshan A. Composition and antimicrobial properties of essential oil of *Foeniculum vulgare*. African Journal of Biotechnology. 2008;7(24):4364-4368.
22. Akin M, Aktumsek A, Nostro A. Antibacterial activity and composition of the essential oils of eucalyptus *Camaldulensis dehn* and *Myrtus communis* L. growing in northern cyprus. African Journal of Biotechnology. 2010;9(4):531-535.
23. Egbuonu ACC, Osakwe ON. Effects of high monosodium glutamate on some serum markers of lipid status in male Wistar rats. Journal of Medicine and Medical Sciences. 2011;2(1):653-656.
24. Egbuonu ACC, Obidoa O, Ezeokonkwo CA, Ezaeanyika LUS, Ejikeme PM. Low dose oral administration of monosodium glutamate in male albino rats may be nephroprotective. Bio-Research. 2009; 7(1):470-473.
25. Omodamiro OD, Umekwe JC. Evaluation of anti-inflammatory, antibacterial and antioxidant properties of ethanolic extracts of *Citrus sinensis* peel and leaves. Journal

of Chemical and Pharmaceutical Research. 2013;5(5):56-66.

26. Harbone JB. Phytochemical methods. A guide to modern techniques of plant analysis. 2nd Edition. Chapman and Hall, London New York; 1973.
27. Pearson DA. The clinical analysis of food (7th edn.). Churchill Livingstone, Edinburgh; 1976.
28. Balayopalan S, Sham S, Priscilla DH. Alkaline picrate colorimetric method of hydrogen cyanide determination. Ind. J. Pharma. Sci. Res. 1988;1(10):430-434.
29. Oberleas D. Phytate In: Toxicants occurring naturally in food (Strong F. ed) National Academy of Sciences. Washington D.C. 1978;363-371.
30. Association of Official Analytical Chemists (AOAC). Official methods of analysis of the Association of Official Analytical Chemists International 17th Ed. Published by the Association of Official Analytical Chemists International, Suite 400 2200 Wilson Boulevard, Arlington, Virginia USA. 2000; 22201-3301.
31. Bougatef A, Naima NA, Manni L, Rozenn R, Barkia A, Guillachan D, Nasri M. Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinella aurita*) by-products. Food Chem. 2009;114:254-261.
32. Ladda W, Supatch K, Chockchai T. Antioxidant activity of rice bran protein extract, its enzymatic hydrolysates and its combination with commercial antioxidants. Pakistan Journal of Nutrition. 2015;14(10): 647-652.
33. Shad MA, Pervez H, Nawaz H, Khan H, Aman UM. Evaluation of biochemical and phytochemical composition of some groundnut varieties grown in arid zone of Pakistan. Pak. J. Bot. 2009;41(6):2739-2749.
34. Nzewi D, Egbuonu ACC. Effect of boiling and roasting on the proximate properties of asparagus bean (*Vigna sesquipedalis*). African Journal of Biotechnology. 2011;10(54):11239-11244.
DOI: 10.5897/AJB11.452
35. Nzewi D, Egbuonu ACC. The effect of soaking on the proximate and anti-nutritional properties of asparagus bean (*Vigna sesquipedalis)* flour. Journal of Science Engineering and Technology. 2012;19(1):10580-10592.
36. Muhammad AS, Haq N, Muhammad Y, Beena Y. Phytochemical composition and antioxidant properties of rhizomes of *Nilumbo nucifera.* Journal of Medicinal Plants Research. 2012;6(6):972-980.
DOI: 10.5897/JMPR11.510
37. Diwan FH, Abdel-Hassan IA, Mohammed ST. Effect of saponin on mortality and histopathological changes in mice. East Meditter. Health J. 2000;6(2/3):345-351.
38. Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm and Pharmacol. 2003;55:131-142.
39. Boskou D. Sources of natural phenolic antioxidants. Trends Food Sci. Technol. 2006;17:505-512.
40. Omar NF, Siti AH, Umi KY, Nur APA, Puteri EMW, Uma RS. Phenolics, flavonoids, antioxidant activity and cyanogenic ggycosides of organic and mineral-base fertilized cassava tubers. Molecules*.* 2012; 17:2378-2387.
DOI: 10.3390/molecules17032378
41. Perez-Lopez AJ, del Amor FM, Serrano-Martinez A, Fortea MI, Nunez-Delicado E. Influence of agricultural practices on the quality of sweet pepper fruits as affected by maturity stage. J. Sci. Food Agr. 2007; 87:2075–2080.
42. Kumari M, Gupta S, Lakshmi AJ, Prakash J. Iron bioavailability in green leafy vegetables cooked in different utensils. Food Chem. 2004;86:217-222.
43. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology. 2006;5(11):1142-1145.
44. Lu Y, Khoo TJ, Wiart C. Antioxidant activity determination of *Citronellal* and crude extracts of *Cymbopogon citratus* by 3 different methods. Pharmacology & Pharmacy. 2014;5:395-400.
Available:http://dx.doi.org/10.4236/pp.2014.54047
45. Kang MH, Lee MS, Choi MK, Min KS, Shibamoto T. Hypoglycemic activity of *Gymnema sylvestre* extracts on oxidative stress and antioxidant status in diabetic rats. Journal of Agricultural and Food Chemistry. 2012;60:2517-2524.
Available:http://dx.doi.org/10.1021/jf205086b
46. Moure A, Cruz JM, Franco D, Dominguez JM, Sineiro J, Dominguez H, Nunez MJ,

Parajo JC. Natural antioxidants from residual sources. Food Chem. 2001;72: 145-171.

47. Ajibola CF, Fashakin JB, Fagbemi TN, Aluko RE. Effect of peptide size on antioxidant properties of African yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. International Journal of Molecular Science. 2011;12(10):6685-6702.

48. Zou Y, Lu Y, Wei D. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum L. in vitro.* J. Agric. Food Chem. 2004;52:5032-5039.

49. Krasuska U, Gniazdowska A. Nitric oxide and hydrogen cyanide as regulating factors of enzymatic antioxidant system in germinating apple embryos. Acta Physiol Plant. 2012;34:683–692.
DOI: 10.1007/s11738-011-0868-8

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14336