



Comparative Proximate, Minerals and Antinutrient Analysis of Selected Nigerian Leafy Vegetables

O. B. Ajayi¹, T. J. Bamidele¹, O. I. Malachi^{2*} and A. A. Oladejo²

¹*Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria.*

²*Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Author OBA designed the study and wrote the protocol. Authors OIM and AAO managed analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Author TJB performed the spectroscopy analysis, managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2018/26666

Editor(s):

(1) Shahira M. Ezzat, Professor, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

Reviewers:

(1) Alli Lukman Adewale, University of Abuja, Nigeria.

(2) B. P. Mishra, Mizoram University, India.

(3) Mustapha Balarabe Idris, Jodhpur National University, India.

Complete Peer review History: <http://www.sciencedomain.org/review-history/22898>

Original Research Article

Received 27th February 2016

Accepted 6th June 2016

Published 27th January 2018

ABSTRACT

Aims: To evaluate the contributions of Nigerian leafy vegetables to nutrition and health.

Methodology: Eight leafy vegetables were comparatively analyzed for proximate, mineral and antinutrient compositions.

Results: The proximate composition showed that the vegetables are good sources of protein, energy and fiber. *Brassica oleracea* has the highest protein content while *Brassica oleracea* and *Trianthema portulacastrum* are the richest in fiber. The result showed that the vegetables are good sources of iron, manganese, magnesium, potassium and calcium. *Trianthema portulacastrum* has the highest levels of minerals. The Recommended Dietary Allowance for manganese, magnesium and calcium can be met by a 100 g per day servings of the vegetables; while up to 60% of the Recommended Dietary Allowance for iron and potassium can be met by a 100 g per day servings of the vegetables. The vegetables were poor sources of zinc. The level of sodium in the vegetables was very low. The antinutrients in the investigated vegetables were much lower than safe limits and

*Corresponding author: E-mail: malachiseyi@gmail.com;

levels reported for other plant sourced foods including nuts, roots, beans and berries.

Conclusion: The investigated vegetables are good sources of essential nutrients and are low in antinutrients.

Keywords: Leafy vegetables; proximate analysis; minerals; antinutrients; Nigeria.

1. INTRODUCTION

Green leafy vegetables are widely succulent plants parts grown in gardens and consumed as a side dish or soup with starchy staples among the tribes in Nigeria [1]. The importance of dietary components of leafy vegetables is significant in African population since they are comparatively rich in fiber, which cereals; root vegetables and other foodstuff are relatively poor sources [2]. In Nigeria, just as in most other African countries where diet is dominated by starchy staple foods, vegetables are considered the cheap source of energy. They are very rich sources of nutrients such as carotene, protein, vitamins, calcium, iron, ascorbic acid and tangible concentration of trace minerals [3,4].

Vast species of vegetables abound in the world and they are characterized with addition of flavor, taste and color to diet which would have otherwise been monotonous. The invaluable food nutrients contained in vegetables can be used for body building, protective and regulatory material as well as sources of energy for the body. The numerous minerals represented in vegetables provide alkalizing effects by neutralizing the acidity produced by other foods during the digestion process [5]. Vegetables also contain plants secondary metabolites which act as antinutrients [6]. These secondary metabolites include: tannins, phytate, oxalate and cyanide; all of which have different detriments to nutrition and health [7]. A good leafy vegetable is the one that contains a great deal of these beneficial nutrients and a very minute amount of antinutrients. The concentrations of the nutrients and antinutrients vary from one leafy vegetable species to another, hence a need for comparative investigation of the level of these nutrients and antinutrients in leafy vegetables. This study thus compares the proximate, mineral and antinutrient composition of some leafy vegetables consumed in Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Materials

Sample of fresh vegetables leaves were collected from two roadside farmlands in Ado-

Ekiti and Isaba-Ekiti, Ekiti State, Nigeria. Authentication of the plants was carried out at the Department of Plant Science and Forestry, Ekiti State University, Nigeria. The leaves were detached from the stalk, rinsed with distilled water and were oven-dried at 40°C. The dried samples were ground into fine powder and sieved through 2.0 mm mesh prior to analysis.

Table 1. Selected leafy vegetables identified in Ekiti State

Scientific name	Common name	Traditional name (Yoruba)
<i>Brassica oleracea</i>	Cabbage	----
<i>Solanum macrocarpon</i>	African Eggplant	Igbagba
<i>Ocimum gratissimum</i>	Scent leaf	Efinrin
<i>Amaranthus cruentus</i>	Amaranth	Arowojeja
<i>Amaranthus viridis</i>	Amaranth	Tete Adayeba
<i>Cnidoscolus aconitifolius</i>	Tree Spinach	Iyana-ipaja
<i>Solanum nigrum</i>	Black nightshade	Odu
<i>Trianthema portulacastrum</i>	Giant pigweed	Esisan

2.2 Proximate Analysis

Moisture, ash, crude fat and crude fiber were analyzed in the fresh samples in accordance with the methods described by Association of Official Analytical Chemist [8], while nitrogen was determined by the micro-Kjeldahl method [9] and the percentage of nitrogen was converted to crude protein by multiplying by 6.25. Carbohydrate was determined by difference [10].

2.3 Mineral Analysis

Wet ashing method was used in releasing the investigated metals in the samples from biological complexes before evaluating them with atomic absorption spectrophotometer (PinAAcle™ 900H) and flame photometer (Gulfex FP6410). The wet ashing was achieved by heating the sample with a digestion mixture containing concentrated nitric, perchloric and

sulphuric acids in a Kjeldahl flask [8]. Calcium, Iron, Manganese, Magnesium, Zinc were determined by atomic absorption spectrophotometer; Sodium and Potassium by Flame photometry while Phosphorus was determined by Molybdate method using hydroquinone as a reducing agent [8].

2.4 Analysis of Anti-nutrients

Total oxalate was determined according to the procedure of Day and Underwood [11] while Phytate was determined by the anion exchange method as described by Harland and Oberlas [12] using KH_2PO_4 as standard. Hydrocyanic acid content was determined using the alkaline titration method of AOAC [8].

3. RESULTS AND DISCUSSION

The results of the proximate analysis of the leafy vegetables are represented in Table 2. Generally, the results indicate that the vegetables are good sources of protein, carbohydrates and crude fiber. Protein content was much higher in *Brassica oleracea* (24.3%) compared to other five vegetables. The high protein content of *Brassica oleracea* is in accord with the reports of Shittu et al. [13] and Shittu and Ogunmoyela [14]. *Brassica oleracea* meets the requirement of providing more than 12% of its calorific value from protein and can thus be considered a good source of protein according to Pearson [9]. The high protein content of *Brassica oleracea* however does not present it as a good source of protein as the quality of a protein is dependent on its ability to supply all the essential amino acids in the required amount [15]. Hence, protein quality assessment is necessary to ascertain the ability of *Brassica oleracea* to meet dietary protein need.

Dietary fiber was well represented in the vegetables; with *Brassica oleracea* and *Trianthema portulacastrum* having values much higher than others. Dietary fiber is the indigestible portion of food which acts by changing the nature of the contents of the gastrointestinal tract, thus affecting how other nutrients and chemicals are absorbed [16]. Dietary fiber is known to provide bulking, thereby easing defecation [17]. It also hinders the absorption of cholesterol in the gastrointestinal tract thus helping in the therapy of hypercholesterolemia [18]. Dietary fiber is associated with reduced diabetes risk, although the mechanism by which this occurs is not fully

understood [19]. Current recommendations from the United States National Academy of Sciences, Institute of Medicine, suggest that adults should consume 20–35 grams of dietary fiber per day [20]. This has been difficult to meet as average daily intake of dietary fiber is only 12–18 grams [21]. However, with very high fiber contents as presented in Table 2, *Brassica oleracea*; *Solanum macrocarpon* and *Trianthema portulacastrum* will meet the requirement at servings slightly higher than 100grams daily. *Ocimum grattisimum* also presented high fiber content and can also be regarded as a good source of fiber. The ash represents the mineral content of the leaves. Significantly high content of ash in *Brassica oleracea*; *Solanum macrocarpon* and *Trianthema portulacastrum* therefore shows high levels of minerals in the vegetables.

The mineral compositions of the investigated vegetables (Table 3) reveal that the vegetables contain different levels of essential minerals. *Trianthema portulacastrum* has higher levels of all the minerals than other vegetables investigated. This is consistent with its high ash content presented in Table 2. Hussain and co workers [22] also reported that *Trianthema portulacastrum* has the highest mineral content of all the vegetables consumed in Kohat region of Pakistan. With iron content higher than 110 ppm as shown in Table 3, *Trianthema portulacastrum* and *Solanum macrocarpon* at servings of 100 g per day will supply 60% of USDA Recommended Dietary Allowance of 18 mg/d for iron [23]. Other vegetables rich in iron include: *Solanum nigrum*, *Amaranthus cruentus* and *Solanum nigrum*, all of which will supply 40% of USDA Recommended Dietary Allowance (RDA) for iron [23] at servings of 100 g per day. Although phosphorus is well represented in the vegetables, especially *Trianthema portulacastrum* and *Solanum macrocarpon*; its ubiquity in protein rich foods makes its deficiency unlikely in protein sufficient diet [24,25]. The concentration of sodium in the vegetables is so small that even a serving of up to 2 kg per day of *Trianthema portulacastrum*, which has the most sodium concentration, will still contribute less than the upper limit standard of sodium. The upper limit standard of sodium is 2.3 grams per day [26]. Intake exceeding this level has been associated with hypertension [27]. With zinc concentrations ranging from 2.20ppm to 6.68 ppm, the investigated leafy vegetables at servings of 100 g thrice daily cannot meet the zinc recommended dietary allowance of 8

mg/day for women and 11 mg/day for men [28]. Hence the vegetables cannot be regarded as good sources of zinc. Manganese is well represented in the vegetables in concentrations much lower than the estimated tolerable upper limit of 11 mg per day [26] at 100 g per day serving. *Trianthema portulacastrum* and *Solanum macrocarpon* at servings of 100 g per day will supply the dietary reference intake of 2.3 mg per day [26].

The macroelements: potassium, magnesium and calcium are also well represented in the vegetables. Although the Dietary Reference Intake (DRI) of 4,700 mg of potassium [29] cannot be met by any of the vegetables at 100g per day serving, the vegetables are nevertheless good sources of potassium as supplementation with other food components will make up for the potassium need. Epidemiological studies indicate that diets high in potassium can reduce the risk of hypertension and possibly stroke by a mechanism independent of blood pressure [30, 31]. Green leafy vegetables are rich sources of magnesium because of the presence of chlorophyll in them. The investigated vegetables thus present magnesium concentrations that meet the recommended daily values for magnesium of 300 mg [32] at a serving of 100g per day. The essentiality of magnesium is owing to its interaction with phosphate in stabilizing nucleic acids. Also, more than 300 enzymes require magnesium ions for their catalytic activities [33]. The vegetables, as presented in Table 3, are also rich in calcium. 100g per day serving with any of the vegetables will meet the calcium recommended adequate intake of 1200 mg/day [34].

Table 4 shows the antinutrient composition of the investigated vegetables. The use of many plants for food is often limited by the composition of antinutrients in them, as the antinutrients pose

dangerous effects in both man and animals [35]. Phytate, a saturated cyclic acid, is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytate is present in the investigated vegetables at different concentrations. The antinutrient potential of phytate is owing to its strong binding affinity for essential minerals like zinc, iron and calcium. Binding to these minerals leads to the formation of insoluble precipitates that are far less absorbable in the intestines; thereby reducing their bioavailability [36,37]. Phytate also chelates the vitamin niacin, the deficiency of which is known as pellagra [38]. The safe limit of phytate in food thus depends on the availability of these nutrients the food. The levels of phytate in all the investigated vegetables are however much lower than the reports for cereals, beans and nuts [39,40,41,42]. Oxalate is a dianion that is synthesized in plants by the incomplete oxidation of carbohydrates. The toxicity of oxalate is due to the fact it combines with divalent metallic cations such as calcium (Ca^{2+}) and iron (II) (Fe^{2+}) to form crystals of the corresponding oxalates which are then excreted in urine as minute crystals. These oxalates crystals can form larger kidney stones that can obstruct the kidney tubules; leading to kidney disease [43]. Spinach and amaranth are leafy vegetables that have been reported to contain relatively high levels of oxalate [44]. The toxicity of oxalate is usually evident in individuals with kidney disorders, gout, rheumatoid arthritis and vulvodinia [45]. The observed levels of oxalate in the investigated vegetables are much lower than the reports for rhubarb, buckwheat [46], Beetroot greens, Purslane leaves, Rhubarb, Spinach, Beet, Chard and Cocoa oxalate [44]. Tannins are astringent plant polyphenolic compounds that bind to and precipitate proteins thereby reducing the bioavailability of dietary proteins [47]. The safe limit of tannins in food thus depends on the availability of protein the food. The level of tannins observed in the

Table 2. Proximate composition of Nigerian leafy vegetables

Leafy vegetable	Protein (%)	Fat (%)	Ash (%)	Crude fiber (%)	Carbohydrate (%)	Moisture content (%)
<i>Brassica oleracea</i>	24.32	0.89	8.26	18.35	37.16	11.02
<i>Solanum macrocarpon</i>	8.44	0.96	12.36	17.69	52.59	7.69
<i>Ocimum grattisimum</i>	13.38	1.85	5.68	14.33	55.42	9.35
<i>Amaranthus cruentus</i>	11.32	15.88	2.15	8.25	43.55	18.85
<i>Amaranthus viridis</i>	8.56	10.50	0.89	4.69	55.51	19.68
<i>Solanum nigrum</i>	15.06	6.38	1.23	9.56	51.04	16.73
<i>Cnidocolus aconitifolius</i>	9.33	8.31	3.02	6.98	57.41	14.95
<i>Trianthema portulacastrum</i>	14.75	0.87	14.23	19.45	41.34	9.36

Table 3. Mineral composition of Nigerian leafy vegetables

Leafy vegetables	Na (ppm)	K (%)	Ca (%)	Mn (ppm)	Mg (%)	Fe (ppm)	Zn (ppm)	P(ppm)
<i>Brassica oleracea</i>	0.26	1.20	2.38	17.59	0.93	75.91	3.88	60.39
<i>Solanum macrocarpon</i>	0.39	1.78	3.41	26.33	1.39	112.85	5.80	90.23
<i>Ocimum grattisimum</i>	0.18	0.83	1.74	12.10	0.65	51.58	2.67	41.46
<i>Amaranthus cruentus</i>	0.26	1.19	2.38	17.57	0.93	75.23	3.84	60.23
<i>Amaranthus viridis</i>	0.15	0.69	1.49	9.99	0.54	42.82	2.20	34.24
<i>Solanum nigrum</i>	0.30	1.38	2.71	20.36	1.08	87.28	4.49	69.95
<i>Cnidoscolus aconitifolius</i>	0.22	1.01	2.06	14.87	0.79	63.73	3.28	50.95
<i>Trianthema portulacastrum</i>	0.45	2.05	3.87	30.31	1.60	129.92	6.68	103.88

Table 4. Anti-nutrient composition of Nigerian leafy vegetables

Leafy vegetables	Tannins (mg/100 g)	Phytate (mg/100 g)	Oxalate (mg/100 g)	Cyanide (mg/100 g)
<i>Brassica oleracea</i>	6.88	2.55	5.08	0.04
<i>Solanum macrocarpon</i>	3.30	1.81	4.61	0.05
<i>Ocimum grattisimum</i>	4.73	1.75	3.50	0.03
<i>Amaranthus cruentus</i>	6.88	2.54	5.08	0.04
<i>Amaranthus viridis</i>	3.91	1.45	2.89	0.02
<i>Solanum nigrum</i>	7.97	2.95	5.88	0.05
<i>Cnidoscolus aconitifolius</i>	5.82	2.15	4.30	0.03
<i>Trianthema Portulacastrum</i>	1.86	2.39	3.76	0.04

investigated vegetables is however lower than those reported for nuts, berries [48,49] and legumes [50]. The cyanide levels in the vegetables were found to be much lower (0.02 - 0.07 mg/100 g) than the levels that results in acute and chronic toxicity [51].

4. CONCLUSION

The investigated vegetables are rich in protein, fiber and essential minerals. *Trianthema portulacastrum* presented the most nutritious profile in terms of the nutrients investigated. *Solanum macrocarpon* and *Brassica oleracea* also presents higher nutrient profiles than other investigated vegetables. The antinutrients in the leafy vegetables are much lower than the safe limits and the reports for other plant sourced foods, including nuts, roots, beans and berries. A serving of 100 g per day of the investigated vegetables, complemented by other nutrient sources, will provide the RDA of the essential minerals investigated. While a serving of up to 2 kg per day of the vegetables does not contribute antinutrients higher than the safe limits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Guarino L. Traditional African vegetables: Proceedings of the ICGRI International Workshop on Genetic Resources of Traditional Vegetables in Africa. 1995;29-31.
2. Brain AF, Allan GC. Food science. A Chemical Approach, 15th ed Edward Arnold publishers Ltd London. 1986;210-211.
3. Prakash D, Pal M. Nutritional and antinutritional comparison of vegetable and grain *Amaranthus* leaves. J. Sci. Food Agric. 1991;57:573-585.
4. Jimoh FO, Oladiji AT. Preliminary Studies on *Piliostigma thonningii* seeds: Proximate analysis, mineral composition and phytochemical screening. Afr. J. Biotech. 2005;4(12):1439-1442.
5. Fayemi PO. Nigerian vegetables. Heinemann Educational Books, Nigeria. 1999;1-8.
6. Akindahunsi AA, Salawu SO. Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. African Journal of Biotechnology. 2005;4(6):497-501.
7. Ekholm P, Liisa V, Maija Y, Liisa J. The effect of phytic acid and some natural

- chelating agents on the solubility of mineral elements in oat bran. *Food Chemistry*. 2003;80(2):165–70.
DOI:10.1016/S0308-8146(02)00249-2
8. A.O.A.C. Official method of analysis. Association of Official Analytical Chemist. 1990;15:910-28.
 9. Pearson D. Chemical analysis of foods. (7th ed) churchchill, Livingstone, London. 1976;7-12.
 10. McDonald P, Edwards RA, Greenhalgh JFD. Animal Nutrition. Edinburgh: T and A Constable Ltd. 1973;2-5.
 11. Day RA, Underwood AL. Quantitative Analysis. 1986;5:701.
 12. Harland BF, Oberleas D. Anion exchange method for determination of phytates in food: Collaborative study. *J. Assoc. Off. Anal. Chem.* 1986;69:667-670.
 13. Shittu TA, Ogunmoyela OA, Sanni LO. Nutrient retention and sensory characteristics of dried leafy vegetables. Proceedings: 23rd Annual Conference of the Nigerian Institute of Food Science and Technology, Abuja. 1999;130-32.
 14. Shittu TA, Ogunmoyela OA. Water blanching treatment and nutrient retention in some Nigerian leafy vegetables. Proceedings: 25th Annual Conference of the Nigerian Institute of Food Science and Technology, Lagos. 2001;64-65.
 15. Pellett PL, Young VR. Nutritional evaluation of protein foods. *Food and Nutrition Bulletin*. 1980;Suppl. 4.
 16. Eastwood M, Kritchevsky D. Dietary fiber: how did we get where we are?. *Annu Rev Nutr.* 2005;25:1–8.
DOI:10.1146/annurev.nutr.25.121304.131658. PMID 16011456.
 17. Anderson JW, Baird P, Davis RH. Health benefits of dietary fiber. *Nutr Rev.* 2009;67(4):188–205.
DOI:10.1111/j.1753-4887.2009.00189.x. PMID 19335713
 18. Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: A meta-analysis. *Am J Clin Nutr.* 1999;69(1):30–42. PMID 9925120.
 19. Weickert MO, Pfeiffer AF. Metabolic effects of dietary fiber consumption and prevention of diabetes. *J Nutr.* 2008; 138(3):439–42. PMID 18287346.
 20. Lustig RH. The 'skinny' on childhood obesity: How our western environment starves kids' brains. *Pediatr Ann.* 2006; 35(12):898–902, 905–7. PMID 17236437.
 21. Suter PM. Carbohydrates and dietary fiber. *Handb Exp Pharmacol. Handbook of Experimental Pharmacology.* 2005; 170(170):231–61.
DOI:10.1007/3-540-27661-0_8. ISBN 3-540-22569-2. PMID 16596802.
 22. Hussain J, Rehman NU, Khan AL, Hamayun M, Hussain SM, Shinwari ZK. Proximate and essential nutrients evaluation of selected vegetables species from Kohat region, Pakistan. *Pak. J. Bot.* 2010;42(4):2847-2855.
 23. NAS. Dietary Reference Intakes: Recommended Intakes for Individuals National Academy of Sciences. Institute of Medicine. Food and Nutrition Board; 2001. Available:[http://www.nationalacademies.org/hmd/Activities/Nutrition/SummaryDRIs/~media/Files/Activity%20Files/Nutrition/DRI%20Summary%20Table%20Tables%201-4.pdf](http://www.nationalacademies.org/hmd/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRI%20Summary%20Table%20Tables%201-4.pdf)
 24. Anderson JJB. Calcium, phosphorus and human bone development. *Journal of Nutrition.* 1996;126(4 Suppl.):1153S–1158S. PMID 8642449.
 25. FNB. Food and Nutrition Board, Institute of Medicine. DRI dietary reference intakes for calcium, phosphorus, magnesium, Vitamin D, and Fluoride. Washington, DC: National Academy Press; 1997.
PMID:23115811
Available:www.ncbi.nlm.nih.gov/pubmed/23115811
 26. FNB. Dietary Reference Intakes: Water, Potassium, Sodium, Chloride, and Sulfate. Food and Nutrition Board, Institute of Medicine, United States National Academies; 2004.
Available:<http://www.nationalacademies.org/hmd/Reports/2004/Dietary-Reference-Intakes-Water-Potassium-Sodium-Chloride-and-Sulfate.aspx>
 27. Geleijnse JM, Kok FJ, Grobbee DE. Impact of dietary and lifestyle factors on the prevalence of hypertension in Western populations. *European Journal of Public Health.* 2004;14(3):235–239.
DOI:10.1093/eurpub/14.3.235. PMID 15369026.

28. Bales CW, Ritchie CS. Handbook of Clinical Nutrition and Aging. Springer. 2009;151. ISBN 978-1-60327-384-8.
29. PDRIEW. Panel on Dietary Reference Intakes for Electrolytes and Water, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition (2005). DRI, dietary reference intakes for water, potassium, sodium, chloride, and sulfate. Washington, D.C.: National Academies Press. 2005; ISBN 0-309-53049-0.
30. Grim CE, Luft FC, Miller JZ, Meneely GR. Racial differences in blood pressure in Evans County, Georgia: relationship to sodium and potassium intake and plasma renin activity. *Journal of Chronic Diseases*. 1980;33(2):87–94. DOI:10.1016/0021-9681(80)90032-6. PMID 6986391
31. Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, Klag MJ. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. *JAMA*. 1997;277(20):1624–32. DOI:10.1001/jama.1997.03540440058033. PMID 9168293.
32. Wester PO. Magnesium. *Am. J. Clin. Nutr.* 1987;45(5 Suppl):1305–12. PMID 3578120.
33. Romani AMP. Chapter 3. Magnesium in Health and Disease. In Astrid Sigel; Helmut Sigel; Roland K. O. Sigel. *Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences 13*. Springer. 2013;49–79. DOI:10.1007/978-94-007-7500-8_3.
34. Ross CA, Jones G, Abrams SA, Kovacs CS, Aloia JF, Manson JE, Brannon PM, Mayne ST, Clinton SK, Rosen CJ, Durazo-Arvizu RA, Shapses SA, Gallagher JC, Gallo RL. Dietary reference intakes for calcium and vitamin D. Institute of Medicine, National Academy of Science; 2010. Available:http://www.nationalacademies.org/hmd/~media/Files/Report%20Files/2010/Dietary_Reference-Intakes-for-Calcium-and-Vitamin-D/Vitamin%20D%20and%20Calcium%20010%20Report%20Brief.pdf
35. Kubmarawa D, Andenyand IFH, Magomya AM. Amino acid of two non conventional leafy vegetables: Sesamum and *Balanites aegyptical*. *Afr. J. Biotechnol.* 2008;7(19): 3502-3504.
36. Prom-U-Thai C, Huang L, Glahn R, Welch RM, Fukai S, Rerkasem B. Iron (Fe) bioavailability and the distribution of anti-Fe nutrition biochemicals in the unpolished, polished grain and bran fraction of five rice genotypes. *Journal of the Science of Food and Agriculture*. 2006;86(8):1209–15. DOI:10.1002/jsfa.2471.
37. Dendougui F, Schwedt G. In vitro analysis of binding capacities of calcium to phytic acid in different food samples. *European Food Research and Technology*. 2004;219(4):2-7. DOI:10.1007/s00217-004-0912-7.
38. Anderson EN. *Everyone eats: Understanding food and culture*. New York: New York University Press. 2005;47–8. ISBN 0-8147-0496-4.
39. Gordon DT, Chao LS. Relationship of components in wheat bran and spinach to iron bioavailability in the anemic rat. *The Journal of Nutrition*. 1984;114(3):526–35. PMID 6321704.
40. MacFarlane BJ, Bezwoda WR, Bothwell TH, Baynes RD, Bothwell JE, MacPhail AP, Lamparelli RD, Mayet F. Inhibitory effect of nuts on iron absorption. *The American Journal of Clinical Nutrition*. 1988;47(2):270–4. PMID 3341259.
41. Reddy NR, Sathe SK. *Food Phytates*. Boca Raton: CRC. 2001; ISBN 1-56676-867-5.
42. Phillippy BQ, Bland JM, Evens TJ. Ion chromatography of phytate in roots and tubers. *Journal of Agricultural and Food Chemistry*. 2003;51(2):350–3. DOI:10.1021/jf025827m. PMID 12517094.
43. Coe EW. Kidney stone disease. *The Journal of Clinical Investigation*. 2005; 115(10):2598–608. DOI:10.1172/JCI26662. PMC: 1236703. PMID 16200192.
44. Resnick MI, Pak CYC. Urolithiasis, a medical and surgical reference. W.B. Saunders Company. 1990;158. ISBN 0-7216-2439-1.
45. Penniston KL. Dietary oxalate and calcium oxalate stones: A theoretical or real concern? Chapter 2. *Practical controversies in management of stone*

- diseases. Pearle M. S.; Nakada S. Y. (Eds.). 2014; ISBN: 978-1-4614-9574-1.
46. Streitweiser A, Heathcock CH. Introduction to Organic Chemistry, Macmillan. 1973; 737.
47. Katie EF, Thorington RW. Squirrels: The animal answer guide. Baltimore: Johns Hopkins University Press. 2006;91. ISBN 0-8018-8402-0.
48. Vattem DA, Ghaedian R, Shetty K. Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. Asia Pac J Clin Nutr. 2005;14(2):120–30. PMID 15927928.
49. Puupponen-Pimiä R, Nohynek L, Meier C. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol. 2001;90(4):494–507. DOI:10.1046/j.1365-2672.2001.01271.x. PMID 11309059.
50. Reed JD. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 1995;73(5):1516–28. PMID 7665384.
51. Biller J. Interface of neurology and internal medicine (illustrated ed.). Lippincott Williams & Wilkins. 2007;939. ISBN 0-7817-7906-5., Chapter 163, page 939.

© 2018 Ajayi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history/22898>