

Proximate, vitamin and phytochemical composition of *cucumis metuliferus* seed

Cosmas Ezekaibeya Achikanu¹, Onuabuchi Nnenna Ani², Ebere Immaculata Akpata³

^{1, 2, 3} Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

Abstract

This study was carried out to evaluate the proximate, vitamin and phytochemical compositions of *Cucumis metuliferus* Seed. The analyses were done using standard methods. The proximate analysis showed that the seed contains carbohydrate ($50.24 \pm 0.03\%$), crude fibre ($19.23 \pm 0.28\%$), crude fat ($15.37 \pm 0.14\%$), moisture ($7.31 \pm 0.28\%$), Ash ($5.23 \pm 0.18\%$) and crude protein ($2.63 \pm 0.06\%$). From the result of the phytochemical analysis, the following phytochemicals were detected: tannins ($2.93 \pm 0.08\text{mg/g}$), steroids ($2.62 \pm 0.16\text{mg/g}$), Alkaloids ($2.54 \pm 0.13\text{mg/g}$), Saponin ($1.41 \pm 0.05\text{mg/g}$), phenols ($1.20 \pm 0.04\text{mg/g}$) and flavonoids ($0.97 \pm 0.06\text{mg/g}$) while glycosides and terpenoids were not detected. The result from the vitamin analysis showed that the seed contains high amounts of vitamins with vitamin K as the most abundant vitamin ($2.58 \pm 0.23\text{mg/g}$) while β -carotene as the least ($1.56 \pm 0.04\text{mg/g}$). This study suggests that the seed of *Cucumis metuliferus* can serve as a good source of nutrient and has the potential to be used for therapeutic purposes.

Keywords: *Cucumis metuliferus*, proximate, phytochemical, vitamin, seed

1. Introduction

Different parts of plants such as seeds, leaves and fruits are used as food for nutritional purposes and many of them are believed to possess different medicinal values. Plant materials contains in abundance nutrients required by man daily for proper metabolic and biochemical processes. Fruit and vegetables are considered as important sources for many indigestible constituents and phytochemicals that act in synergy in contributing to the health and nutritional benefits of foods either separately, or in combination [1]. From epidemiological studies it has been shown that a positive association exists between fruit and vegetable intake and reduced cardiovascular diseases and certain cancer [2, 3]. Seeds in particular are good sources of protein, fats, carbohydrate and minerals. Some plant seeds have nutritive and calorific values which make them necessary components of diets [4]. Studies have shown that seeds do not only contain important nutritionally bioactive compounds but are also sources of other phyto-compounds which have therapeutic effects. There is an increase in interest among scientist in searching for biologically active constituents from indigenous plants products for therapeutic use in traditional medicine and for nutritional purposes [5, 6] which is geared towards man's benefits.

Cucumis metuliferus belongs to the family Cucurbitaceae and commonly called African horned cucumber, jelly melon or Kiwano in English. In Nigeria it is called 'bùuràr zaàki', 'nòòn-kuùràà' 'gautar kaji' by the Hausas [7, 8, 9]. It grows naturally in tropical Africa south of the Sahara down to Senegal, Nigeria, Namibia, Botswana, South Africa and Swaziland [10]. The name horned melon came about as its fruit has horn-like spines. Ripe fruit has orange skin and lime green, jelly-like flesh with a refreshingly fruity taste, and texture similar to a passion fruit or pomegranate [11]. Different parts of *Cucumis metuliferus* are used for different culinary and medicinal purposes. The young and green fruits are used as seasoning and the dried fruits are incinerated and used for treatment of sore throat [12]. The seeds and fruits of

the plant are eaten raw as food supplement [13, 14]. In Nigeria, the seeds are ground into fine flour, turned with water into an emulsion, and then eaten to expel parasites from the body [15].

2. Materials and method

2.1 Sample collection and Preparation

Cucumis metuliferus fruits were bought from market in Gboko, Benue State, Nigeria. The fruit was cut open, the pulp scooped out and the seeds were separated from the pulp. The seeds were dried at room temperature for two weeks and thereafter ground into fine powder using an electric grinder. The ground powder were then subjected to the various analysis.

2.2 Proximate Analysis

The proximate composition of the sample was analyzed using the official method of AOAC [16]. The carbohydrate was determined by difference method as reported by Onyeike *et al.* [17] as % Carbohydrate = $100 - (\% \text{ moisture} + \% \text{ crude fiber} + \% \text{ ash} + \% \text{ crude fat} + \% \text{ crude protein})$.

The calorific value, that is, the total energy content was determined by multiplying the values of crude protein, crude fat and total carbohydrates by their respective Atwater factors; 4, 9 and 4. The sum of the products is expressed in kilocalories per 100 g sample as reported by Onyeike and Ehirim [18] thus:

Total energy (Kcal) = $4 \times (\text{Protein} + \text{carbohydrate}) + 9 \times (\text{lipid})$.

2.3 Phytochemical Analysis

The powdered sample was screened for various phytochemical constituents. Qualitative and quantitative phytochemical analysis were carried out.

Qualitative Phytochemical Analysis

Qualitative analysis was carried out to confirm the presence of the various phytochemicals in the powdered sample.

Tannin, alkaloid, flavonoid and phenol were determined using the method of Trease and Evans ^[19] while Saponin, glycoside, terpenoid and steroid were determined according to the method of Sofowara ^[20].

Quantitative Phytochemical Analysis

Alkaloid, terpenoid, glycoside and phenol were determined using the method of Harborne ^[21]. Saponin was determined using the method of Obdoni and Ochuko ^[22]. van-Burden and Robinson method ^[23] was used for the determination of tannins. Flavonoid was determined using the method of Bohn and Kocipal-Abyssan ^[24]. Steroid was determined according to the method of Okeke and Elekwa ^[25].

2.4 Vitamin analysis

The various vitamins were determined spectrophotometrically using the method of AOAC ^[16].

2.5 Statistical Analysis

Data was analyzed using ANOVA and values are presented as mean \pm standard deviation of duplicate analyses.

3. Result

3.1 Proximate Composition

Table 2: Qualitative and quantitative phytochemical composition of *Cucumis metuliferus* seed powder

Phytochemicals	Qualitative composition	Concentration (mg/g)	Concentration (%)
Alkaloids	+++	2.539 \pm 0.130	21.751
Flavonoids	+	0.973 \pm 0.051	8.326
Saponin	++	1.411 \pm 0.054	12.076
Steroids	+++	2.624 \pm 0.158	22.447
Tannins	+++	2.934 \pm 0.083	25.113
Glycosides	ND	ND	ND
Terpenoids	ND	ND	ND
Phenols	++	1.202 \pm 0.041	2.288

Values are mean \pm standard deviation. Key: +++ (highly present); ++ (moderately present); + (present in low amount); ND (Not detected).

3.3 Vitamin Composition

The result of the vitamin analysis shows that the sample is very rich in vitamins K, B1, B9, E and D. Others are present in appreciable amounts as shown in table 3.

Table 3: Vitamin Composition of *Cucumis metuliferus* seed powder

Vitamins	Concentration (mg/g)	Concentration (%)
Vitamin A	2.22 \pm 0.03	12.351
B-Carotene	1.56 \pm 0.04	8.674
Vitamin B1	2.195 \pm 0.02	12.208
Vitamin B2	1.715 \pm 0.04	9.537
Vitamin B9	2.095 \pm 0.06	11.648
Vitamin C	1.720 \pm 0.07	9.573
Vitamin D	1.855 \pm 0.04	10.315
Vitamin E	2.05 \pm 0.17	11.390
Vitamin K	2.575 \pm 0.23	14.305

4. Discussion

Plant products play important roles in meeting the nutritional and therapeutic needs of man and animal. This has attracted interest in search of plant materials which could meet these needs. The result of the proximate analysis of the seed of *Cucumis metuliferus* in table 1 shows that the seed is rich in carbohydrate (50.24%) with appreciable amount of energy (384.55Kcal/100g). The carbohydrate content is comparable with that of *Cucumis sativus* (50.1%)

The result of the proximate analysis is presented in table 1. The seed contained highest amount of carbohydrate and least amount of crude protein. It also contained appreciable amount of energy.

Table 1: Proximate Composition of *Cucumis metuliferus* seed powder

Parameters	Composition
Moisture Content (%)	7.31 \pm 0.29
Ash content (%)	5.23 \pm 0.18
Crude Fat (%)	15.37 \pm 0.14
Crude Fibre (%)	19.23 \pm 0.28
Crude Protein (%)	2.63 \pm 0.06
Carbohydrate (%)	50.24 \pm 0.03
Energy (Kcal/100g)	384.55

Values are mean \pm standard deviation

3.2 Phytochemical Composition

Table 2 shows the result of the qualitative and quantitative phytochemical analysis. The result indicated that alkaloids, flavonoids, saponin, steroids, tannins and phenols are present. Tannin was the highest constituent found while flavonoid was the lowest constituent found. Glycosides and terpenoids were not detected.

but higher than the reported values of other seeds such as *Citrullus lanatus* (7.22%), *Cucurbita maxima* (5.183%), *Cucurbita pepo* (28.03%) ^[26, 27, 28, 29] which are all from the same Cucurbitaceae family. With this high amount of carbohydrate, the seed of *Cucumis metuliferus* can be considered as a potential source of energy. The energy value is also comparable with that of seed of *Cucurbita maxima* (311.54Kcal/100g) ^[28] but lower than the energy value of *Citrullus lanatus* (582.05Kcal/100g) ^[27]. The fibre content was found to be 19.23% which is higher than those reported for *Cucumis sativus* (3.00%) ^[26], *Citrullus lanatus* (6.005%) ^[27] and *Cucurbita pepo* (1.00%) ^[29] but lower than that of *Cucurbita maxima* (46.647%) ^[28]. Fibre aids in digestion and thereby reduces constipation ^[30]. The crude fibre content in this study showed *Cucumis metuliferus* has the ability to maintain internal distension for a normal peristaltic movement of the intestinal tract which is one of the major physiological roles that crude fibre plays in the living system. However, excessive intake can cause increase in production of intestinal gas which can cause bloating ^[31]. Consumption of more than 30g per day could lead to malabsorption of essential minerals such as calcium, magnesium and phosphorus ^[32]. The crude fat content was 15.37%. This is high enough but lower than that of *Citrullus lanatus* (49.05%) ^[27] and *Cucurbita maxima* (23.447%) ^[28] and higher than that of *Cucumis sativus* (0.70%) ^[26]. The

value of fat obtained for *Cucumis metuliferus* seed suggests that the seed cannot be referred to as oil seed. However, it could provide the body with enough amount of energy and aid in various metabolic processes such as absorption of vitamins. More so, consumption of the seed of *Cucumis metuliferus* will not predispose one to obesity which arises from consumption of food with high fat content. The moisture content obtained from this study was 7.31%. Moisture is the amount of water in a material. It is an index of shelf life of a food stuff. The moisture content of *Cucumis metuliferus* is comparable with that of *Citrullus lanatus* seed flour (7.10%)^[27] but lower than that of seed of *Cucumis sativus* (12.3%) and *Cucurbita maxima* (56.740%)^[26, 28]. The moisture content of *Cucumis metuliferus* shows that the seed has a relatively high shelf life and could be stored for a long time without being susceptible to microbial attack. The ash content of *Cucumis metuliferus* as shown in table 1 was 5.23% which is lower than that *Cucumis sativus* seed (9.34%) but higher than that of *Cucurbita maxima* seed (3.537%)^[26, 28]. Ash is an index of mineral content of a material. The ash content of *Cucumis metuliferus* is relatively high and this value shows the percentage of inorganic mineral elements present in the seeds. It has been reported that high mineral elements in foods enhances growth and development, and also catalyzes metabolic processes in human body^[27]. The crude protein content obtained in this study was 2.63%. This is very low and lower than most of the seeds in the same family such as *Cucurbita maxima* (21.313%)^[28], *Cucumis sativus* (24.5%)^[26], *Citrullus lanatus* (30.63%)^[27] and *Cucurbita pepo* (27.48%)^[29]. This shows that the seed of *Cucumis metuliferus* may not be a good source of protein but could be combined with other protein sources for complete protein requirement.

The result of the qualitative and quantitative phytochemical analysis in table 2 shows the presence of alkaloids, flavonoids, saponin, steroids, tannin and phenols. Tannin (2.934mg/g) was the most abundant followed by steroid (2.624mg/g), and then alkaloid (2.539mg/g). The least was flavonoid (0.973mg/g). Tannins are polyphenols which are found in many plants. They have been implicated in the speeding up blood clotting processes, reduction of blood pressure, modulation of immune-response and in reduction of plasma lipid^[33]. Steroid containing compounds are important in pharmacology due to their relationship with sex hormones^[34]. Alkaloids are nitrogen-containing compounds that occur naturally and are reported to possess antimalarial, anticancer, antiasthma^[35], antiarrhythmic, vasodilatory^[36], analgesic, hypoglycemic, antibacterial activities^[37]. Saponins are glycosides which are amphipathic in nature and are used as adjuvants in the treatment of cancer^[38]. They form complex with dietary cholesterol in the intestinal walls, preventing their uptake, and hence lowering the amount of circulating cholesterol^[31]. They also act as surfactants, aid in the uptake of macromolecules, such as proteins; by cells through the membrane^[31]. Phenols play a vital role in scavenging of free radicals. They possess antioxidant activities and may possibly have anti-carcinogenic properties^[39]. Flavonoids also possess antioxidant properties and are reportedly involved in several physiological activities, such as anti-inflammatory, anti-allergic, anti-oxidant^[40], anti-microbial, anti-diarrheal and anti-cancer^[41]. From the result of the phytochemical screening, the seed of *Cucumis metuliferus* could be utilized

for therapeutic purposes and in drug formulations.

From the result of Vitamin analysis, the seed of *Cucumis metuliferus* is rich in vitamins all of which have important roles to play. Vitamins are micronutrients which play protective roles in the body and need to be obtained from diets. Vitamin K was the most abundant with a concentration of 2.575mg/g which is 14.305% of the total mineral content of the seed. Vitamin K is involved in blood clotting. Vitamins B1, B2 and B9 which are the B-complex vitamins were also in high amount and they are involved in macronutrient metabolism acting as co-enzymes. Vitamin B1 is known as anti-beriberi vitamin. Vitamin B2 helps in production of red blood cells and essential for growth, healthy body, skin, eye and nervous system. Vitamin B9 plays a role in the synthesis of purines and pyrimidines which are required for erythropoiesis and DNA production^[42]. Vitamin A was the second highest vitamin content. Vitamin A helps to provide good vision, healthy immune system and cell growth. Vitamin A possess anti-cancer property through inhibition of DNA synthesis in cancer cells. It also delays tumor growth and inhibits division of leukemia cells^[43]. In the same vein, beta carotene, a precursor of vitamin A is very important in strengthening the immune system. Diets that are rich in beta carotene said to aid in slowing down aging and may also repair and protect DNA^[44]. Vitamin C and E were also in abundance from the study. They are very significant antioxidants which protect the cell membranes from oxidative stress/damage caused by free radicals^[45]. Vitamin C has different mechanism of action which depends on the conditions and hence can act as a pro-oxidant, an antioxidant, a metal chelator, an oxygen scavenger or a reducing agent^[1]. Vitamin C is required for wound healing, maintenance of normal connective tissues, promotes the absorption of dietary iron from the intestine and prevents development of scurvy^[46]. Vitamin E known as anti-sterility vitamin is crucial in the development and normal functioning of the red blood cell and muscles^[42, 48]. Vitamin D, although known as sunshine vitamin was also found in high amount (1.855mg/g) in the seed of *Cucumis metuliferus*. Vitamin D is responsible for increase intestinal uptake of phosphate, magnesium and calcium as well as many other biological effects^[49].

5. Conclusion

The findings from this study have shown that *Cucumis metuliferus* seeds contain rich amount of nutrients and phytochemicals. The nutritional qualities indicate that the seed could be used in food supplements especially for carbohydrate, fat and fibre. The rich phytoconstituents could be sources of therapeutic compounds in prevention and management of various illnesses. The high vitamin content shows that the seed could be a source of antioxidants for management of free radical related diseases.

6. References

1. Okogeri O, Onu R. Nutritional and phytochemical profiles of three neglected fruit seeds from Ebonyi state. International Journal of Food Science and Nutrition. 2016; 1(3):42-44.
2. Hu FB. Plant-based foods and prevention of cardiovascular diseases: An overview. American Journal of Clinical Nutrition. 2003; 78(3):544S-551S.
3. Ikram EHK, Eng KH, Jalil AMM, Ismail A, Idris S,

- Azlan A *et al.* Antioxidant capacity and total phenolic content of Malaysian Underutilised fruits. *Journal of Food Composition and Analysis*. 2009; 22(5):388-393.
4. Lawhorn T, Carter U. The International Dairy Food Association: Microbiological and Nutritional foods from meat, fish and dairy products, 1971, 30-32.
 5. Oktay M, Gülçin I, Küfrevioğlu OI. Determination of invitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Journal of Advanced Scientific Research*. 2003; 36:263-271.
 6. Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. *Food Chemistry*. 2004; 88:293-297.
 7. Burkill HM. Useful Plants of West Tropical Africa. 2nd ed. Royal Botanic Gardens, London. 1985; 2:570-605.
 8. ANON. Mienkie Welman National Herbarium, Pretoria. <http://www.plantzafrica.com/planted/cucumismet.htm>. 2009.
 9. Wannang NN. Aqueous fruit extract of *Cucumis metuliferus* E Mey. Ex Naud (Cucurbitaceae) alters behavioural activities in chicks. *PAT*. 2011; 7(1):84-89.
 10. Usman JG, Sodipo OA, Kwaghe AV, Sandabe UK. Uses of *Cucumis metuliferus*: A Review *Cancer Biology*. 2015; 5(1).
 11. Kiwano information. Exotic fruits; It's almost like a sour berry. Nature's Pride. www.naturespride.eu. Retrieved 2020-04-21.
 12. Berhaut J. (Ed.) Flore Illustrée du Sénégal, Imprimerie Maisonneuve: Paris, 1975, 634.
 13. Bruecher H. Cucurbitaceae. In: *Tropische Nutzpflanzen*. Springer verlag, Berlin, 1975, 258-297.
 14. Keith ME, Renew A. Notes on some edible plants found in the Kalahari. *Gemsbok Park*. Koedoe. 1975; 18:1-12.
 15. Chiej R. *Encyclopaedia of Medicinal Plants*. MacDonald, 1984.
 16. AOAC. Official method of Analysis 16th Edition, Association of official Analytical Chemists, Washington D.C US. 2000, 200-210.
 17. Onyeike EN, Olungwe T, Uwakwe AA. Effect of heat treatment and defatting on the proximate composition of Some Nigerian local soup thickeners. *Food Chemistry*, 1995, 173-175.
 18. Onyeike EN, Ehirim FC. Chemical and Sensory Evaluation of Melon fungus (*Pleurotostuberregium*) and Melon fungus Cake. *Journal of Biochemistry & Molecular Biology*. 2001; 16(1):77-81.
 19. Trease GE, Evans MC. *Textbook on Pharmacognosy* (13th Edn). Bailliere Tandal and Causse, London, 1989; 144-148.
 20. Sofowora A. Screening Plants for Bioactive Agents. In: *Medicinal Plants and Traditional Medicines in Africa*. 2nd ed. Spectrum Books Ltd., Sunshine House, Ibadan, 1993, 81-93.
 21. Harbone JB. *Phytochemical Methods. A Guide to Modern Technique of Plant Analysis*. Chapman and Hall Ltd, London, 1973, 49-188.
 22. Obdoni BO, Ochuko PO. Phytochemical Studies and Comparative Efficacy of the Crude Extract of some Homostatic Plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*. 2001; 8b:203-208.
 23. Van-Burden TP, Robinson WC. Formation of complexes between protein and Tannic acid. *Journal of Agricultural Food Chemistry*. 1981; 1:77.
 24. Bohn BA, Kocipai-Abyazan R. Flavonoids and Condensed Tannins from Leaves of *Hawaiian vaccinium vaticulatum* and *V. calycynium*. *Pacific Science*. 1994; 48:458-463.
 25. Okeke CU, Elekwa I. Phytochemical study of the extract of *Gongronema latifolium* Benth. *Journal of Health and Visual Sciences*. 2003; 5(1):47-55.
 26. Oluwagbenle HN, Adesina AJ, Aremu OI. Comparative Assessment of the Proximate, Mineral Composition and Mineral Safety Index of Peel, Pulp and Seeds of Cucumber (*Cucumis sativus*). *Open Journal of Applied Sciences*. 2019; 9:691-701.
 27. Jacob AG, Etong DI, Tijjani A. Proximate, Mineral and Anti-nutritional Compositions of Melon (*Citrullus lanatus*) Seeds. *British Journal of Research*. 2005; 2(5):142-151.
 28. Amin MZ, Islam TM, Uddin R, Uddin MJ, Rahman MM, Satter MA. Comparative study on nutrient contents in the different parts of indigenous and hybrid varieties of pumpkin (*Cucurbita maxima* Linn.). *Heliyon*. 2019; 5:e02462.
 29. Elinge CM, Muhammad AI, Atiku FA, Itodo AU, Peni IJ, Sanni OM *et al.* Proximate, Mineral and Anti-nutrient Composition of Pumpkin (*Cucurbitapepo* L) Seeds Extract. *International Journal of Plant Research*. 2012; 2(5):146-150
 30. Erhirhie EO, Ekene NE. Medicinal Values of *Citrus lanatus*. *International Journal of Research in Pharmaceutical and Biomedical Sciences*. 2013; 4(4):1305-1312.
 31. Onyegeme-Okerenta BM, Nwosu T, Wegwu MO. Proximate and phytochemical composition of leaf extract of *Senna alata* (L) Roxb. *Journal of Pharmacognosy and Phytochemistry*. 2017; 6(2):320-326.
 32. Abdulwaliyu L, Arekemese SO, Bala S, Ibraheem AS, Dakare AM, Sanodare R *et al.* Nutritional properties of *Senna alata* (L) Roxb Linn leaf and flower. *International Journal of Modern Biology and Medicine*. 2013; 4(1):1-11.
 33. Chung KT, Wong TY, Wei C, Huang Y, Lin Y. Tannins and Human Health: A Review. *Critical Reviews in Food Science and Nutrition*. 1998; 38(6):421-64.
 34. Okwu, DE. Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global Journal of Pure Applied Science*. 2001; 7(3):455-459.
 35. Kittakoop P, Mahidol C, Ruchirawat S. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Current Topics in Medicinal Chemistry*. 2014; 14(2):239-252.
 36. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A. Multitarget drugs of plants origin acting on Alzheimer's disease. *Current Medicinal Chemistry*. 2013; 20(13):1686-93.
 37. Qiu S, Sun H, Zhang AH, Xu HY, Yan GL, Han Y *et al.* Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chinese Journal of Natural Medicine*. 2014; 12(6):401-406.
 38. Sun H, Xie Yong, Ye Y. Advances in saponin-based adjuvants. *Vaccine*. 2009; 27(12):1787-1796.

39. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and humans. *Journal of Medicinal Plants Research*. 2011; 5(31):6697-6703.
40. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF- κ B pathway in the treatment of inflammation and cancer. *Journal of Clinical Investigation*. 2001; 107(2):135-42.
41. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio R *et al*. Flavonoids: Prospective Drug Candidates. *Mini-Reviews in Medicinal Chemistry*. 2008; 8(13):1429-1440.
42. Lukaski HC. Vitamin and Mineral Status: Effects on Physical Performance. *Nutrition*, 2004; 20:632-644.
43. Brett, J. Health vitamins and supplements facts, 2013. www.howstuffworks.com.
44. Anon. HCDA (Horticultural Crops Development Authority). Practical Note Series No.2: Kiwano, 2014. <http://www.hcda.or.ke/downloads/>.
45. Guyton C, Hall JE. Textbook of medical physiology. Elsevier publisher, Philadelphia, India. 2006; 11:113-115.
46. Button KC. Prescription for nutritional healing. Penguin Putnam. 2004; 4(5):478-479.
47. Olusanya JO. Essentials of food and nutrition. 1st edition, Apex Books limited, Lagos, 2008, 36-77.
48. Onwuka GI. Food analysis and instrumentation (Theory and practice). Istedn, Napthal prints, Surulere, Lagos – Nigeria, 2005, 140-160.
49. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *The American Journal of Clinical Nutrition*. 2004; 80(6):1678S-88S.