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Determination of the ascorbic acid content of selected Nigerian local vegetables used as food and medicine

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Abstract
Vegetables are storehouses for vitamins and minerals. Sixteen different vegetables used in Nigeria as food and medicine were selected for ascorbic acid quantitative analysis based on their availability and agronomic desirability in the six different geopolitical zones of Nigeria. The determination of the ascorbic acid content was conducted using Iodometric titration. Data were analysed by comparison of the results obtained with the WHO daily requirement standard for ascorbic acid content in food. The results showed that all the sixteen (16) vegetables contain substantial amounts of ascorbic acid. These vegetables should be added to diets in the right amount that could add up to meet the daily ascorbic acid content needed by the body for normal and healthy growth and for the protection against diseases.

Keywords: Nigeria, vegetable, food, medicine, ascorbic acid

Introduction
The local use of vegetables as food and medicine is largely part of the culture and traditions of every tribe in Nigeria, Africa and the World at large [1]. Nigeria has rich genetic resources of cultivated, semi-wild and wild species of crops consumed by various ethnic groups for either common or different purposes [2] and are grown all year round by different methods of cultivation [3]. Nigerian local vegetables are storehouses for vitamins like β-carotene, ascorbic acid, riboflavin, etc and minerals such as iron, calcium, phosphorous, etc [3]. The minerals and the vitamins help to build strong bones and teeth, contribute to faster wound healings, convert food to energy, repair cellular damage, help in treating anemia and indigestion [1]. Of all the vitamins, vitamin C is the most easily destroyed by oxidation [3], poor storage conditions [5], overcooking [6] witting and temperature [6,7] and should be given much attention as the vitamin C from green leafy vegetables are reported to protect the human body from degenerative diseases due to their antioxidant properties [8].

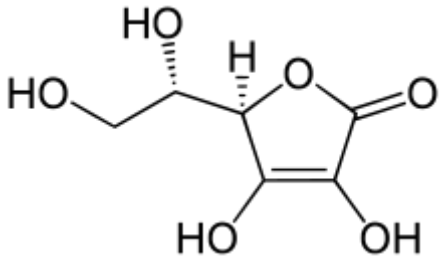

Fig 1: Structure of Ascorbic acid

Materials and methods
Plant collection and identification
Sixteen different fresh, green leafy vegetables consumed in Nigeria were selected from Yola market (North-East), Jos-Terminus market (North-central), Umuahia (South-East), Asaba market (South-South), Ibadan market (South-west) and Birnin-Kebbi market (North-West) between March to October 2018. The vegetables were identified in the various markets using pharmacognostic descriptions in the official books [9, 10] and were authenticated at the Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Nigeria and assigned Voucher Specimen Numbers FHJ 191, FHJ 293, FHJ 331, FHJ 743, FHJ 911, FHJ 401, FHJ 707, FHJ 511, FHJ 744, FHJ 332, FHJ 333, FHJ 334, FHJ 621, FHJ 106, FHJ 821, and FHJ 822 for *Telfairia occidentalis* Hook.F., *Gongronema latifolium* Bentham,

Corchorus olitorius L., *Ocimum gratissimum* L., *Amaranthus hybridus* Linn., *Moringa oleifera* Lam, *Murraya koenigii* L., *Talinum Triangulare* (Jack) wild, *Mentha piperita* L., *Adansonia digitata* L., *Hibiscus sabdariffa* L., *Abelmoschus esculentus* L., *Allium fistulosum* L., *Brassica oleracea* Var., *Lactuca sativa* L., and *Vernonia amygdalina* Del. respectively by Mr. J.J. Azila. The leaves were dried at room temperature under shade until a constant weight for each of the vegetable samples was obtained for three weeks. The plant was reduced to powder using local mortar and pestle and sieved with a mesh of size-20 and then stored in air-tight containers labeled: A, B, C, D, E, F, G, H, I, J, K, L, M, N, O & P respectively to match the different vegetable samples. All the foreign matters were removed and the leaves properly washed with water and made ready for the extraction process.

Plants extraction

The method by Olotu *et al*., 2020 [8] was used. The vegetables were separately reduced into pieces and blended with 50 ml distilled water using '*Calamansi*' juice extractor. The juices were filtered into separate beakers and poured into separate graduated cylinders and the solutions were diluted to 100 ml in each case and stored in a refrigerator at the temperature of 5 ̊C until when required for use.

Chemicals and reagents

Analytical grade chemicals purchased from Sigma-Aldrich Company (St. Louis, MO, USA) were used including: 1% starch solution, 2% Iodine solution and Standard ascorbic acid solution (the stock standard solution was prepared by dissolving 0.25 g of ascorbic in 100 ml of distilled water and then made up to 250 ml in a volumetric flask).

Standardization of iodine solution

Vitamin C standard solution (25 ml) was measured and transferred into 125 ml Erlenmeyer flask and 10 drops of 1% starch solution was added and mixed with it. A burette was rinsed with a small volume of iodine solution and then filled with an iodine solution. The initial volume of the iodine solution was noted. The iodine solution was then titrated against the standard Vitamin C solution until a blue color that persisted for 20 seconds of swirling was observed. The final volume of the iodine solution in the burette was recorded and subtracted from the initial volume to determine the volume of the iodine used. The procedure was repeated two more times and the average was determined. The concentration of iodine solution was then determined using the equation:

$$\text{Mg (Vitamin C)} = \frac{\text{Mass (Vitamin C)} \times 1 \text{ mole (Vitamin C)} \times 1000 \text{ ml/L}}{176.12 \text{ g} \times \text{volume (iodine solution)}}$$

Where M is the concentration of iodine solution and 176.12 g is the molar mass of Vitamin C.

Procedure for determination of vitamin C

The methods described by Olotu *et al*., 2020 [8] was used. Sample solution (25 ml) was measured and poured into 125 ml Erlenmeyer flask and 10 drops of 1 % starch solution was added and mixed with it. A burette was rinsed with a small volume of iodine solution and then filled with an iodine solution. The initial volume of the iodine solution was noted. The iodine solution was then titrated against the standard Vitamin C solution until a blue color that persisted for 20 seconds of swirling was observed. The final volume of the iodine solution in the burette was recorded and subtracted from the initial volume to determine the volume of the iodine used. The procedure was repeated two more times and the average was determined to find the amount of Vitamin C present in the different samples of the fresh vegetables using the equation:

$$\text{Mg (Vitamin C)} = \text{M (Iodine solution)} \times \text{ml (Iodine solution)} \times 176.12 \text{ g/mole}$$

Where Mg is the concentration of Vitamin C, M is the concentration of iodine solution; ml is the volume of iodine solution used and 176.12 g/mole is the molar mass of Vitamin C.

Data analysis

The Ascorbic acid content in the vegetables were expressed as mean ± standard deviation and were compared with the WHO daily requirement standard for Vitamin C (45 mg/day).

Results

Table 1: Scientific identification of the vegetable plants

Sample	Botanical Name	Authority	Family	Voucher Number
A	*Telfairia occidentalis*	Hook.F.	Cucurbitaceae	FHJ 191
B	*Gongronema latifolium*	Bentham	Asclepiadaceae	FHJ 293
C	*Corchorus olitorius*	L.	Malvaceae	FHJ 331
D	*Ocimum gratissimum*	L.	Lamiaceae	FHJ 743
E	*Amaranthus hybridus*	Linn.	Amaranthaceae	FHJ 911
F	*Moringa oleifera*	Lam	Moringaceae	FHJ 401
G	*Murraya koenigii*	L.	Rutaceae	FHJ 707
H	*Talinum Triangulare*	(Jacq) wild	Portulacaceae	FHJ 511
I	*Mentha piperita*	L.	Lamiaceae	FHJ 744
J	*Adansonia digitata*	L.	Malvaceae	FHJ 332
K	*Hibiscus sabdariffa*	L.	Malvaceae	FHJ 333
L	*Abelmoschus esculentus*	L.	Malvaceae	FHJ 334
M	*Allium fistulosum*	L.	Amaryllidaceae	FHJ 621
N	*Brassica oleracea*	Var.	Brassicaceae	FHJ 106
O	*Lactuca sativa*	L.	Asteraceae	FHJ 821
P	*Vernonia amygdalina*	Del.	Asteraceae	FHJ 822

Table 2: English & Vernacular Names of the Vegetable Plants

Sample	English Name	Igbo Name	Hausa Name	Yoruba Name	Location
A	Fluted Pumpkin	Ugu	Gammon Fataakee	Ewe Elegede	Umuahia
B	Bush Buck	Utazi	Utaxi-Genyen Ibo	Arokeke/Madumaro	Umuahia
C	Jute Leaf	Ahinghara/Kerenkeren	Ayoyo/Rama	Ewedu	Ibadan
D	Scent Leaf	Nchuanwu	Daddooya	Effirin	Umuahia
E	African Spinach	Inine	Allayyafoo	Efo-tete	Jos
F	Moringa	Okwe Oyibo/Odudu Oyibo	Zogele	Igi Iyanu/Ewe Igbale	Yola
G	Curry Leaf/Sweet Neem	Corri Liif	Cori Lipf	Effirin Oso	Asaba
H	Water Leaf	Mgbolodi	Allerun-ruwa	Efo Gbure	Asaba
I	Mint Leaf	Mint Liif	Na'a Naa	Ewe Minti	Asaba
J	Baobab	Ose	Kuka	Igiose	Birnin-Kebbi
K	Roselle	Zobo	Yakwuwa/Zoborodo	Amukan/Ishapa	Yola
L	Okra	Okwuru	Kubewa	Ila	Jos
M	Spring Onion	Alibasa	Albasa	Alubosa Elewe	Jos
N	Cabbage	Kabeeg	Kabeeji	Kabiji	Jos
O	Lettuce	Akwuwo-Nkowa-Okwu	Letal	Orisi Ewe	Jos
P	Bitter Leaf	Bita Liif	Bita Lipf	Ewuro	Birnin-Kebbi

Table 3: Folklore Uses of the Vegetable Plants

Sample	Folklore Uses
A	The leaf is used to prepare blood tonic for weak patients and also for easier digestion of food
B	The leaf is used to prepare soup for mothers newly put to bed, where it is believed to stimulate appetite, reduce post-partum contraction and enhance the return of their menstrual cycle
C	The leaf is used to prepare blood tonic for anemic patients and the cold infusion is believed to restore appetite and strength, correct irregular menstrual cycle, aid delivery in difficult labor and milk secretion in lactating mothers
D	The squeezed juice from the leaf is used to treat stomach related diseases like cholera, dysentery, diarrhoea, worms, etc and to treat flu, cold and catarrh and convulsion in children
E	The leaf is used to prepare soup to improve digestion, stimulate appetite, improve strength, prevent the early onset of ulcer and cancer and as a tonic for anemic patients
F	The salad of the leaf is eaten for the treatment of hypertension and diabetes mellitus, improve strength and stimulate appetite
G	The leaf is used to make soup to improve appetite and digestion and for the management of diabetes
H	The leaf is cooked as food and eaten for building strong bones, improve strength, stimulate appetite, improve digestion and as a tonic for the weak and anemic patients
I	The macerated leaf is used for the treatment of stomach pain, indigestion, flatulence, gastritis and intestinal colic
J	The soup preparation is taken as a treatment for malaria, tuberculosis, fever, infections, diarrhoea, dysentery, toothache, anemia and HIV
K	The preparation of the juice is taken as a treatment for constipation, anemia, cardiac diseases, hypertension, cancer and as a diuretic
L	Salad from the young leaf and tender fruit is eaten as a cure for kidney stones, constipation, vaginal discharges, excess involuntary ejaculation, diabetes, jaundice, ulcer and anemia
M	The decoction of the leaf and bulb is taken as a diuretic and for the management of arteriosclerosis and hypertension
N	The salad is eaten as a preventive treatment for obesity, diabetes, cancer, osteoporosis, hypertension and for the management of inflammation
O	The leaf is eaten as a salad for the prophylactic management of diabetes, indigestion, hypertension and cancer
P	The macerated juice from the leaf is taken as antidiabetic and antimalarial agents and for the induction of labor in overdue pregnant women and for the treatment of constipation

Note: This information was provided by the market traders

Table 4: Result of the standardization of the iodine solution

Mass of Ascorbic used (g)	Initial Volume of Iodine Solution used (ml)	Final Volume of Iodine Solution used (ml)	Volume of Iodine Solution Used (ml)	Concentration of Iodine Solution used (m)
0.250	66.800	53.600	13.200	0.108
0.250	53.900	40.800	13.100	0.109
0.250	40.100	26.900	13.200	0.108

Table 5: Result of the Concentration of Ascorbic acid in Vegetables

Sample	Vegetable	Amount of Ascorbic acid (mg/100 ml)
A	*Telfairia occidentalis*	259 ± 0.90
B	*Gongronema latifolium*	217 ± 1.55
C	*Corchorus olitorius*	290 ± 0.90
D	*Ocimum gratissimum*	291 ± 0.90
E	*Amaranthus hybridus*	280 ± 0.90
F	*Moringa oleifera*	235 ± 0.90
G	*Murraya koenigii*	201 ± 1.55
H	*Talinum Triangulare*	171 ± 1.25
I	*Mentha piperita*	281 ± 1.25

J	*Adansonia digitata*	225 ± 0.90
K	*Hibiscus sabdariffa*	299 ± 0.90
L	*Abelmoschus esculentus*	175 ± 1.55
M	*Allium fistulosum*	135 ± 0.98
N	*Brassica oleracea*	129 ± 1.55
O	*Lactuca sativa*	112 ± 0.90
P	*Vernonia amygdalina*	165 ± 0.90

Discussion

Ascorbic acid can exist in food either in the reduced form or in the oxidized form as dehydroascorbic acid. It is known to play essential physiological roles in the human body and it is therefore important to determine its concentrations in vegetables which are a major source of this vitamin for humans. The Ascorbic acid content of sixteen (16) vegetables consumed in Nigeria both as food and medicine were analyzed based on their geographical distribution and agronomic desirability and were found to be relatively appreciable when the values were compared with the World Health Organisation recommended intake of 45 mg/day [11]; with *Hibiscus sabdariffa* having the highest ascorbic acid content and *Lactuca sativa*, with the least content. The ascorbic acid contents of these vegetables could be responsible for the reason for the poly- folklore uses and the high rate of their consumption as soups, spices and salads. The body requires ascorbic acid for normal physiological and biochemical functions. Ascorbic acid helps the body in the synthesis and metabolism of aromatic amino acids such as tyrosine, folic acid and tryptophan, hydroxylation of glycine, proline, lysine, carnitine and catecholamine [12], thus reduces the accumulation of excess acids in the stomach and could be responsible for the use of these vegetables as preventive and treatment of constipation, colic pain, ulcer, gastritis, flatulence, indigestion, diarrhoea and dysentery. Ascorbic acid increases the absorption of iron in the gut by reducing ferric to a ferrous state of iron [12] and could be the reason for the use of these vegetables in the management of anemia and weak patients. As an antioxidant, ascorbic acid protects the body from various deleterious effects of free radicals, pollutants and toxins [12], and should be the reason why the vegetables were used for the prevention and treatment of common cold, cancer, inflammation, diabetes, atherosclerosis, catarrh, stroke, heart diseases, macular degeneration and infections. Ascorbic acid is the cofactor for hydroxylation and activity of mono-oxygenase enzymes in the synthesis of collagen, carnitine and neurotransmitters. It accelerates hydroxylation reactions by maintaining the active forms of metal irons in a reduced stage for optimal activity of enzymes- hydroxylase and oxygenase. Thus, is crucial in the maintenance of collagen which represents about one-third of the body total protein. Ascorbic acid is also essential for maintaining the enzyme, prolyl and lysyl hydroxylase in an active form. Deficiency results in reduced hydroxylation of proline and lysine, thus affecting collagen synthesis. Collagen constitutes the principal protein of skin, bone, teeth, cartilage, tendons, blood vessels, heart valves, intervertebral discs, cornea, and eye lens [12], and so could be attributed to the use of these plants in the prevention and treatment of strong bones, nerves and muscles, eye and diseases and for healthy growth. The synthesis and accumulation of collagen and subsequent cross-linking of the fibre by ascorbic acid to give new tensile strength to the damaged tissues [12], explained the use of these plants in wound healings. Ascorbic acid is essential in the structural and functional integrity of androgen-dependent reproductive organs. Low concentration of ascorbic acid in the seminal plasma causes degenerative changes in the testes, epididymis and vas deferens [12], and could be the reason for the use of these vegetables to prevent early infertility problem in males. Ascorbic acid causes the reduction of blood cholesterol levels, triglycerides, lipids and peroxidation and increases the high-density lipoproteins (HDL) in the plasma. Dietary inadequacy of it causes dyslipidemia and atherosclerosis [12], and could be responsible also for the use of the Nigerian vegetables for the prevention, treatment and prophylactic management of atherosclerosis.

Conclusion

The study has shown that the sixteen (16) Nigerian vegetables selected had an appreciable quantity of ascorbic acid and when consumed in the right proportion, would add hugely towards meeting the nutritional requirement for normal growth and protection against diseases since deficiency is often associated with anemia, infections, bleeding gums, scurvy, poor wound healing, capillary hemorrhage, muscle degeneration, atherosclerotic plaques and neurotic disturbances. This explained the reasons for the high use of these vegetables as food and medicine in Nigeria.

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