HPLC Analysis of Fat-Soluble Vitamins in some Vegetables and Fruits in Lagos, Nigeria: an Appraisal of Psycho-Physiology of Wellness

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ABSTRACT
Background: Fat-soluble vitamins play a crucial role in maintaining the body healthy and psycho-physiological functioning, from immune system, muscle and heart function, easy flow of blood as well as eye health. They are critical to health and wellness of humans.
Objective: The objective of the research is to use High Performance Liquid Chromatography (HPLC) analysis to quantify the amount of fat soluble vitamins (Vitamin A, D, E and K) in some vegetable and fruit samples purchased from Mushin market in Lagos, Nigeria.
Methods: The vitamins were extracted with hexane and analyses were performed by HPLC using an analytical reversed phase C-8 column and coupled to a UV detector.
Results: The concentration of vitamin A ranges from 31.768 mg/kg in wild banana to 678.2024 mg/kg in fluted pumpkin leaf. The content of vitamin D ranged from 4.653 mg/kg in wild banana to 228.407 44 mg/kg in scent leaf. Vitamin E concentration is lowest in wild banana (4.411 mg/kg) and highest in scent leaf (1,657.710 mg/kg) while the concentration of vitamin K ranged from 6.691 mg/kg in wild banana to 14,087.313 mg/kg in fluted pumpkin leaf.
Conclusion: Hence, the green leafy vegetables and fruits tested may be considered as a dietary source of fat-soluble vitamins. The study concludes inter-alia that as a people in developing nations we tend to focus more on our physical health than our psycho-physiological health. And it is clear that our collective inadequate diet has consequences for our well being.

Keywords: Fat-soluble, Vitamins, Fruits, HPLC, Psycho-physiological.

INTRODUCTION

Eating well in the workplace and at home can have a significant influence on our overall health and well-being as human beings. This is because nutritious foods can improve concentration and cognitive functions thereby boosting performance. It is documented that employees well-being leads to higher creativity, performance and productivity (1). The World Health Organisation (WHO) has found that optimal nourishment can raise national productivity levels by 20%. According to Institute of Health Matrics, poor nutrition has nearly three times influence on health, resulting in low fitness. In rural and surprisingly urban centres in Lagos State greater percentage of people are going about their daily jobs struggling with nutritional deficiencies with serious health problems and so the need for better, more accessible nutrition education has become a matter of utmost importance. One of such health education is the general advocacy for

consumption of Vitamins and fruits particularly.

Vitamins may be described as organic compounds occurring in minimum amount in diverse natural foods and needed for development and maintenance of acceptable health in human beings. Vitamins are vital food factors, which are necessary for the regular utilization of proximate principles of food like carbohydrates, proteins and lipids (2).

Based on the solubility of vitamins and their application in human nutrition, they can be divided into fat-soluble and water-soluble vitamins (3). The former includes vitamin A (Retinol), D (Calciferol), E (Tocopherol) and K (Phylloquinone), while the latter includes the vitamin B-complex and C. The fat- soluble vitamin is essential components of human diets and is important to health and psycho physiological functioning. The fat-soluble vitamins are required in complex metabolic reactions linked to main biological functions such as vision, healthy immune system and mucous membrane (Vitamin A), calcium absorption (vitamin D), antioxidant protection of cell membranes (vitamin E) and correct blood clotting (vitamin K), among other functions (4). The deficiencies of these vitamins are associated with conditions that are not compatible with good health conditions. Unfortunately, despite the fact that they are readily available in several food sources, people still fail to consume them thereby making them susceptible to ailments associated with their deficiencies. The reason for the poor eating habits is not unconnected to the nature of people daily activities that makes it virtually impossible to eat right and partly due to lack of adequate knowledge of good dietary habits. The dietary source of vitamin A (retinol) includes animal sources: liver, butter, fortified margarine, fortified milk, eggs, cream, cheese, and plant sources (beta-carotene): dark orange, vegetables (sweet potatoes, pumpkin, carrots, and winter squash), fruits (cantaloupe, apricots), and dark green leafy vegetables. Vitamin D includes fortified milk, fortified margarine, egg yolks, fatty fish, liver, the skin can also produce vitamin D when it is exposed to sunlight. Vitamin E includes egg yolks, nuts and seeds, liver, wholegrain products, wheat germ, leafy green vegetables and polyunsaturated plant oils. Vitamin K includes vegetables from the cabbage family, leafy green vegetables, milk; it is also produced in the intestinal tract by the bacteria (5).

Since 2010, high performance liquid chromatography (HPLC) has become the most common method for the determination of vitamins, because of its high sensitivity and wide linear range. Presently, reverse-phase (RP)-HPLC is the most widely used analytical method for vitamins: long-established RP-HPLC with ultraviolet (UV), photodiode array (PDA) and a fluorescence (FL) detector is still widely used for the regular quantification of vitamins in different types of samples (6-8). All of these applications are quantitative but not confirmatory, as they cannot give direct verification of the structure or composition of a substance. Ultraviolet detection is the most economical and flexible method, but the least selective and sensitive, while fluorescence detection is much more sensitive and selective (9). The huge majority of chromatographic separations of vitamins have been achieved with traditional silica-based reversed phase columns (mainly C18) with spherical sorbent particles, 3–5 μm in diameter (10). The rate of the analysis can be increased through the use of a high temperature or ultra-high pressure system (11, 12). Momenbeik *et al* showed an HPLC method for the determination of vitamins A, D3, E and K. A Zorbax-eclipse XDB-C8 column (150 × 4.6 mm, 5 μm) was applied in the experiment, with wavelength at 285 nm. This method has been validated and revealed to be useful for routine analyses, but the analysis is too long-30 min (13). These methods are able to detect both fat- and water-soluble compounds and are of significant interest to analytical laboratories due to their, high sample throughput, simplicity and cost-effectiveness.

The Problem: Rationale for the analysis within the context of psychology and behaviours

It is a common knowledge among urban dwellers that many people usually focus attention on physical activities in their wellness and fitness activities, and so they do not prioritise the other essential aspects of the wellness activities such as nutrition. Eating well is all about eating with intention and eating with intention requires thought and focus, and it can be so hard and difficult when there are so many life stresses competing for attention and mental energies. How many times do people starve themselves after a long, stressful day and do not have enough energy to think about what foods or dinner would make them feel the best? Or perhaps, tried to stick to a certain diet and gotten confused or overwhelmed by all the conflicting nutritional information out there. Most often than not, People feels like the best they can do is just to get something in our bellies and survive the day which is typical of an average Lagosian. One prominent health conscious behaviour that is capable of reversing this ugly trend of pervasive nutritional deficiencies is to encourage "intentional eating" behaviour: the act of purposefully choosing food that nourish and

energize the body such as vitamins and fruits. Unfortunately, for both the lower and middle class people one of the biggest inhibitor of intentional eating is the workplace. The reason for this is not farfetched: work for most people is stressful and mentally draining, because schedule can be very tight and unpredictable, and are most often surrounded by people who and inevitably influencing their behaviour. Particularly, most humans are creatures of habits, preoccupied with daily routines centered on work demands. This is attribute is a prime opportunity to be explored to improve out international eating habits to engender wellness and optimal psycho-physiological functioning.

Hence, the objective of the research is to use HPLC analysis to quantify the amount of fat soluble vitamins in some vegetables and fruits purchased from Mushin market in Lagos, Nigeria.

MATERIALS AND METHODS

Collection and authentication of samples

Samples were obtained randomly from different stores at Mushin market, Mushin Local Government Area, Lagos State. Samples were authenticated at the department of botany, University of Lagos, Nigeria. The samples were authenticated by assigning voucher number to each of them. The samples were then sun dried and homogenized with the aid of blender into fine powder, expect for avocado pear which did not completely dry.

The experiment was carried out on six different vegetable and two fruits samples for the determination of their vitamin A, vitamin D, vitamin E and Vitamin k content. The samples include bitter leaf (*Vernonia amygdalina*) (LUH 8605), Scent leaf *(Ocimum gratissimum)* LUH 8606), Bush bock leaf (*Gongronema latifolium*) LUH 8607), Fluted pumpkin leaf (*Telfairia occidentalis)* LUH 8611, Lettuce (*Lactuca sativa*) LUH 8610, Wild banana (*Musa acuminata*) *(8608))*, Avocado pear (*Persea american) (8609)* and African spinach (*Celosia argentea*) LUH 8612.

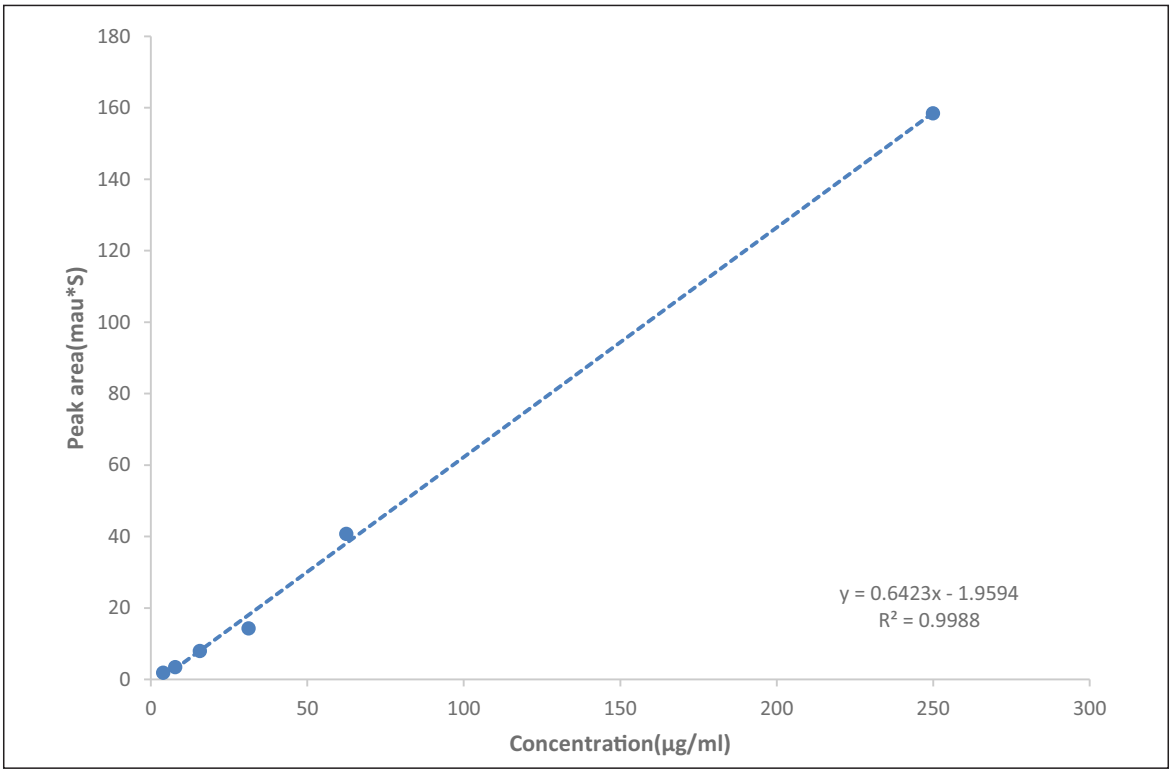

Figure 1: The calibration curve of standard vitamin A

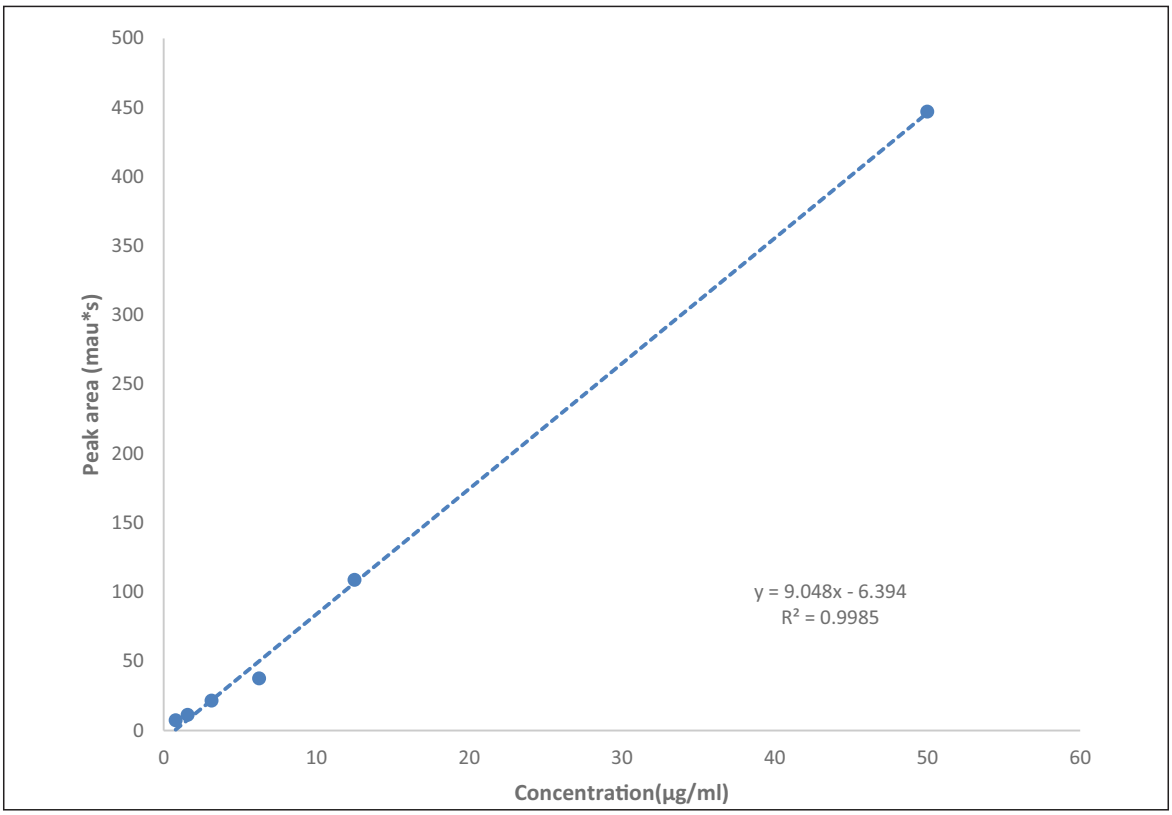

Figure 2: The calibration curve of standard vitamin D

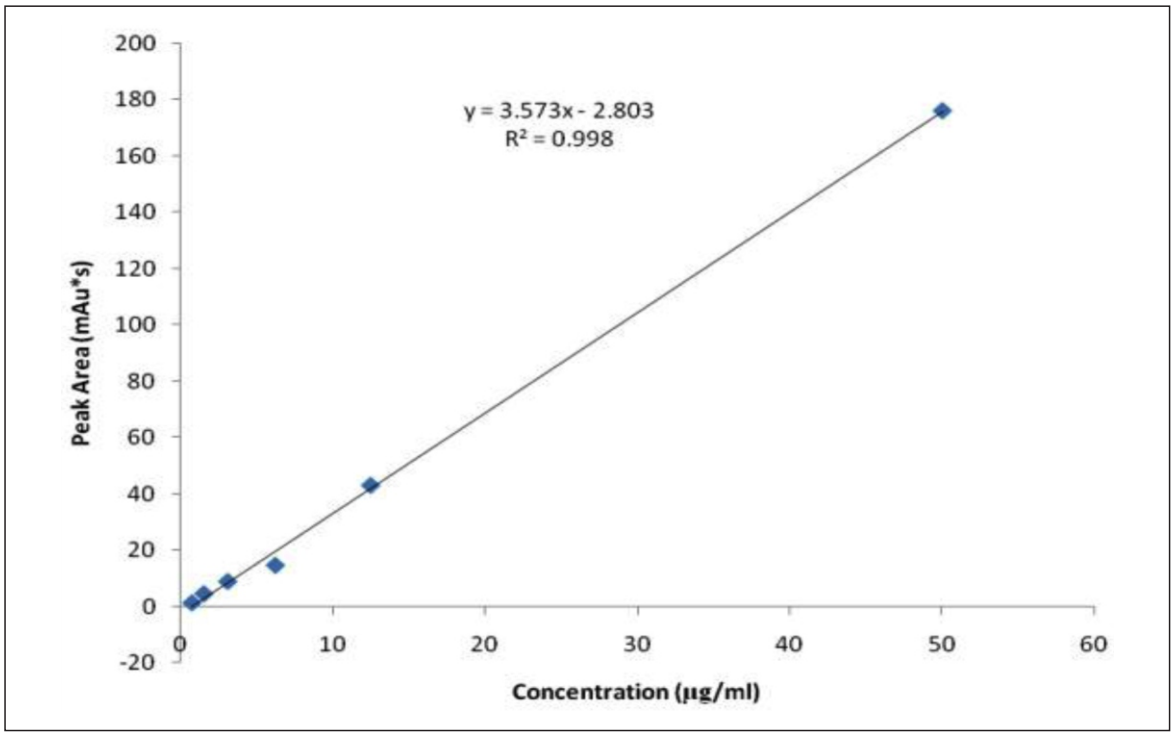

Figure 3: The calibration curve of standard vitamin E

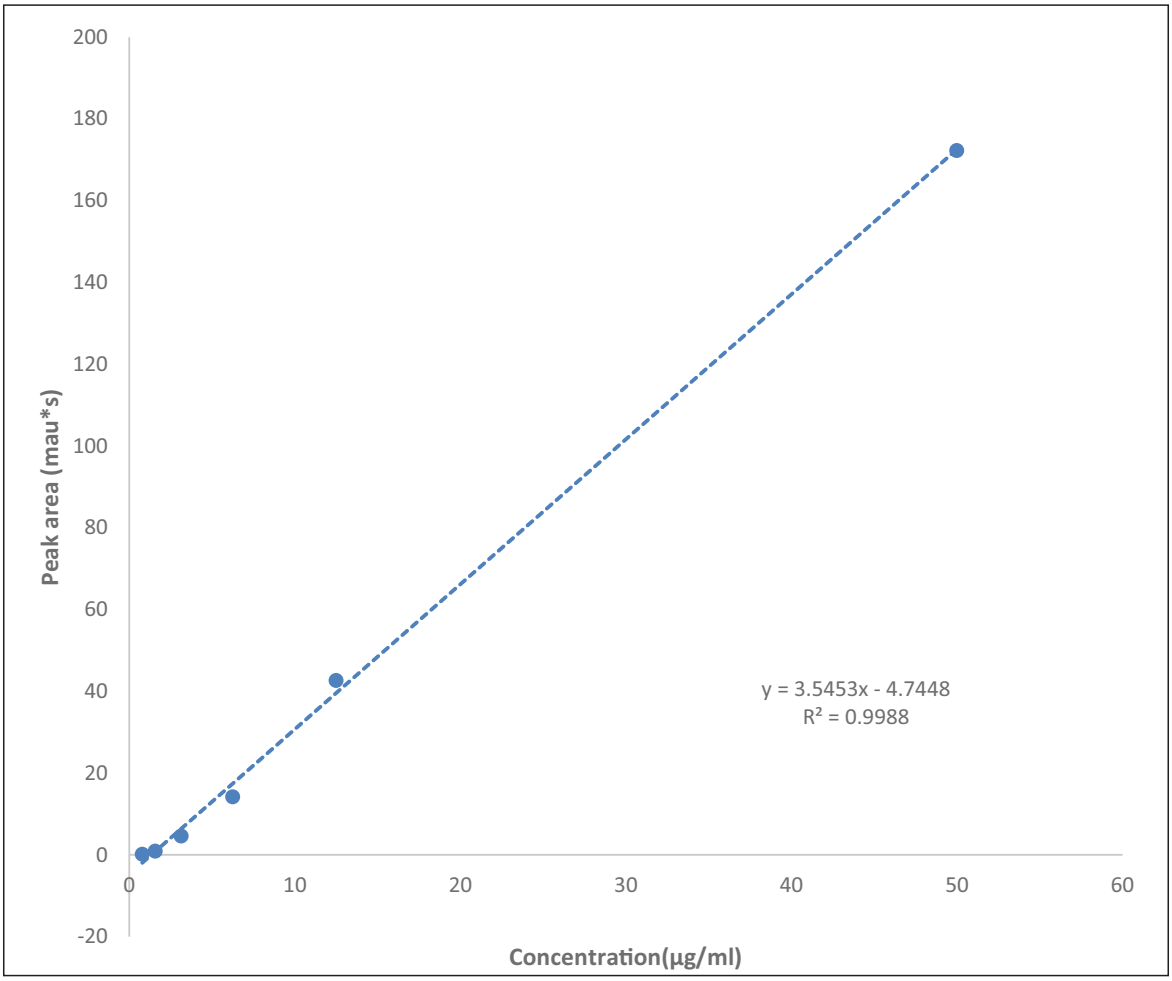

Figure 4: The calibration curve of standard vitamin K

Table 1: Concentration (mg/kg) of Fat soluble vitamins in some fresh vegetables

Common name	Botanical name	Family name	Vitamin A (Beta carotene)	Vitamin D (Calceferol)	Vitamin E (Tocopherol)	Vitamin K (Phylloquinone)
Biter leaf		Asteraceae	47.036	167.455	1,217.584	24.032
Scent leaf	Vernonia amygdalina Del.	Lamiaceae	490.541	228.407	1,657.710	6.691
Bush bock leaf	Ocimum gratissimum	Apocynaceae	33.716	22.435	22.688	9.888
Fluted pumpkin leaf	Gongronema latifolium Benth.	Curcibitaceae	678.202	44.785	239.010	14,087.313
Lettuce	Telfairia occidentalis Hook.F.	Asteraceae	40.336	38.703	137.355	7.802
Spinach	Lactuca sativa	Amaranthaceae	56.028	16.469	499.324	21.576
	Celosia argentea Linn					

Table 2: Concentration (mg/kg) of Fat soluble vitamins in some fresh fruits

Common name	Botanical name	Family name	Vitamin A (Beta carotene)	Vitamin D (Calceferol)	Vitamin E (Tocopherol)	Vitamin K (Phylloquinone)
Wild banana	Musa acuminate Colla.	Musaceae	31.768	4.653	4.411	6.691
Avocado pear	Persea mericana Miller.	Lauraceae	51.332	15.580	4.975	6.747

Preparation of plant extracts

Ten (10 g) each of the dried milled samples was weighed using an analytical balance and was transferred into the beaker. Fifty milliliter (50mL) of ethanol was added to the beaker and stirred gently with stirrer. The beaker was covered with an aluminum foil, stored in the dark and allowed to stand for 24 hrs. This was then filtered with filter paper (whatman) through a glass funnel. The filtrate was partitioned with 50mL n-hexane and was transferred into the separating funnel, the organic layer was collected and was passed through sodium sulphate to remove water. The sample extracts were then filtered using a membrane filter and aliquots of the extract were injected through a syringe filter into the HPLC.

High Performance Liquid Performance (HPLC) analysis

All vitamin standards were of chromatography grade and were obtained from Sigma Chemical Co. (Poole, Dorset). A calibration curve was gotten for the standard (vitamins A, D, E and K), and concentrations for the sample were obtained. Agilent HPLC system coupled to UV detector was used for the analysis. The HPLC system was operated with; Column Zorbax eclipse XDB C8 150x4.6mm, 5μm, Mobile phase (HPLC grade): MeOH: 0.0.01%,TFA: THF 93:5:2)%. Temperature: Ambient, Flow rate: 0.5ml/min, VWD: 280nm.

The calibration plots for the reference standards (vitamin A, D, E and K) were linear with a good correlation coefficient as shown in Figures 1 - 4.

DISCUSSION

The consumption of fruits and vegetables is increasing on daily basis as the people are getting more aware about the nutritional importance of fruits and vegetables. The concentration of fat-soluble vitamins in fruits and vegetables vary depending on the rate of the maturity of the vegetables and fruits as the early reaping are a common practice of the local farmers to get more economical benefits. The recommended dietary allowance (RDA) for vitamin A for adult men and women is 900 and 700 μg. For children, it ranges from 300 to 600 μg (14). Therefore consuming 1 kg of the fruits and vegetable samples in a day is higher than the RDA with the concentration of vitamin A ranges from 31.768 mg/kg in wild banana to 678.2024 mg/kg in fluted pumpkin leaf. High doses of vitamin A may lead to hypervitaminosis A, which is associated with various symptoms and consequences such as fatigue, headache, irritability, stomach pain, joint pain, lack of appetite, blurred vision, skin problem and inflammation of the mouth and eyes. Higher amounts, 900 mg may cause acute hypervitaminosis A in adult (15). However, consumption of 1 kg of any single samples tested in a day may not lead to hypervitaminosis A.

For children and adults, the RDA for Vitamin D is 15 μg. The amount is slightly higher for elderly adult, at 20 μg (16). Therefore consuming 1 kg of the fruits and vegetable samples in a day is higher than the RDA with the content of vitamin D ranged from 4.653 mg/kg in wild banana to 228.407 44 mg/kg in scent leaf. Higher amounts, ranging from 1000 μg – 2500 μg per day, may cause symptoms of toxicity in adults when taken daily for one or two months. However, consumption of 1 kg of any single samples tested in a day may lead to hypervitaminosis D. The main consequence of toxicity is hypercalcemia, a condition characterized by excessive amounts of calcium in the blood (17).

Among adults, RDA for vitamin E is 15 mg. For children and adolescence, the RDA ranges from 6 to 15 mg depending on the age group. Vitamin E appears to be less toxic at high doses than Vitamin A and D. However, at high doses of more than 1000 mg per day, vitamin E may have pro-oxidant effect. That is it can become the opposite of an antioxidant, potentially leading to oxidative

stress (18). The fruits (banana and avocado pear) showed lower Vitamin E content per Kg when compared to the RDA. Bitter leaf and scent leaf may act as a pro-oxidant due to their high vitamin E content of more than 1000 mg (Table 1 and 2).
The adequate intake of vitamin K is 90 μg for women and 120 μg for men. For children and adolescents, the adequate intake ranges for 30-75 μg, depending on the age group. The maximum safe dosage of vitamin K is unknown and no symptoms of toxicity have been identified (19). Therefore, consuming 1 kg of the fruits and vegetables of any sample in a day is higher than the RDA with the concentration of vitamin K ranged from 6.691 mg/kg in wild banana to 1, 4087.313 mg/kg in fluted pumpkin leaf. The entire group of water-soluble vitamins (Vitamins A, D, E, and K) as well as water-soluble vitamins B and C-vitamins seems to crucial for mental health. Retinol, Thiamine, niacin, folate, and biotin are just a few of the B-vitamins that are necessary for brain function (vision etc), and deficiencies in most these essential vitamins can lead to poor immune system, headaches, depression, confusion, and fatigue (20 - 22). Luckily, both water-soluble and water-soluble vitamins are available in substantial quantities in many food sources including most whole grains, spinach, carrots, leafy green vegetables, and fish. Vitamin D has two main roles in the body: it maintains bone health and supports the immune system, dietary sources included oily fish and fish oil, fortified dairy products, plant- based milks, and cereals etc.

CONCLUSION

Fat-soluble vitamins are vitamins A, D, E, and K. They are present in foods containing fats. The body absorbs these vitamins as it does dietary fats. They do not dissolve in water. Studies have shown that they serve several functions in body such as: as an antioxidant, to boost the immune system, to dilate blood vessels and help prevent clotting. Deficiencies of these vitamins have profound effects on physiological processes with implications for certain behavioral manifestations that are not consistent with sound health e.g. fatigue, memory loss, and cognitive functions). From the findings of this study, fat-soluble (vitamin A, vitamin D, vitamin E and Vitamin K) were revealed to be present in a significant amount in the fruits and vegetables tested. HPLC method of analysis was adequate in the quantification of these vitamins content in the samples. This study concludes that though most fat-soluble and water-soluble vitamins occur naturally in the human body, they are present in some dietary sources. Hence, it is recommended that green leafy vegetables and fruits tested may be considered as a dietary source of fat soluble vitamins, this can be applicable in the household nutrition and food industry.

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