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Evaluation of Fat and Water Soluble Vitamins Composition of Mature (Yellow) *Carica papaya* Leaf

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ABSTRACT: Vitamins are a broad group of molecules with unique chemical, biological, and structural characteristics that are necessary for a normal metabolic process. This research evaluated the fat and water-soluble vitamin composition of mature (yellow) *Carica papaya* leaf using standard analytical techniques. The results obtained from the analyses revealed that mature (yellow) *Carica papaya* leaf contained the following fat-soluble vitamins: vitamins A (5.08 mg/100g), Vitamin D (3.14 mg/100g), Vitamin E (15.47 mg/100g), and Vitamin K (8.03 mg/100g), while the water-soluble vitamins include Vitamin B1 (0.019 mg/100g), Vitamin B2 (0.018 mg/100g), Vitamin B3 (0.610 mg/100g), Vitamin B6 (0.0231 mg/100g), Vitamin 12 (4.415 mg/100g), and Vitamin C (60.41 mg/100g). The study has shown that the mature (yellow) leaf of *Carica papaya* is a rich source of both fat-soluble and water-soluble vitamins, which may be used in the formulation of multivitamin food supplements.

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Vitamins are class of specialized chemical compounds that the body needs to function normally (Avrămia *et al*., 2024). They are broad group of molecules with unique chemical, biological, and structural characteristics that are necessary for a normal metabolic process (Chromatography, 2020). They are required in small amounts and are obtained primarily through diet and various vitamin supplements (Altria, 2013). Because they are very essential for several metabolic processes and vital bodily reactions, they should be taken in tiny doses with food or as supplements. (Skowik-Borowiec *et al*., 2023). Vitamins are categorized into two groups: fat-soluble and water-soluble vitamins. Examples of water-soluble vitamins include Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6 (Pyridoxine),Vitamin B12 (Cyanocobalamin), Vitamin C (Ascorbic Acid), and others, while examples of fat-soluble vitamins include Vitamin A (Retinol), Vitamin A acetate (Retinol Acetate), Vitamin D2 (Ergocalciferol), Vitamin D3 (Cholecalciferol), Vitamin E (Tocopherol), and Vitamin K (Phylloquinone) (chromatography, 2020). A study by Kendy (2016) revealed that the B vitamins are involved in vital and closely connected functions in cellular activity, acting as helper molecules in both

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catabolic and anabolic reactions known as coenzymes. Ascorbic acid, also known as Vitamin C, a member of the water-soluble vitamins, is considered the most suitable antioxidant found in diet as it is naturally occurring in huge quantities in plant-based foods (Sami *et al.,* 2014). Even though the majority of vitamins come from plants, foods with animal origins, such as meat, dairy, and eggs, are frequently an indirect source of these nutrients because they are higher up the food chain (Hashim *et al*., 2021).

Vitamin A belongs to several groups of chemical compounds referred to as carotenoids, retinol, and its derivatives (Zasada and Budzisz, 2019). The two main sources of vitamin A are from both plant and animal origin. Animal sources include the liver, egg, fish, milk, cheeses, meat, and butter. Vitamin A from plant origins includes papaya, spinach, broccoli, kale, cabbage, peach, apricot, carrots, spinach, mango, parsley leaves, and a ton of other vegetables (Skowik-Borowiec *et al*., 2023). Vitamin A plays a lot of significant physiological roles, aiding vision in poor light. Vitamin A also has an advantageous impact in growth, procreation, embryogenesis, control of cell differentiation, and immunity. Vitamin A derivatives, retinoids, control the division of skin cells and the process of epithelial keratinization in the skin. As such, the vitamin is employed in the management of skin conditions. Vitamin A has also been reported to be successfully used for the treatment of cancers and measles (Ross *et al*., 2014; Beckenbach *et al.*, 2015; Skowik-Borowiec *et al*., 2023). Tocopherol is found in a variety of food products. A large quantity of vitamin E is found in vegetable oils as they contain all the homologs of the vitamin in numerous proportions; smaller amounts of vitamin E can be found in fish. Nuts and seeds such as almonds and hazelnuts are also good sources of vitamin E. In addition, vitamin E is also found in fruits such as mango, avocado, papaya, and asparagus (Rizvi *et al*., 2014; EFSA, 2015). Vitamin E serves as an antioxidant that scavenges free radicals and protects the whole body system against many illnesses and health problems related to oxidation. By inhibiting lipid peroxidation, vitamin E fortifies blood vessel walls, guards against the damaging effects of polyunsaturated fatty acids (PUFA), stops platelet hyper-aggregation, and shields the body against cardiovascular illnesses. It also plays a role in hormone synthesis. (Riza *et al*., 2014; Niki and Abe, 2019). Vitamin K found in nature can be divided into two categories: K1 (phylloquinone), which is primarily found in plants, and K2 (menaquinones), which is produced by bacteria. The vitamin K-dependent carboxylase requires vitamin K as a cofactor. Sources of vitamin K include green leafy vegetables, cheese, fish meat, grains, meat, and milk, among others (Weber, 2001). Vitamin D is crucial for maintaining the health of the musculoskeletal system. Because it controls the metabolism of calcium and phosphorus, it is very crucial for people's health. The primary source of it is sunshine that contains enough ultraviolet B radiation, which causes it to be synthesized in the skin. Additionally, foods like milk, meat, fish, and other dietary supplements can provide it (SACN, 2023).

In general, plants contain a lot of bioactive components with medicinal characteristics, although proper assessment is needed to ascertain their safety before usage as drugs (Mmuo and Okoli, 2022). *Carica papaya* is a perennial herbaceous plant, recognized for a variety of biological activities. It has previously been claimed that the plant parts, including the seeds, leaves, fruits, peels, roots, and stems, offer nutritional and therapeutic qualities. This plant has low calories and a high nutritional value due to its natural mineral and vitamin content. Furthermore, all parts of this plant have been used historically to regulate childbirth as well as for wound dressing, antimicrobial, and anthelminthic properties, among other medical purposes (Alara *et al*., 2020). In some local languages in Nigeria and West Africa, the plant is known as mbuwe in Tiv, gwanda in Hausa, and ohuo in Bini. Phytochemically, the whole plant contains enzymes (papain), lycopene, carotenoids, alkaloids, mono-terpenoids, flavonoids, minerals, and vitamins. The types of vitamins reported to be present in papaya include vitamin thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, folate, vitamin B12, vitamin A, vitamin E, vitamin K, and carotene (Gunde *et al*., 2016). A lot of studies have reported the vitamin profile of young (green) leaves of *Carica papaya*; hence, the objective of this paper is to evaluate the fat- and water-soluble vitamin composition of vitamin profile of mature (yellow) *Carica papaya* leaf.

MATERIALS AND METHODS

Plant Collection and Preparation: The mature (yellow) *Carica papaya* leaves were collected in Benin City, Edo State, and were authenticated by a taxonomist. A herbarium specimen was deposited, and a voucher number was given. The leaves were prepared as described by Igbashio *et al*. (2023).

Evaluation of Vitamin A: Vitamin A was evaluated by the technique of Bayfield and Cole (1980). The test was based on the spectrophotometeric estimation of the color yielded by vitamin A acetate or palmitate with TCA. Vitamin A content was expressed as mg/100 g.

Evaluation Vitamin E: Vitamin levels in the sample samples were evaluated using the Emmerie-Engel reaction. The Emmerie-Engel reaction was based on the reduction of ferric to ferrous ions by Vitamin E, which, with 2, 2'-dipyridyl, forms a red color. Vitamin E and carotenes are first sampled with xylene and read at 460 nm to measure carotenes. A correction was made for this after adding ferric chloride and reading at 520 nm (Emmerie and Engel 2010).

Evaluation of Vitamin C: Spectrophotometric approach was used to analyze vitamin C.

Principle: Absorbate was converted to dehydro-ascorbate after treatment with activated charcoal. This reacted with 2, 4-dinitro-phenyl hydrazine to form osazones. The osazones produces an orange-coloured substance when dissolved in tetraoxosulphate (VI) acid, in which the absorbance was measured using spectrophotometric technique at a wavelength of 540nm (Roe and Keuther 1943).

Evaluation of Vitamin D and K: Vitamin D and K were estimated by the method described by AOAC, (2005). The principle was based on the formation of a yellow color by reaction of the vitamin with a chloroform solution of trichloroacetic acid. The yellow color was read spectrophotometrically at 450 nm and 503 nm, respectively, for vitamin D and K.

Evaluation of vitamin B_1 and B_2: The B-vitamins were estimated by methods described by AOAC (2005). About 1 gram of sample was weighed into a conical flask; this was dissolved with 100 ml of deionized water and was shaken thoroughly, heated for 5 minutes, and allowed to cool and filter. The filtrate was poured into a cuvette, and their respective wavelengths for the vitamins were set to read absorbance using a spectrophotometer: Vitamin B_1 at a wavelength of 261 nm and Vitamin B_2 at a wavelength 242 nm.

Calculation:

$$\text{Concentration (mg \%)} = \frac{\text{A x D.F} \times \text{volume of cuvette}}{E} \quad (1)$$

Where A = absorbance; E = extinction coefficient = 25 for B_1 and B_2; DF = dilution factor=5

Evaluation of vitamin B_3: Exactly 5 g of sample was dissolved in 20 ml of anhydrous glacial acetic acid and warmed slightly. 5 ml of acetic anhydride was added and mixed. 2–3 drops of crystal violet solution were added as an indicator. Titrate with 0.1 M perchloric acid to a greenish blue color (AOAC, 2005).

Calculation:

$$\text{Vitamin B3} = \frac{\text{Titre value x 0.0122}}{0.1} \quad (2)$$

*Evaluation of vitamin **B_6**:* Exactly 5 g of sample was dissolved in a mixture of 5 mL of anhydrous glacial acetic acid and 6 mL of 0.1 mL mercury II acetate solution. 2 drops of crystal violet were added as an indicator. Titrate with 0.1 mL perchloric acid to a green color end point. Calculation: each meal of 0.1 M perchloric acid is equivalent to 0.02056 g of $C_8H_{11}NO_3HCL$ (AOAC, 2005).

Evaluation of Vitamin B_{12}: Up to 25 mg of sample was dissolved in 250 mL of deionized water. The absorbance was read at 361 nm (AOAC, 2005)**.**

Calculation:

$$\text{Concentration of Vitamin } B12 \text{ (mg \%)} = \frac{\text{A x D.F} \times \text{volume of cuvette}}{E} \quad (3)$$

Statistical Analysis: Statistical package for social sciences (SPSS), version 25.0, was used to analyze the data obtained. Values were deemed statistically significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Water soluble Vitamins Composition of Mature (yellow) Carica papaya Leaf: Table 1 presents the water-soluble vitamin composition of the plant leaf. From the result, vitamin C has the highest composition (60.41 mg/100 g), followed by vitamin B_{12} (4.415 mg/100 g). Vitamins B_1, B_2, B3, and B_6 recorded very low concentrations in this study.

Table 1: Water soluble Vitamins Composition of Mature (yellow) Carica papaya Leaf

Vitamins	Composition (mg/100g)
Vitamin B1	0.019±0.010
Vitamin B2	0.018±0.000
Vitamin B3	0.610±0.001*
Vitamin B6	0.231±0.001*
Vitamin B12	4.415±0.003*
Vitamin C	60.41±0.55**

Data were obtained in triplicate mean± Standard Error Mean (SEM) was determined.

Fat soluble Vitamins Composition of Mature (yellow) Carica papaya Leaf

Table 2 presents the water-fat vitamin composition of the plant leaf. From the result obtained, vitamin E has the highest composition (15.47 mg/100 g), followed by vitamin K (8.03 mg/100 g). Vitamin D recorded the lowest concentration (3.14 mg/100 g).

Table 2: Fat soluble Vitamins Composition of Mature (yellow) *Carica papaya* Leaf

Vitamins	Composition (mg/100g)
Vitamin A	5.08±0.001*
Vitamin D	3.14±0.05*
Vitamin E	15.47±0.00**
Vitamin K	8.03±0.03*

Data were obtained in triplicate mean± Standard Error Mean (SEM) was determined: Vitamins are a class of varied substances that can exist in a variety of chemically distinct but physiologically interchangeable forms. They are vital nutrients for both human and animal diets. They are relatively small amounts for sustaining life and excellent health (Khaneghah *et al*., 2019). As a result, inadequate vitamin intake can result in severe illnesses. Moreover, the quantity of vitamins necessary for optimal development and required maintenance of bodily functions is not always met by the food that humans eat; in fact, they can be lost due to chemical reactions, extraction, and leaching that occur during food processing and storage; this is frequently the case with water-soluble vitamins (Zhu *et al*., 2020). The vitamin evaluation has revealed that the mature (yellow) *Carica papaya* leaf is a rich source of both fat- and water-soluble vitamins such as vitamin A, C, D, E, K, B_1, B_2, B_3, B6, and B_{12}. This agrees with the work done by Olumide *et al*. (2022). The B-vitamins are important and primarily function as coenzymes in the synthesis of energy. Additionally, Vitamin B1, also known as thiamine, is required for nerve function; Vitamin B2 (riboflavin) is needed for good skin and vision. Vitamin B3 (Niacin) is important for nervous, digestive, systems, and skin health. Vitamin B12 (cyanocobalamin) is needed for the production of new cells and nerve function (Hashim *et al*., 2021). Vitamin B6 influences the appropriate operation of the immunological and endocrine systems and is involved in protein, carbohydrate, and fat metabolism. In addition, it lessens neurotoxicity and is also used to treat hypertension (Ahmad *et al*., 2013). Vitamins A, C, and E act as antioxidants by detoxifying free radicals (Pruteanu *et al*., 2023). Vitamin A is essential in biological processes and also vital for the regulation of proliferation, embryonic development and differentiation numerous cell types. The human body needs vitamin D for a variety of purposes, and deficiencies in it are linked to both skeletal and non-skeletal disorders (Abdelsalam *et al*., 2023). Vitamin E is necessary for several bodily processes, including development, reproduction, disease prevention, and tissue integrity preservation. (Saima *et al*., 2013). The high concentrations of vitamin C and E (60.407 mg/100 g and 15.458 mg/kg), respectively, suggest that plant leaves could be a rich source of antioxidants. Antioxidants are substances that have the potential to fight free radicals that cause oxidative stress. They play a preventive role against diseases associated with oxidative stress; such as cancer, cataracts, cardiovascular diseases, age-related diseases, central neurodegenerative diseases, and diabetes mellitus among others. Vitamin K has been reported to play a role in the biological activity of multiple coagulation factors, including factors II, VII, and IX, as well as proteins C and S (Suttie *et al.*, 2001).

Conclusion: The preliminary study revealed that the mature (yellow) *Carica papaya* leaf contains both fat- and water-soluble vitamins, which may be utilized in the preparation of multivitamins and food supplements.

Declaration of conflict of interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

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