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Research Article

ATR-FTIR AND HPLC SPECTROSCOPIC STUDIES AND EVALUATION OF MINERAL CONTENT OF CARICA PAPAYA LEAVES AND FLOWERS

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ABSTRACT

We applied Fourier transform infrared spectroscopy (FTIR) to study the main constituents in the male *Carica papaya* leaves and flowers in the mid infrared region 4000–650 cm⁻¹. The findings indicated that FTIR spectrum can discriminate and identify various functional groups present in the papaw parts.

Four water-soluble vitamins, thiamine (vitamin B₁), riboflavin (vitamin B₂), niacin (vitamin B₃) and folic acid (vitamin B₉) were analyzed by HPLC. Niacin and folic acid were detected in the leaves at a concentration of 7.08 mg/100 g and 1.00 mg/100 g dry weight, respectively, while only folic acid could be detected in the flowers at a high concentration of 510.34 mg/100 g dry weight. Atomic absorption spectroscopy (AAS) analysis of the samples showed that the leaves and flowers contain elements like K, Na, Mn, Mg, Fe, Zn, Cu, Ca, Cd and Ni in various proportions, with the leaves containing higher concentrations of Mg, Ca, Zn, Fe and Ni than the flowers.

KEY WORDS: Spectroscopic Studies, Carica Papaya

INTRODUCTION

Carica papaya L belongs to the family- Caricaceae. It is a fast growing small tree, 2-10 m, which probably originated in South America (southern Mexico and Costa Rica), but now cultivated in tropical countries, including Nigeria. The plant contains a broad spectrum of phytochemicals and every part of the plant is useful medicinally and economically. The leaves contain alkaloids (carpaine, pseudocarpaine and dehydrocarpaine I and II), choline, carposide, vitamins C and E [1],[2]. Papaya trees may be female or male. The male tree contains only male flowers and will not produce fruit.

The fundamental vibrations of structures (functional groups) of the phytochemicals can be studied by using the mid-infrared energy, approximately in the region between 4000 to 400 cm⁻¹.

Different functional groups adsorb at different IR regions, hence the Fourier Transform InfraRed (FTIR) spectroscopy has been widely used to study, integrally, the main constituents of some medicinal plants [3],[5].

The papaya fruit is known to be rich in natural vitamins and minerals [6]. Vitamins are a broad group of organic compounds which are minor constituents of food.

Although they are minor, they are essential and required for normal growth, self-maintenance and functioning of human and animal bodies. Vitamins are relatively unstable. They can be lost in food either during storage or processing [7]. The preferred technique for vitamin separation is the high performance liquid chromatography (HPLC) because of its high selectivity [8].

There have been other reports of elemental contents of some medicinal plants or plant extracts [9],[11]. The concentrations of these elements vary in different parts of the plants (leaves, seeds and roots) and it has been established that a number of mineral elements play an important role in the metabolism as well as in the cure of some diseases [12],[13].

The literature contains several reports on research on the *Carica papaya* tree, but there is no report on the attenuated total reflectance-Fourier transform infrared (ATR-FTIR), high performance liquid chromatography (HPLC) and mineral content analyses of *Carica papaya* leaves and flowers.

Despite the enormous amounts of work done on the carica papaya tree, little or no studies have been carried out on the leaves and flowers of the *carica papaya* male tree. The present paper describes mineral, vitamin and infrared analyses of fallen leaves and flowers of male *Carica papaya* collected from Ile-Ife, Nigeria

MATERIALS & METHODS

Plant Materials

In the present study, fresh fallen leaves and flowers of male *Carica papaya* were collected from an area at the Obafemi Awolowo University, Ile-Ife, Nigeria. The leaves and flowers were

Separated, cleaned and dried at room temperature (r.t.) and subjected to mortar grinding to obtain a fine powder. The powdered samples were kept in a lyophilizer to remove moisture. All analyses were carried out at the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria.

Spectroscopic Analysis (FTIR)

Spectra were obtained with the aid of a diamond attenuated total reflectance (ATR) accessory on an Agilent Cary 630 FTIR spectrometer, with a scanning range of 4000 to 650 cm^{-1} , at a resolution of 4 cm^{-1} and 32 scans.

(HPLC).

Liquid chromatography was performed with an Agilent 1260 infinity liquid chromatographic system (Agilent Technologies, Santa Clara, USA) fitted with variable (200-800 nm) ultraviolet-visible detector and a quaternary pump. The column was Hypersil ODS (C₁₈) 3.5 μm , 4.6 X 100 mm reversed phase stainless steel type (Agilent Technologies, Santa Clara, USA).

All the reagents used were HPLC grade and the chemicals were of analytical grade. The reagents used were methanol (sigma Aldrich), acetonitrile (sigma Aldrich), acetic acid, hydrochloric acid and phosphoric acid. Chemicals: Sodium hydroxide and potassium dihydrogen phosphate. The vitamin standards (niacin (Vitamin B3), thiamin (Vitamin B1), riboflavin (Vitamin B2) and folic acid (Vitamin B9)) from Sigma (Sigma-Aldrich, Deisenhofen-Germany) were used without further purification. Water was distilled using Autostill® distiller.

An extraction solution was prepared by mixing 50 ml of acetonitrile with 10 ml of acetic acid in a 1L flask. The mixture was then made up to the mark with distilled water.

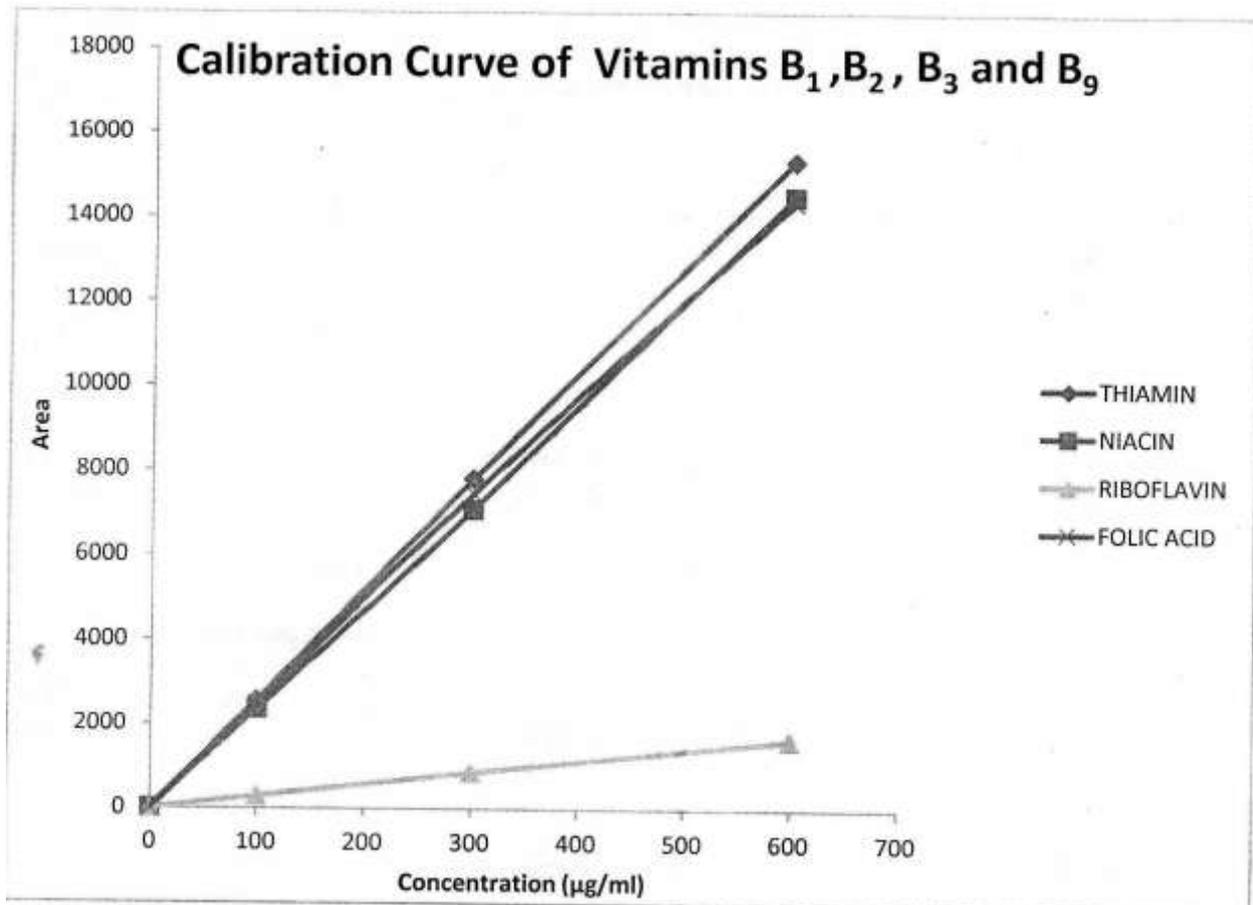
0.15g of each of the powdered samples was weighed (analytical balance (Mettler Toledo)) and transferred to a conical flask. 10 ml of the extraction solution was added to each flask and heated over a water bath at 70°C for 40 min. Thereafter, the mixtures were allowed to cool, filtered and made up to 50 ml with extraction solution in a volumetric flask.

Standard stock solutions of niacin and thiamin were prepared by dissolving 0.6 mg of each in 1ml of 0.1M HCl and standard stock solutions of riboflavin and folic acid were prepared by dissolving 0.6 mg of each in 1ml 0.1M NaOH. Working standards of other concentrations were prepared from these.

0.1M Phosphate buffer solution was prepared using potassium dihydrogen phosphate in a volumetric flask and the pH adjusted to 3.3 using phosphoric acid. The mobile phase consisted of 0.1M phosphate buffer, methanol and acetonitrile in the ratio 90:2:8.

20 μl of each of the prepared standard solutions were injected into the liquid chromatographic system using the mobile phase prepared above at a flow rate of 0.3ml/min and wavelength of 260nm. The retention times were noted. A calibration plot was prepared for each vitamin. Correlation coefficients for niacin, thiamine, folic acid and riboflavin on the basis of plots of concentration ($\mu\text{g mL}^{-1}$) against peak area (mAU) were found to be > 0.999 (Figure 1).

Figure 1: Calibration curve for Vitamins B₁, B₂, B₃ and B₉



(AAS)

Na, K, Ca, Fe, Mg, Mn, Ni, Zn, Cd, Cu and Cr in the plant samples were analyzed using PerkinElmer A Analyst 400 Atomic Absorption Spectrometer, under standard conditions for atomic absorption measurement.

RESULTS & DISCUSSION

It is now common to use attenuated total reflection (ATR) accessories in conjunction with Fourier transform infrared (FTIR) spectrometers. This accessory provides for the non-destructive infrared measurement of both transmittance and absorbance spectra of samples with little or no preparation. The FTIR spectrum can be used to identify the functional groups of the active components based on the absorption band values in the region of infrared radiation.

The spectra obtained in this study showed that the plant samples are devoid of moisture (H₂O) as indicated clearly by the absence of any vibrational band at around 1635 cm⁻¹ associated with moisture. As shown in Figure 2 for the powdered leaves, the strong, broad absorption band at 3392 cm⁻¹ can be assigned to the O-H stretching vibration, while the peaks at 2919 and 2851 cm⁻¹ belong to the C-H stretching vibration of methyl (CH₃) and methylene (CH₂) (which are the typical absorption of lipophilic components) [14]

The strong band centered at 1619 cm⁻¹ can be attributed to C=C stretching of aromatic rings in lignin (? and NH₂ asymmetric deformation). The vibration band around 1421 cm⁻¹ could be due to aliphatic and aromatic (C-H) groups in the plane deformation vibrations of CH₃, CH₂ and OCH₃ groups [15], while band at 1377 cm⁻¹ can be assigned to C-H bending mode of CH₂ [16] and the strong band at 1317 cm⁻¹ can be ascribed to CH₂ wagging vibrations in cellulose. The peak at 1244 cm⁻¹ may indicate the presence of structural carbohydrate such as cellulose [17] and predict the presence of ester carbonyl group. The absorption band at 1148 cm⁻¹ (shoulder) is assignable to C-O stretching vibration (alcohols, esters) and anti-symmetric bridge C-O-C stretching vibration [18]. Ir absorption bands around 1028 cm⁻¹ are attributed to C-O and C-C stretching vibrations and C-O-H deformation of starch [17].

The FTIR spectrum of the powdered flowers of *C. papaya* is shown in Figure 3. The spectrum is different from that of the leaves (Figure 2). The obvious differences are the bands at 3276 cm⁻¹,

1734 cm⁻¹ and the peak situated at 1393 cm⁻¹. The absorption band at 3276 cm⁻¹ can be associated with N-H stretching of amide A stretching mode and intermolecular O-H molecules, while the peak present at 1734 cm⁻¹ can be attributed to the ester C=O stretching of phospholipids [19].



The band at 1393 cm^{-1} probably represents C=O symmetric stretching of COO^- and assigned to lipids or aminoacids [20],[21]. The peak occurring at around 780

cm^{-1} in the flowers and leaves of the plant can be attributed to out-of-plane N-H wagging, primary and secondary amide.

Figure 2: FTIR spectrum of *Carica papaya* male leaves

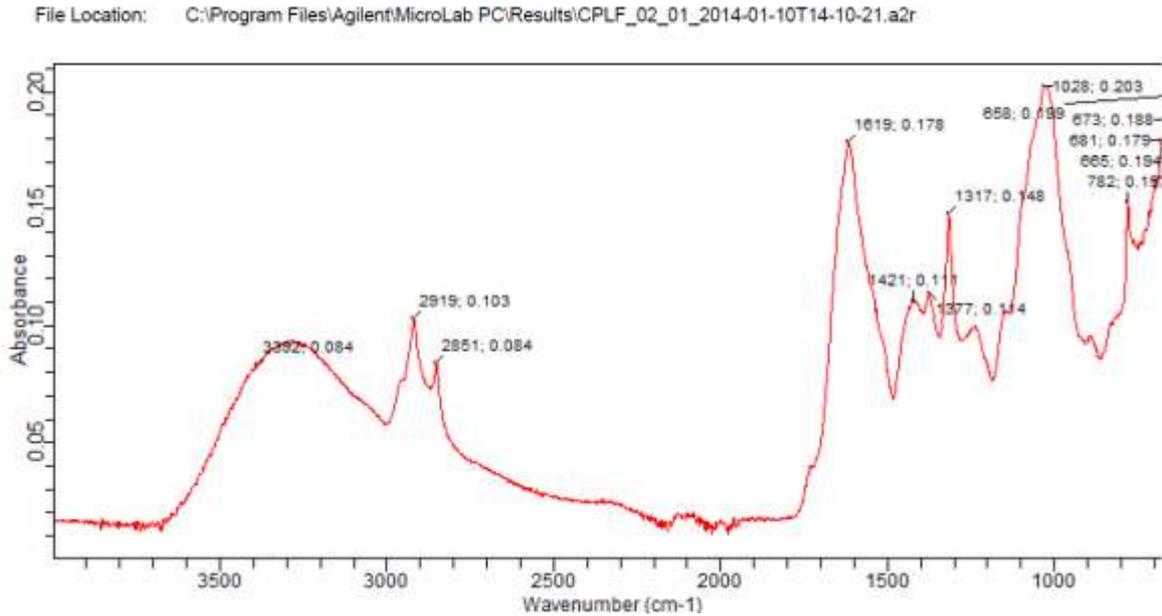
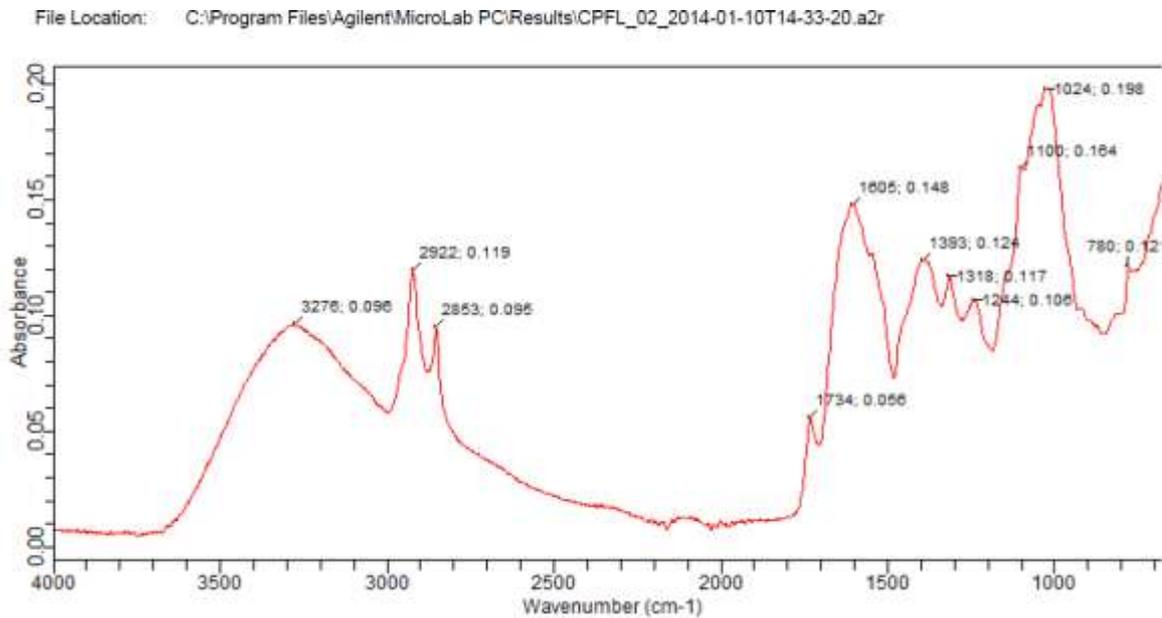


Figure 3: FTIR spectrum of *Carica papaya* male flowers



To the best of our knowledge, there are no published research studies on HPLC determination of water-soluble vitamins in male *C. papaya* leaves and flowers. The calibration curves for the four studied vitamins are shown in Figure 1, with coefficients of determination (r^2) greater than 99.97% for the four vitamins. The solvent system used for the analysis produced good separation of the peaks. The HPLC chromatogram showing separation of water soluble vitamins from *C. papaya* leaves and flowers are depicted in Figure 4 and Figure 5, respectively and the calculated average vitamin contents are shown in Table 1.

Table 1: Vitamin content ($\mu\text{g}/100\text{ g}$ dry weight) of leaves and flowers of male *Carica papaya*

SAMPLE				
	$B_1 \pm \text{SD}$	$B_2 \pm \text{SD}$	$B_3 \pm \text{SD}$	$B_9 \pm \text{SD}$
Leaves	0.00	0.00	7078.68 ± 0.28	1003.37 ± 0.43
Flowers	0.00	0.00	0.00	510339.28 ± 0.74

SD = standard deviation

Niacin (B_3) and folic acid (B_9) were only detected in the leaves at a concentration of $7.08\text{ mg}/100\text{ g}$ and $1.00\text{ mg}/100\text{ g}$ dry weight, respectively. On the other hand, only folic acid could be detected in the flowers at a high concentration of $510.34\text{ mg}/100\text{ g}$ dry weight. The other peaks in the chromatograms were not identified. The result shows that the male *Carica papaya* flowers can be sources of folate intake.

Figure 4: HPLC chromatogram of male *Carica papaya* leaves

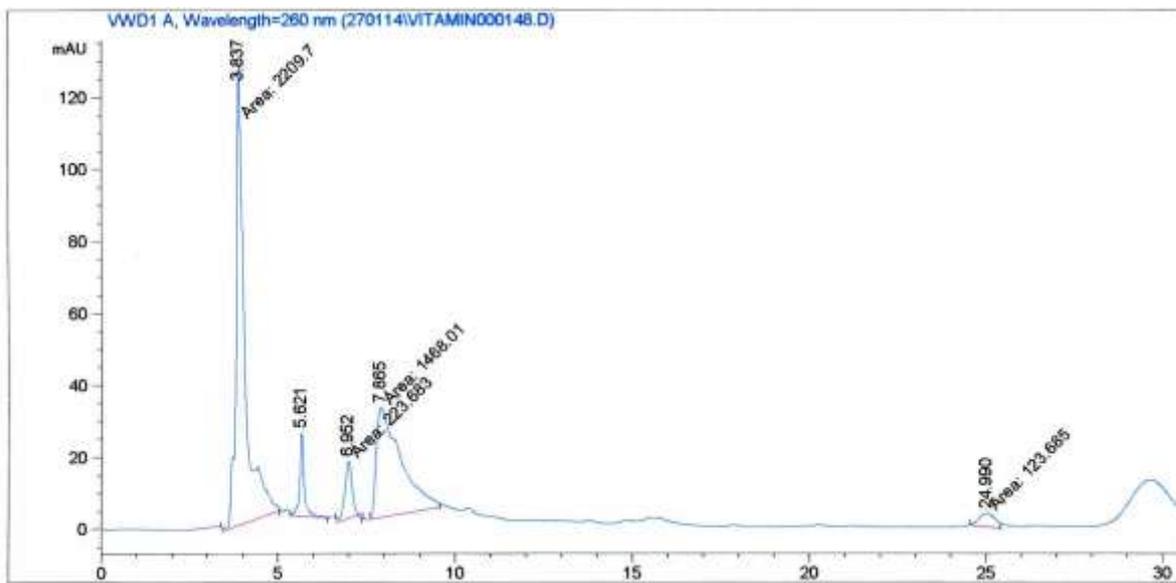
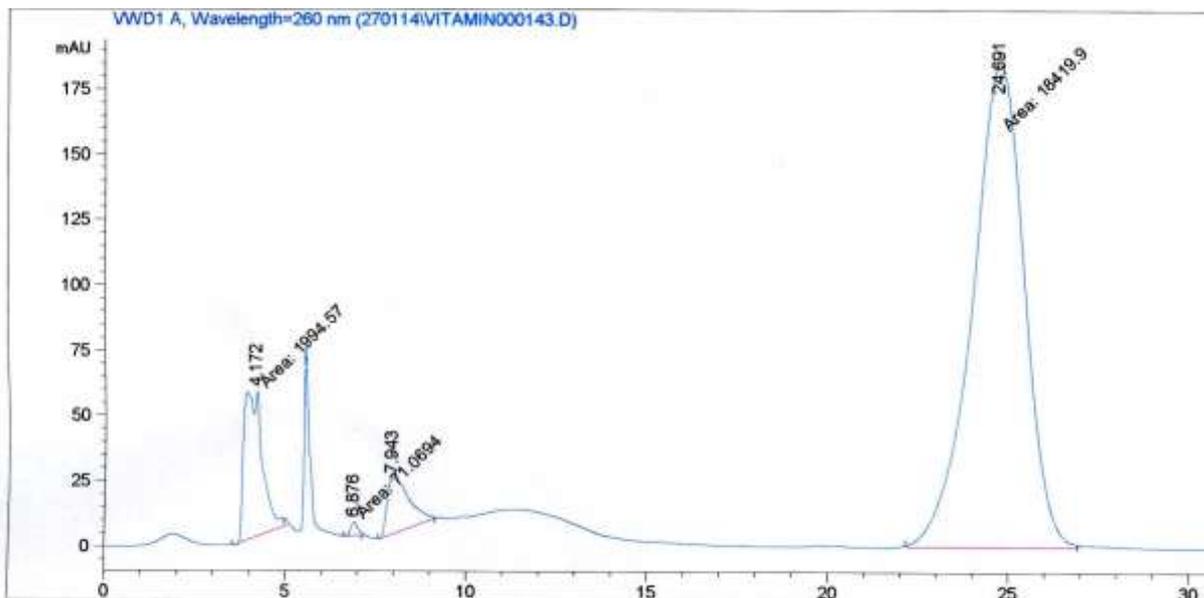


Figure 5: HPLC chromatogram of male *Carica papaya* flowers



An examination of the data from Table 2, shows that the leaves and flowers contain elements like K, Na, Mn, Mg, Fe, Zn, Cu, Ca, Cd and Ni in various proportions. The result shows that K content is high in flowers of the male *Carica papaya* (46.29 mg/g), but relatively low in the leaves (2.75 mg/g). On the other hand, the Na contents are low in the leaves and flowers at 0.032 mg/g and 0.047 mg/g, respectively. Potassium is an essential nutrient; it helps in the protein and carbohydrate metabolism [22]. Sodium is essential in regulating the osmotic pressure of the body and assists in acid-base maintenance and water balance of the body.

The leaves have the higher levels of concentration in Mg (16.18 mg/g) than the flowers with a value of 7.64 mg/g. Magnesium is one of the six essential primary elements that are used by plants in relatively large amounts [23] and its deficiency can affect virtually every organ system of the human body.

The calcium contents were high, with the leaves having 32.73 mg/g, while the flowers gave a value of 9.32 mg/g. Calcium is essential for healthy teeth, bones and blood [24].

The high calcium content in the leaves and flowers of male pawpaw suggests their possible use to treat Ca deficiency (which causes osteomalacia, rickets and scurvy).

Zn and Fe are micro nutrients for living organisms. From the results in Table 2, it is observed that the concentration

of Zn is higher again in the leaves (0.046 mg/g) than in the flowers (0.015 mg/g). The relatively high concentration of zinc in the leaves suggests its possible use in treatment of problems associated with zinc deficiency (such as bleeding, infertility, insect bites, skin disease, wounds, etc).

The Fe content in the leaves was 0.46 mg/g, while it was 0.13 mg/g in the flowers. The most observed nutritional deficiency in humans is Fe deficiency (associated with anemia) [25].

Iron, associated with hemoglobin in the body helps in the transfer of oxygen from lungs to the tissue cells [26].

Nickel is essential for plant growth and development [27], but the concentration in the majority of plant species is very low (0.05 – 10 mg/kg dry weight) [28]. In the present study, the nickel concentrations in the leaves and flowers are 6.4 mg/kg and 3.4 mg/kg, respectively. The amount of Cr and Cd are negligible in the plant parts.

From the results, the trend of element concentration in the pawpaw plant samples is:

For leaves; Ca > Mg > K > Fe > Mn > Cu > Zn > Na > Ni > Cd

For flowers; K > Ca > Mg > Fe > Na > Mn > Zn > Cu > Ni > Cd.

Table 2: Elemental Analysis of *Carica papaya* male leaves and flowers by AAS Technique

Metals (g/kg)	C.P LEAVES	C.P FLOWERS
Cu	0.0623 ± 0.0021	0.0082 ± 0.0010
Na	0.0325 ± 0.0090	0.0473 ± 0.0050
Mg	16.1767 ± 0.0040	7.6400 ± 0.0060
Mn	0.1193 ± 0.0150	0.0430 ± 0.0020
Zn	0.0465 ± 0.0040	0.0155 ± 0.0000
Ni	0.0064 ± 0.0007	0.0034 ± 0.0020
K	2.7467 ± 0.0050	46.2900 ± 0.0049
Ca	32.7267 ± 0.0100	9.3167 ± 0.0060
Cd	0.0008 ± 0.0002	0.0003 ± 0.0001
Cr	ND	ND
Fe	0.4584 ± 0.0100	0.1311 ± 0.0120

6 (±) Standard deviation, ND = Not Detected

CONCLUSION

The present study indicates that the leaves and flowers of the male *Carica papaya* contain the essential nutrients like minerals and some vitamins required in the body.

Therefore the male *Carica papaya* leaves and flowers can be utilized in some herbal formulations for the treatment of various diseases.

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