



Phytochemicals and Minerals Composition of Matured (Yellow) *Carica Papaya* Leaves

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Abstract

Carica papaya Linn. belong to the family Caricaceae. It has long been a popular plant used in folk medicine around the world to manage a variety of ailments. It is also known for its food and nutritional value. Matured (yellow) *Carica papaya* leaves were analysed for various phytochemicals and minerals. The extraction of plant contents was carried out using 70% ethanol. The qualitative test revealed: saponins++, phenolics+, eugenols+, terpenoids+, steroids+, alkaloids++, flavonoids+ and tannins+. But glycosides was absent. Quantitative phytochemical analysis showed the percentage of phytochemicals: phenols 5.60 ± 0.001 , terpenoids 4.451 ± 0.000 , cyanogenic glycosides 2.70 ± 0.005 , cardiac glycosides ± 0.001 , steroids 5.451 ± 0.000 , saponins 8.776 ± 0.001 , alkaloids 19.990 ± 0.0033 , tannins 4.776 ± 0.000 , and eugenols 4.432 ± 0.000 . Elemental-analysis was conducted using atomic absorption spectrophotometry (AAS); the elemental values were given in ppm, thus: iron 1.983 ± 0.001 , mercury 0.011 ± 0.001 , lead 0.029 ± 0.000 , zinc 0.486 ± 0.045 , sodium 6.464 ± 0.026 , cadmium 0.003 ± 0.000 , potassium 7.522 ± 0.003 , magnesium 2.952 ± 0.003 , arsenic 0.043 ± 0.001 , manganese 0.643 ± 0.003 , selenium 0.107 ± 0.000 , calcium 8.407 ± 0.000 , copper 0.187 ± 0.000 , nickel 0.004 ± 0.000 , and phosphorus 8.898 ± 0.000 . The study suggests that the matured (yellow) *Carica papaya* leaves are a rich source of essential phytochemicals and minerals)

1. Introduction

Plants have long been utilised to manage a variety of medical conditions in addition to serving as food and nutritional supplements for living things. The medicinal benefits of plants are crucial for maintaining human health [1]. Numerous chemical constituents found in various parts of plants have significant physiological potentials, such as anxiolytic, antimalarial, antimicrobial, aphrodisiac, antiviral, uterine contractile, anti-asthmatic, and anti-cancer properties [2].

Their medical value comes from the bioactive elements in them that result in defined physiological effects in the human body [3]. In contrast to synthetic drugs, medicinal plants are abundant in a variety of biologically active elements with strong antibacterial properties [4]. For many disorders, synthetic medications used to be the first line of treatment. However, studies looking at the use of

alternative medicines in the treatment and prevention of diseases have expanded significantly due to the negative consequences exhibited by long or even short-term usage [5].

Carica papaya Linn., a member of the family Caricaceae, has long been a popular herb used in folk medicine around the world to cure a variety of ailments[6]. The pace of growth of the plant is rapid [7]. *Carica papaya* is known as gwanda in Hausa, ohuo in Bini, mbuwe in Tiv, okwuru beke in Ibo, and pawpaw in English. The tall herbaceous succulent plant has leaves that are only restricted to the top of the trunk and are arranged in a spiral around it. The lowest part of the trunk is where the leaves and fruits are found. The size of the leaves is enormous and palmate with a stem. The trees are dioecious, but monoecious species are also available in nature. There are no branches emerging from the main trunk. The flowers are small, and a wax-like appearance is seen on their petals. They appear on the axils of the leaf and develop into fruit after getting fertilised [8]. The herbaceous plant *Carica papaya* is used medicinally for its seeds, fruits, leaves, and latex. The fruit has a delicious flavour and is loaded with antioxidant vitamins and minerals like B- vitamins, carotene, vitamin C, flavonoids, folate, potassium, and magnesium. These biological enzymes, chymopain and papain, are found in the fruits, stems, and leaves [4].

The papaya plant produces a variety of phytochemicals in its leaves that are useful in treating a wide range of illnesses. Vitamins like riboflavin, thiamine, and ascorbic acid are present in the plant leaf. High concentrations of calcium, magnesium, sodium, potassium, manganese, and iron can be found in it. While the dying brown, pawpaw leaf works best as a tonic and blood purifier, the fresh, green, antiseptic pawpaw leaf is also an antiseptic. The ripe pawpaw fruit's seeds can be chewed to relieve nasal congestion. Due to its antibacterial properties, the unripe green pawpaw has therapeutic potential. It eliminates bacteria from the intestines to such an extent that only a healthy bowel can absorb vitamins and minerals, including vitamin B₁₂. The tea made from green papaya leaves aids in the treatment of conditions including arteriosclerosis, high blood pressure, chronic indigestion, overweight and obesity, and weakness [7]. It also stimulates digestion. The anti-oxidation activity of the older yellow leaves was higher than that of the younger green leaves [9].

"Mature yellow leaves" refers to leaves that have reached their full growth and development stage and have turned yellow naturally as part of their aging process. Several studies have been done to ascertain the biological-activities of various parts of the *carica papaya* plant, such as the shoots, leaves, fruits, seeds, roots, etc. The fresh (green) leaves of *carica papaya* have been proven to contain many bioactive components with anti-oxidant, anti-anaemic, anti-diabetic, and anti-hyperlipidemic activities. Due to the high cost of synthetic drugs and their adverse effects, there has been a surge in the study of medicinal plants, which are very affordable and have fewer adverse effects. To date, in vitro studies have focused much on the phytochemical and mineral analysis of the fresh (green) *carica papaya* leaves; there are no many studies done on the matured (yellow) leaves of *carica papaya*, which has triggered interest in carrying out this research work. The aim of this research is to determine the photochemical constituents and elemental composition of mature (yellow) *carica papaya* leaves.

2. Materials and Methods

2.1 Materials

2.1.1 Collection of the Plant

The mature (yellow) *carica papaya* leaves were harvested around the Ovia, North East Local Government Area in Edo State. The matured (yellow) leaf was identified and authenticated at the Department of Plant Biology and Biotechnology (PPB), University of Benin, Nigeria. The herbarium specimen was deposited with voucher number UBH-C505/2023 (*Carica papaya* Linn.).

2.1.2 Preparation of Plant and Extraction

The leaves were thoroughly washed under running water to remove adhering dirt and shed-dried for 14 days. Thereafter, the dried leaves were pulverised using an industrial blender and stored in an airtight container. 70% ethanol was used for the extraction of the crude powdered sample. The filtered water was concentrated to dryness with the aid of a rotary evaporator at a reduced pressure. The concentrated extract was afterward freeze dried using a freeze dryer and weighed, and the percentage yield was calculated.



Figure 1: *Carica papaya* tree showing a mature (yellow) leaf



Figure 2: Harvested mature (yellow) *Carica papaya* leaves



Figure 3: Dried Shredded, mature (yellow)



Figure 4: Powdered mature (yellow)

2.2 Methods

2.2.1 Qualitative Phytochemical Analysis

Photochemical screening was performed using standard procedures described by Okereke *et al.* [1].

2.2.2 Quantitative Phytochemical Analysis

2.2.2.1 Alkaloids Determination

200 ml of 20% acetic acid in ethanol was poured into a beaker containing 5g of the sample. It was capped and then left to stand for 4 hours at 250 °C. The filtrate was concentrated to one-fourth of the original volume using a water-bath after being filtered. Until the precipitate was fully formed, conc. ammonium hydroxide (NH₄OH) was added to the extract in drop-wise. The solution was allowed to settle, and the precipitate (ppt) was collected, rinsed with weak NH₄OH, and filtered with filter paper. The residue left on the filter paper was placed in an oven set to 800 °C to dry. The alkaloid content was calculated and the result, expressed in percentage [10, 11].

Calculation of Alkaloids

$$\% \text{ weight of alkaloid} = \frac{\text{Filter paper weight with remain} - \text{Filter paper weight}}{\text{Weight of sample examined}} \times 100 \dots \dots \dots (1)$$

2.2.2.2 Flavonoids Determination

10g of the Pulverized mature (yellow) *carica papaya* leaves was extracted several times in 100 ml of 80% aqueous methanol at 20°C. The solution was filtered afterward using Whatmann filter paper. The filtrate was then transferred into a crucible, evaporated in water- bath, and weighed [12].

Calculation of Flavonoids

$$\% \text{ Flavonoids} = \frac{(\text{Crucible weight} + \text{Remain}) - (\text{Weight of crucible})}{\text{Sample weight}} \times 100 \dots \dots \dots (2)$$

2.2.2.3 Saponins Determination

A water bath heated at a temperature of 500 °C was used to hold 5g of the pulverized mature (yellow) *carica papaya* leaves in 20% acetic-acid in ethanol for precisely 24 hours. It was then filtered, and the extract, concentrated to a quarter of the initial sample volume using water bath. The extract was gradually treated with concentrated NH₄OH until the precipitate was fully formed. After allowing the solution to settle, the precipitate was filtered, collected, and weighed. The saponin content was calculated in percentages [11].

Calculation of Saponins

$$\% \text{ Saponin content} = \frac{(\text{weight of filter paper} + \text{remain}) - (\text{weight of filter paper})}{\text{Sample weight}} \times 100 \dots \dots \dots (3)$$

2.2.2.4 Tannins Determination

The method described by Pearson [13], was used to determine the presence of tannins. Petroleum-ether 100ml) was placed in a conical flask containing 20g of powdered mature (yellow) pawpaw leaves, and the flask was capped for 24 hours. After filtration, the sample was let to stand for 15 minutes. In order for the petroleum- ether to evaporate. It was again soaked in 10% acetic acid in ethanol for four hours. The sample was filtered and the filtrate mixed with 25ml of NH₄OH to precipitate the tannins.

Calculation of Tannins

(C₁V₁ = C₂v₂) molarity.

Data

C₁ = conc. of Tannic Acid

C₂ = conc. of Base

V₁ = Volume of Tannic acid

V₂= Volume of Base

Therefore $C_1 = \frac{C_2 V_2}{V_1}$

$$\% \text{ of tannic acid content} = \frac{C_1 \times 100}{\text{Weight of sample examined}} \dots \dots \dots (4)$$

2.2.2.5 Phenols Determination

Phenol was determined using the spectrophotometry method. The pulverised leave sample was heated along with CH₃CH₂O (50 ml) for 15 minutes. 5 ml of the sample was transferred into flask containing 10 ml of distilled water, 2 ml of NH₄OH solution and 5 ml of conc. CH₃(CH₂)₃CH₂OH were added to the solution and left to stand for 30 minutes for colour development. The wavelength was measured at 505nm.

2.2.2.6 Terpenoids Determination

5g of the pulverised sample was boiled in 100 ml of 2 MHCl for 30 minutes. Utilising Whatmann filter paper, the hydrolysate was filtered. After transferring the filtrate into the separation funnel, an equal volume of ethylacetate was added, stirred, and allowed to divide into two layers. While the aqueous layer was discarded, the ethylacetate layer was recovered. A steam bath was used to dry the extract. The anthocyanin was subsequently extracted from the dry extract using 10 ml of strong amyl alcohol. After filtration, the alcohol extract was dried. The weight of terpenoids was determined and expressed as a percentage of the original sample [10].

Calculation of Terpenoids

$$g (\%) = \frac{\text{weight of terpenoids}}{\text{Weight of original sample}} \times 100 \dots \dots \dots (5)$$

2.2.2.7 Determination of Steroids

100 ml of distilled water and 1 gram of the powdered material were placed in a conical flask and filtered afterward. 0.1N ammonium hydroxide solution was used to elute the filtrate. 2 ml of the eluent and chloroform were combined in a test-tube. 3 ml of acetic anhydride was poured over the liquid. Two drops of a standard sterol-solution containing 200 mg/dl were produced and used as the

test's blank. The spectrophotometer was calibrated with a blank at 420 nm to measure the absorbance of the standard and test.

Calculation of Steroids

$$(mg/100ml) \frac{\text{Absorbance of test} \times \text{standard concentration}}{\text{Standard absorbance}} \dots\dots\dots(6)$$

2.2.2.8 Cynogenic Glycoside Determination

Acid Titration Method

10g of sample was placed in an 800 ml Kjeldahl flask, and 100 ml of water was added. It was then macerated at room temperature for 2 hours. 100 ml of water was again added and steam distilled. The distillate was collected in 20 ml of 0.02N AgNO₃ acidified with 1 ml of HNO₃. AgNO₃ was titrated in combination with the filtrate and washed with 0.02N KCN. Iron-alum was used as an indicator.

2.2.2.9 Cardiac Glycosides Determination

Dinitrosalicylic acid (in methanol) and 1 ml of 5% aqueous sodium hydroxide were heated for 2 minutes along with the sample until brick-red ppt was observe. The sample was boiled and filtered. The filtrate was dried in 50 °C heated oven. The percentage of the cardiac glycoside was calculated.

Calculation of Cardiac Glycosides

$$\% \text{ Cardiac glycoside} = \frac{(\text{Filter paper weight} + \text{Remain}) - (\text{Filter paper weight}) \times 100}{\text{Weight of sample examined}} \dots\dots\dots(7)$$

2.2.3 Method for Elemental Analysis

Elemental analysis was conducted using an Agilent FS240AA atomic absorption spectrophotometer according to the methodology of APHA 1995 (American Public Health Association) [14].

2.2.3.1 Sample Digestion

20 ml of conc. HNO₃, 80 ml of concentrated perchloric acid, and 20 ml of concentrated H₂SO₄ was added to 2g of the sample, which was then heated until a digest was produced. The digest was then diluted with distilled water until it reached the 100ml mark [15].

2.2.4 Statistical Analysis

Descriptive statistical analysis and ANOVA was conducted using IBM SPSS, version 25.0

3. Result and Discussion

Table 1: Phytochemical Constituents of Matured (Yellow) *Carica papaya* leaves

Phytochemicals	Tests	Inference
Glycosides	General test	+
Saponins	Frothins	++
Phenolics	Ethanol/ferric chloride	+

Eugenols	Ethanol/ferric chloride	+
Terpenoids	Salkowski	+
Steroids	Acetic/H ₂ SO ₄	+
Alkaloids	Wagner	++
Flavonoids	Lead acetate	+
Tannins	Ferric chloride	+

Key: Present: +, Largely present: ++, Absent: -

Table 2: Quantitative Analysis of Phytochemicals

Phytochemicals	(%) Composition
Phenols	5.604±0.001
Terpenoids	4.451±0.000
Steroids	5.451±0.000
Saponins	8.776±0.001
Cyanogenic glycoside	2.7±0.005
Cardiac glycoside	1.347±0.001
Flavonoids	6.181±0.001
Alkaloids	19.900±0.033
Tannins	4.776±0.000
Eugenols	4.432±0.000

Mean± Std. Error Mean of the sample triplicate

Table 3: Elemental Composition of Matured (Yellow) *Carica Papaya* Leaves

Elements	Composition (ppm)
Iron	1.983±0.001
Mercury	0.011±0.001
Lead	0.029±0.000
Zinc	0.486±0.045
Sodium	6.464±0.026
Cadmium	0.003±0.000
Potassium	7.522±0.003
Magnesium	0.043±0.001
Arsenic	2.952±0.001
Manganese	0.643±0.003
Selenium	0.107±0.000
Calcium	8.407±0.000
Copper	0.187±0.000
Nickel	0.004±0.000
Phosphorus	8.898±0.000

Mean ±Std. Error Mean of the sample triplicate

Phytochemical screening of the pulverised matured (yellow) *Carica papaya* leaves showed a handful of phytochemicals such as alkanoids, phenols, flavonoids, steroids, eugenols, tannins, saponins, and terpenoids; as shown in Table 1. Phytochemicals are naturally occurring bioactive compounds in plants [16]. Table 2 shows the percentage composition of the quantitative phytochemical analysis of the plant leaf. Alkaloids have the highest percentage (19.900%), followed by **saponins (8.776%)** Ayoola and Adeyeye [7], reported that alkaloids are used as basic medicinal agents due to their analgesic, antibacterial, and antiplasmodic properties. Alkaloid is believed to

contribute to the plant's antimalarial properties. Some studies have explored its potential for inhibiting the growth of malaria parasites [17]. The presence of saponins indicates that the matured (yellow) pawpaw leaves have cytotoxic effects [16]. Saponins have several potential health benefits, such as antioxidant, anti-inflammatory, and anticancer properties [18]. It has been demonstrated that flavonoids support cardiovascular health by reducing blood pressure and cholesterol levels and preserving blood vessels, they may reduce the risk of heart disease [19]. According to Gani *et al.* [20], tannins have anti-inflammatory effects. Yadav and Agarwala [21] reported that, phenolic compounds have proven antiaging, anticarcinogenic, antiapoptosis, antiarterosclerotic, and cardiovascular protection. Cardiac glycosides has been reported to support heart muscle. Cyanogenic glycosides have anti-inflammatory effect according literatures. Eugenols have a wide range of pharmacological and bioactive activities with practical industrial uses. The anti-inflammatory and antioxidant properties of eugenol protect neuron cells and lessen illness caused by oxidative stress [22]. Terpenoids and steroids are known for anti-inflammatory and anti-bacterial properties respectively.

Matured (yellow) *Carica papaya* leaves are abundant in both micro- and macronutrients, as shown in Table 3. The discovered elements may be extremely beneficial to the living system in terms of physiology, food, and pharmacology. Phosphorus had the highest value (8.898 ppm) among the elements examined, followed by calcium (8.407 ppm). Calcium is essential for the development and maintenance of healthy bones and teeth, as well as hemostasis [7, 23]. Potassium is a crucial element that the body needs in order to function; it also aids in the contraction of muscles and the regulation of cardiac rate. Phosphorus supports repair of tissue and waste filtration. ATP, protein synthesis, and bone and tooth development are also facilitated by phosphorus. Magnesium helps keep the heart's rhythm regular. During respiration, iron helps with erythropoiesis and oxygen delivery. Antioxidant such as zinc and manganese support a stronger immune system. Sodium, copper supports the operation of muscles and nerve impulses as well as the preservation of the body's water and acid-base balance within cells. Iron and selenium support haemoglobin and immune function [7,23].

Matured (yellow) *carica papaya* leaves may be safe because of the low content of heavy metals (Nickel, Asernic mercury, lead, and cadmium). Heavy metals are poisonous, non-biodegradable elements that build up in soil and the environment. These elements can be consumed by humans and animals through food crops, vegetables, and other plants [24]. Statistical analysis (analysis of variance) of the quantitative phytochemicals and minerals revealed that there was a significant difference between the parameters at $P < 0.01$ and $P < 0.05$.

4. Conclusion

This preliminary study suggests that matured (yellow) *Carica papaya* leaves are a rich source of phytochemicals and minerals.

5. Recommendation

This research requires future studies such as water-soluble and fat-soluble vitamins, proximate analysis as well as full characterization and structural elucidations of the various phytochemicals revealed by the preliminary studies.

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