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PHYTOCHEMICAL SCREENING, PROXIMATE COMPOSITION AND NUTRIENT CONTENT OF THE LEAVES AND ROOTS OF PAWPAW (*Carica papaya*)

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Abstract

Carica papaya (male and female) leaves and roots were collected, dried and reduced to powdered form by mechanical grinder separately. These parts were analyzed to identify the phytochemicals present as well as determine their proximate and mineral content. There is the presence of glycosides, saponins, steroids and terpenoids in both male and female leaves of the aqueous extract. Methanolic extract of the male leaves revealed the presence of glycosides, eugenols and terpenoids. The male and female leaves indicated the presence of moisture (%), (13.9/12.6), ash (5.6/3.9), protein (21.1/18.5), fibre (5.7/4.3), fat (4.7/5.0) and carbohydrate (64.5/66.5), while male and female roots indicated the presence of moisture (%), (13.0/10.6), ash (7.9/6.9), protein (10.2/12.4), fibre (6.0/6.3), fat (3.3/2.9) and carbohydrate (73.4/70.3) respectively. The mineral nutrient content had high potassium ($mg.100g^{-1}$) (379.7/355.2) for male and female leaves and (354.8/ 342.9) for male and female roots. However, in calcium, the male and female leaves had 105.0 and 98.02 while the male and female roots were low (49.6 and 49.0 $mg.100g^{-1}$). The functional group detected for the female leaves were observed at 3320.13cm^{-1} (O-H stretch) of carboxylic acid derivative, 1640.73cm^{-1} (C=C stretch) of unsaturated group and 1103.18 cm^{-1} of (C-O-C stretch) of ethers. The proximate and mineral analyses may provide both nutritive and therapeutic values to human and animals.

Keywords: *Carica papaya, mineral* nutrient, presence, phytochemical, proximate

Introduction

Carica papaya (Pawpaw) commonly called Ibepe in Yoruba, Gwanda in Hausa, Okwuru Beke in Ibo and Ohuo in Bini is a fast growing tree-like herbaceous plant in the family *Caricaceae* with four genera in the world. The genus *Carica linn* is the most common of which *Carica papaya linn* is the most widely cultivated and best known species, (Krishna *et al.*, 2008). *Carica papaya* is widely believed to have originated from the lowlands of Eastern Central America (Nakasone and Paull 1998). Presently, *Carica papaya* grows in all tropical countries and many sub-tropical regions of the world. *Carica papaya* is soft-wooded perennial plant that has a life span of about 5-10 years, although commercial plantations are usually replanted sooner. *Carica papaya* is an erect, usually unbranched tree or shrub with a crown and large palmate leaves emerging from the apex of the trunk (Dick 2003). The soft, hollow, cylindrical trunk ranges from 30cm in diameter at the base to about 5cm in diameter at the crown. Under normal conditions, *Carica papaya* trees can reach 8-10 meters in height, though in cultivation, suitable heights for easy harvesting is of the desired target (Garret, 1995).

Carica papaya is an interesting tree in that some are either male (staminate) or female (Pistillate), (Bruce and Peter 2008) whereas others have both male and female flowers on the same plant. Fruits are produced on the female through cross- pollination. *Papaya* is reported to tolerate drought, high pH insects, laterites, myobacteria, slope and virus (Dick 2003). Ripe papaya fruits have smooth, thin yellow-orange colored skin of about 1.5 to 4cm thick, usually sweet and juicy. Maturated fruits contain numerous grey-black spherical seeds about 5mm in diameter each enclosed in a gelatinous membrane (Nakasone and. Paull 1998). The fruit bearing trees are usually less than 18 months old (Krishna *et al*, 2008). Economically, *Carica papaya* is the most important species within the *caricaceae*, being widely cultivated for consumption as either fresh drinks, Jams, and Candies, or as dried and crystallized fruit. The green fruit, leaves and flowers are also cooked as vegetable (Nakasone and Paull 1998).

Information available in literature are generally about the plant without a distinction of whether it is male or female and it is conjectured that the male and female plants may differ in their phytochemical composition. This conjecture is quantitatively supported by the report of acute toxicity of the leaves of the male plants to snail (private communication). It is therefore imperative to do a comparative phytochemical analysis of both male and female plant sourced in the same environment.
The aim of the research is to examine the leaves and roots of the *Carica papaya* as well as determine their proximate and mineral content for their phytochemical and proximate composition and mineral nutrient content.

Materials and Methods

Materials

Collection of plant materials

Fresh leaves and roots of male and female *Carica papaya* were collected from a farmland in University of Benin. Ugbowo campus.

Sample Preparation

Carica papaya plant parts (leaves and roots) collected were washed under running water, cut into small pieces and air dried in the laboratory for two weeks. After drying, the plant materials were ground into powder and stored in air tight bottles prior to use for analysis.

Extraction

Aqueous extract

For the preparation of aqueous extract, 1kg of dried powder of experimental material of both the male and female (leaves and roots) were soaked in 10,000ml of water (in the ratio 1:10) for 72hrs. The mixture was then filtered through the whatman No 1 filter paper to ensure that no particles were present in the solution and the extract was collected.

Methanolic extract

For the preparation of methanolic extract, 300g of dried powder of both the male and female (leaves and roots) were soaked in 3L of ACS grade 99.0% methanol and kept shaking for 72hrs. The mixture was then filtered with whatman No 1 filter paper and the extract was collected.

Phytochemical Screening

Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, eugenol, tannins and terpenoids (Akindele and Adeyemi 2007).

Phytochemical screening was performed using standard procedures by Sofowora (1993), Trease and Evans (1987).

Test for Alkaloids

The methanol and aqueous crude extract (0.2g) of the various plant parts were transferred into various test tubes. 2m1 of Picric acid was added to the test tubes and shaken. A yellowish precipitate is a positive test for the presence of alkaloids (Egan 2019).

Test for Terpenoids (Salkowski test)

Chloroform (2ml) was added to 0.2g of the methanol extract and also to the aqueous extract. Then, 3ml of concentrated sulphuric acid was carefully added down the side of the inner wall of the test tubes to form a layer. A reddish brown coloration of interphase is required for the presence of terpenoids.

Test for Steroids

Methanol crude extract and aqueous extract (0.2g) were dissolved in 2ml chloroform respectively. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish- brown color at the interphase indicated the steroidal nucleus (Egan 2019).

Test for Eugenol

The methanol crude extract and aqueous extract (0.2g) were mixed with 5ml of 5% potassium hydroxide (KOH) solution. The aqueous layer was separated and filtered with filter paper, few drops of dilute HCl was added to the filtrate. A pale-yellow precipitate is indicative of positive test for eugenol (Edema and Osarumwense 2007).

Test for Tannin

Distilled water (10ml) was added to 0.2g of the methanol extract and aqueous extract, boiled for 5 minutes and then filtered. Ferric chloride ($FeCl_3$) solution was added to the filtrate; formation of bluish precipitate is required for tannin (Egan 2019).

Test for Phenolics

Methanol crude extract and aqueous extract (0.2g) were dissolved in distilled water and few drops of ferric chloride solution were added to the solution. Blue-black or brown coloration is an indication of the presence of phenol.

Test for Flavonoids (Shinoda's test)

Methanol crude extract and aqueous extract (0.2g) were dissolved in dilute NaOH solution. A yellow solution which turns colorless on addition of hydrochloric acid indicates the presence of flavonoids.

Test for Saponins

The methanol crude extract and aqueous extract (0.2g) were shaken with distilled water in a test tube and observed for frothing which persisted for more than 15 minutes on warming indicated positive test for saponins (Egan 2019).

Test for Glycosides

The methanol crude extract and aqueous extract (0.2g) was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution followed by the addition of 1ml of conc. H_2S0_4 by the side of the test tubes to form a lower layer; a brown ring obtained indicates the presence of glycoside (AOAC 2018).

Proximate Analysis

Proximate analysis is partitioning of compounds in a feed into six groups based on the chemical properties of the compounds. The leaves and roots were pulverized to a particle size of 355µm using mechanical grinder and analyzed for moisture, protein, ash, fat, fiber and nitrogen free extract by method of AOAC (2018).

Determination of Moisture

Moisture was determined by oven drying method. 1.5g of well- mixed sample was accurately weighed in clean, dried crucible (W_1). The crucible was placed in an oven at 100-105°C for 8 hours to dry until a constant weight was obtained. Then the crucible was placed in the desiccators for 30mins to cool. After cooling, it was weighed again (W_2). The percent moisture was calculated using the formula in equation 1.

$$\%Moisture = \frac{W_1 - W_2}{W_t\ of\ Sample} \times 100 \quad (2.1)$$

Where W_1 Initial weight of crucible + sample, W_2 = Final weight of crucible + Sample

Note: Moisture free samples were used for further analysis.

Determination of Ash

Clean empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in desiccators and weighed (W_1). One gram of moisture free sample was taken in crucible (W_2). The sample was ignited over burner with the help of blowpipe, until it was charred. Then the crucible was placed in a muffle furnace at 550°C for 2-4 hours. The appearance of grey white ash indicates complete oxidation of all organic matter in the sample. The crucible was cooled and weighed (W_3). Percent ash was calculated using the formula in equation 2.

$$\%\ Ash = \frac{Difference\ in\ Wt\ \ Ash}{W_t\ of\ Sample} \times 100$$

Difference in wt. of Ash = W_3 —W_1

Determination of Crude Protein

Crude protein was determined by measuring the nitrogen content of the sample and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crude protein was determined by modified

kjeldahl method as reported by Ikpe and Akpabio (2013). The method involved digestion, distillation and titration.

Determination of Crude Fiber

A moisture free extracted sample of crude fiber made of cellulose was first digested with dilute (0.128M, 7.1 ml in 992.9 ml distilled H_2O) H_2SO_4 and then with dilute (0.223M, 12.5 g in 1dm^3) KOH solution. The undigested residue collected after digestion was ignited and loss in weight after the ignition was registered as crude fiber. 1g sample (W_0) was weighed and the circuit for heating was kept in "OFF" position. 150 ml of pre heated H_2SO_4 solution and some drops of foam suppresser (acetone) were added to each column. Then the cooling circuit was opened and the heating elements (power at 90%) were turned on. As the mixture started boiling, the power was reduced to 30% and left for 30 minutes. The valves were opened for drainage of acid and were rinsed with distilled water three times to completely ensure the removal of acid from sample. The same procedure was used for alkali digestion using KOH instead of H_2SO_4. The sample was dried in an oven at 150°C for 1 hour. Then the sample was allowed to cool in a desiccator and weighed (W_1). The crucible was transferred into the muffle furnace at 550°C for 3- 4 hours and then cooled in a desiccator and weighed again (W_2). Calculations were done by using the formula in equation 3.

$$\%\ Crude Fiber = \frac{W_1 - W_2}{W_0} \times 100 \quad (2.5)$$

Determination of Crude Fat

Dry extraction method for fat determination was implied. It consisted of extracting dry sample with petroleum ether, since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. were extracted together, therefore, the results are frequently referred to as crude fat. Crude fat was determined by ether extract method using soxhlet apparatus. 1.0 g of the moisture- free sample was weighed and wrapped in a filter paper, placed in a fat- free thimble and then introduced into the soxhlet. Weighed, cleaned and dried receiving round bottom flask was three quarter filled with petroleum ether and fitted to the soxhlet. A condenser was fitted into the soxhlet with water and heater turned on to start the extraction. After 5 hours, the ether was allowed to evaporate and then the flask was disconnected before the last siphoning. The extract was transferred into clean glass dish rinsed with ether then, the ether was evaporated on water bath. Then the dish was placed in an oven at 105°C for 2 hours and cooled in a desiccator. The percent crude fat was determined by using the formula in equation 4.

$$\%\ Crude\ fat = \frac{W_{res} - W_{ta}}{Weight\ of\ Sample} \times 100 \quad (4)$$

W_{ta} = tare weight of beaker in grams
W_{res} = weight of beaker and fat residue in gram.

Determination of Nitrogen- Free Extract (Carbohydrate)

Nitrogen Free Extract (NFE) was determined by mathematical calculation. It was obtained by subtracting the sum of percentages of all the nutrients already determined in proximate analysis from 100.

NFE = (100- % moisture + %crude protein + %crude fat + %crude fiber + % ash). (5)

Mineral Analysis

Mineral analysis is the measure of the amount of specific inorganic components present in food. The samples are brought to a mesh size of 355µm and were analyzed for iron, calcium, magnesium, zinc, manganese, copper, potassium and sodium by digestion method of AOAC (2018).

Digestion

A sample of 1.0g was weighed into the kjeldahl flask, well clamped to a retort stand above a regulated hot plate inside the fume cupboard. The mixed acid, l0ml was added (HNO_3 and HCl in ratio 1: 3). The flash was heated slowly, later increased until one-third of the digest remained. The digest was diluted with distilled water, filtered and made up to 100 ml volumetric flask. The mineral analysis of the filtrate was determined using atomic absorption spectroscopy and flame photometry.

Results and Discussion

Table 1: Phytochemical screening of *Carica papaya* (male &female)

Phytochemical screening of the crude methanolic and aqueous extract of leaves and roots of *carica papaya* revealed the presence or absence of some secondary metabolites (phytochemical constituents) such as alkaloids, terpenoids, flavonoids, saponins, phenolics, glycosides, tannins as shown in Table 1 and 2 below.

The Phytochemical composition of male and female leaf extract of *carica papaya* is given in Table 1**.**

Table 1: Phytochemical composition of extract of male and female leaves of *Carica papaya*

Constituents	Aqueous extract		Methanolic extract	
	ML	FL	ML	FL
Alkaloids	-	-	-	+
Eugenols	-	+	+	-
Flavonoids	-	-	-	+
Glycosides	+	+	+	+
Phenolics	-	-	-	-
Reducing su	-	-	-	-
Saponins	+	+	-	+
Steroids	+	+	-	-
Tannins	-	-	-	+
Terpenoids	+	+	+	-

Key: + present; - negative; ML male leaf; FL female leaf

The Phytochemical composition of male and female root extract of *carica papaya* is given in Table 2.

Table 2: Phytochemical composition of extract of male and female roots of *carica papaya*

Constituents	Aqueous extract		Methanolic extract	
	MR	FR	MR	FR
Alkaloids	+	+	+	+
Eugenols	+	+	+	+
Flavonoids	-	-	-	-
Glycosides	+	+	+	+
Phenolics	+	+	+	+
Reducing sugar	-	-	-	-
Saponins	+	+	+	+
Steroids	-	-	-	-
Tannins	-	-	-	-
Terpenoids	+	+	+	+

Key: + present; - negative; MR male root; FR female root

The results of the phytochemical analysis on Table 1 showed that the extracts contains glycosides, saponins, steroids and terpenoids in both male and female leaves of the aqueous extract, while glycosides, eugenols and terpenoids were obtained in the methanol extracts. Eugenols was absent in the female leaves analysed. The ethanolic and aqueous extract (Owoh *et al*., 2021) carried out on the male leaves showed the presence of all the tested phytochemicals except glycosides and reducing sugar while the female leaves showed the presence of alkaloids, saponins, steroids and tannins. Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds found in vegetables, fruits and spices. The presence of saponins supports the fact that pawpaw leaf has cytotoxic effects such as permealization of the intestine (Okwu *et al* 2004). It also gives the leaves the bitter taste. Phenolics are also absent in the male and female leaves of the plant but alkaloids and phenolics are seen in both root parts. Glycosides are natural cardio active drugs used in the treatment of congestive heart failure and useful in lowering blood pressure. The presence of cardiac glycosides may support the usefulness of the plant for the treatment of cardiac diseases (W.H.O, 2010). Terpenoids are known to possess a wide range of biological activities such as antimicrobial, antifungal, antiparasitic, and anti-inflammatory are also important in the prevention and therapy of several diseases including cancer (Koffi *et al*., 2009) while Eugenols have been reported to exhibit antioxidant, anti-inflammatory properties for humans (Gupta *et al*., 2014). However it has been reported that eugenol and its derivatives in concentrations as low as 10ppm could be toxic to snails Souza *et al* 1991. Eugenol is present in the methanol extract of the male and also present in the aqueous extract of the female leaves. The forms and concentration of the eugenol needs to be established to help unravel the mortality associated with the male leaves of the plants.

Proximate composition of carica papaya plant parts

The proximate composition of the leaves and roots of Carica papaya is given in Table 3.

Table 3: Proximate composition of leaves/roots of *Carica papaya*

Parameters	Male Leaf	Female Leaf
Ash %	5.68 (7.94)	3.92 (6.92)
Carbohydrate %	64.52 (73.43)	66.53 (70.38)
Crude fat %	4.72 (3.32)	5.01 (2.92)
Crude fibre %	5.72 (6.09)	4.32 (6.32)
Crude protein %	21.12 (10.24)	18.50 (12.42)
Moisture content %	13.92 (13.08)	12.69 (10.63)

Mean ±S.D of the triplicate sample

Values in parentheses are for *Carica papaya* roots

The results in Table 3 show that the levels of crude proteins are about the same order of magnitude, but somewhat higher in the male leaves (21.12%) than in the female leaves (18.5%). These values are lower than the value (29.5%) reported by Joseph B et al 2015 but are comparable with the value (18.68%) reported by Oche et al 2017. Apart from carbohydrate, ash, crude fibre, values for roots are higher than for leaves. There appears to be no previous reports on the comperation of proximate composition of leaves and roots of male and female *Carica papaya.* Crude fiber (5.72%) crude fat (4.72%) and ash (5.68%) were relatively low. Ash is an indication of mineral contents of feed and food stuff (Agu H *et al*, 2014).

Mineral Composition of Female and Male Leaves of Carica papaya.

The results of the mineral content of the leaves and roots of male and female *carica papaya* are given in Table 4.

Table 4: Mineral nutrient content of leaves and roots of *Carica papaya*

Parameters	Male Leaf	Female Leaf
Na (mg. 100g^{-1})	6.490 (9.590)	4.450 (9.050)
K (mg. 100g^{-1})	379.760 (354.890)	355.230 (342.900)
Ca (mg. 100g^{-1})	105.000 (49.634)	98.022 (49.000)
Mg (mg. 100g^{-1})	17.500 (16.723)	17.600 (16.611)
Mn (mg. 100g^{-1})	3.231 (1.000)	1.012 (1.061)
Cu (mg. 100g-1)	0.100 (0.420)	0.310 (0.110)
Fe (mg. 100g-1)	0.650 (0.600)	1.230 BDL
Zn (mg. 100g-1)	2.100 (1.260)	1.100 (2.300)
Cr (mg. 100g-1)	0.020 (0.030)	0.010 (0.040)
Ni (mg. 100g-1)	0.060 (0.010)	0.010 (0.020)

Mean ±S.D of the triplicate sample
Values in parentheses are for *Carica papaya roots*; BDL: Below Detection Limit.

The relatively order of magnitude of the minerals are Mn<Na<Mg<Ca<K. It can be seen that as with proximate composition, the mineral contents in the leaves are markedly higher than in the roots. The results of the mineral composition showed that *Carica papaya* leaves and roots are rich in minerals. The total mineral content of the male leaf and root is higher than the female plant parts. Minerals are essential for the proper functioning of tissues, act as second messengers in some biochemical cascade mechanisms and a daily requirement for human nutrition (Iniaghe *et al*., 2009). Presence of potassium is good, as potassium is known to help lower blood pressure (Otuski *et al*., 2010) which is usually observed in most diabetic patients and it has important interrelationships in the control of arterial resistance (Altura and Altura 1999). When compared to ginger as reported by (Sajid *et al*., 2014), potassium content of papaya leave is lower, ginger extract (410.9mg/100g) compared to (379.760mg/100g and 355.230mg/100g) observed in this study. Potassium is necessary for the muscular weakness which is associated with malaria, and also slows down sclerosis of the vascular system. It contributes to fight against bacteria and cleanses the digestive system (Claude and Paulle, 1979). Potassium and sodium regulate the fluid balance of the body and hence, influence the cardiac output. Calcium is also essential for bone and teeth formation (Okwu 2004). It has been shown that potassium, calcium and magnesium take part in neuromuscular transmission and together with other elements like manganese which are involved in biochemical reaction in the body. The availability of these elements to plant is attributed to composition of soil, water and air composition as well as the permissibility and absorbability of plant for the uptake of these elements.

The importance of these elements cannot be overemphasized because they are required by many enzymes as co-factors to function effectively. Chromium functions as an insulin-performance enhancer. Insulin is a vital hormone that is used in the metabolism and storage of proteins, carbohydrates and fats. Chromium may also be directly involved in the process of metabolism. It stimulates fatty acid and cholesterol synthesis, which are important for brain function and other body process. Chromium also aids in insulin action and glucose metabolism.

Conclusion

A comparison of the phytochemical, proximate and mineral composition of the male and female leaves and roots of *carica papaya* have been reported in this study. The presence of eugenols in the leaves and roots of *carica papaya* suggests limitation in the application of the plant parts as feed for animals on account of the known toxicity at relatively low concentrations of the class of compounds.

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