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Phytochemical Contents, Characterization and Elemental Analysis of Pawpaw Leave Extract (*Carica papaya*)

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Abstract

Traditionally, *carica papaya* (pawpaw) leaves have been used to cure so many diseases which include infertility. The *carica papaya* leaves were subjected to phytochemical screening using standard methods, elemental analysis was conducted using AAS (Atomic Absorption Spectrophometry), FTIR was used to elucidate the compounds detected and GC-MS was used for the qualitative and quantitative detection of the bioactive compounds. The results showed that flavonoids, alkaloids, tannins, steroids, saponins and terpenoids were detected. AAS (Atomic Absorption Spectrometry) analysis in mg/kg showed the presence of zinc (26.19mg/kg), chromium (5.22mg/kg), iron (29.17 mg/kg) and manganese (7.92mg/kg) respectively. The functional groups identified in $\bar{\upsilon}$ (cm-1) are 3354 (O-H stretch); 2924, 2853 (C-H stretch); 1736 (C=O stretch); 1619 (C=C stretch), while the result obtained from GC-MS analysis of methanolic extract of *Carica papaya* leaves revealed Δ -Tocopherol (32.835), γ - Tocopherol (33.671), Vitamin E (34.374), Neryl nitrile (31.817), Curan-17-oic acid, 2, 16-didehydro- 20-hydroxy-19-oxo (30.764). It can be concluded that the *Carica papaya* leaves extracted with methanol contains some elements such as zinc, iron and manganese and compound such as vitamin E along with the phytochemical. which are require for the treatment of infertility.

Keywords: *carica papaya* leaves extract, phytochemical contents, elemental analysis, characterization, AAS, FTIR, GC-MS, infertility.

INTRODUCTION

Natural fruits with high dietary value play a significant role to the and urban communities in the form of food and nutrient enhancement [1,2].

In modern existence, the function and advantageous effects of numerous phytonutrients from plant origins such as fruits and vegetables had drawn the much-needed attention from both the scientists as well as the general public. These phytochemicals are non-synthetic antioxidant which are often promoted owing to the concerns as regards toxicity of the synthetic ones. Asides from scavenging activity of free radicals, antioxidants identified from most of the plants possess health beneficial effects such as antibacterial, antiviral etc. [3].

The wastes generated from food processing (such as seed and peel of some fruits) often contains a potentially higher antioxidant activities than that the edible portion [4].

Carica Papaya is a brief, evergreen plant that grows up to 25 feet tall. Its hollow trunk is noticeable with leaf scars. The leaves grow in a spiraled huddle straight from the upper part of the stem on parallel petioles (leaf stalks) 1 to $3^1/_2$ feet long. The leaves are intensely divided and with an array in width from 1 to 2 feet. Naturally, the male and female flowers are produced on separate plants; however, there are hermaphrodite forms in cultivation which bear both male and female flowers on the same plant. The flowers are fleshy and waxy and have a light scent. The fruit has a taste of a combination of melons and peaches. Although, these trees are

grown primarily for their fruit, however the tree contains latex from which papain, a digestive enzyme is extracted. Papain breaks down protein in meat to make it tender therefore Papaya can be used as a meat tenderizer [5].

It has several uses in this Modern-day including Immuno-modulatory [6], Fiber of *c. papaya* is able to bind cancer-causing toxins in the colon and keep them away from the healthy colon cells, protein enzymes including papain, chymopapain and antioxidant nutrients in papaya including vitamin C, vitamins E, and beta-carotene, reduce the severity of asthma, osteoarthritis and rheumatoid arthritis. It provides the human with protection against inflammatory polyarthritis a form of rheumatoid arthritis involving two or more joints. It helps the lung to be healthy and save life, rubbing the white pulp of raw *c. papaya* expels pimples as well as wrinkles. Papaya works as a good bleaching agent [7].

The ripe papaya fruit contains significant amounts of macro and micro minerals which are Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn [8] . Ripe papaya is most commonly consumed as fresh fruit whereas green papaya as vegetable usually after cooking or boiling [9]

Some of its allergies are link to a latex fluid when it is not ripe, which can cause irritation and provoke allergic reaction in some people, excessive consumption of papaya can cause carotenemia, the yellowing of soles and palms, which is otherwise harmless. However, a very large dose would need to be consumed. Papaya contains about 6% of the level of beta carotene in carrots (the most common cause of carotenemia) [10].

The aim of this research is to determine the phytochemical contents, characterization and elemental analysis of pawpaw leave extract

MATERIALS AND METHODS

Sample treatment and extraction

The leaves of *Carica papaya* were washed with distilled water, air-dried for three weeks, and ground into powder form. A methanol extract was prepared from the powdered *Carica papaya* leaves. 80 grams of the sample was pulverized with 500ml of methanol in a 1000ml beaker for 72 hours at room temperature. Thereafter, the extract was filtered using Whatman No. 1 filter paper (particle retention: 20-25µm) and the filtrate was evaporated to dryness on a water bath. The yield of 3.2g was obtained and calculated using equation 1.

$$\% \text{ yield} = \frac{\text{Final yield}}{\text{Initial yield}} \times 100$$

[equ 1]

Phytochemical (qualitative) contents of the leaf extract were carried out for Alkaloids, Saponins, Terpenoids, Flavonoids, Tannins and Cardiac glycosides using the methods as described by [11]. Steroids were determined by dissolving 1ml of the extract in 10ml of chloroform, and an equal volume of concentration H_2SO_4 was added from the side of the test-tube. The upper layer turns red and the H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids.

The determination of the Phenol was done by adding 4 drops of $FeCl_3$ on 0.2g of the extract. The bluish-black color indicated the presence of phenol.

Functional groups were determined by FTIR (Fourier-transform infrared spectroscopy).

Characterization of the bioactive compounds of *Carica papaya* was done using GC-MS (Gas chromatography-mass spectroscopy).
AAS (Atomic absorption spectroscopy) was used to determine the Elemental analysis.

RESULTS AND DISCUSSION

Results

The percentage yield of the leaf extract at the end of the extraction gave 4.0g of *Carica papaya*. Preliminary phytochemical constituents of the methanol extract of the *Carica papaya* revealed the presence of flavonoids, alkaloids, phenols, tannins, steroid, saponins, and terpenoids while cardiac glycoside was absent in the extract (Table 1). The result obtained from

the AAS in mg/kg showed zinc, chromium, iron and, manganese (Table 2). The result obtained from GC/MS analysis of methanol extract of *Carica papaya* leaves revealed the existence of Δ-Tocopherol (32.835), γTocopherol (33.671), Vitamin E (34.374), Neryl nitrile (31.817), Curran-17-oic acid, 2, 16-didehydro- 20-hydroxy-19-oxo (30.764) (Table3a&b) GC/MS spectra (figure 1). FTIR analysis result for *Carica papaya* show the presence of some functional group (figure 2).

Table 1: Phytochemical screening of the Carica papaya leaf extract

Phytochemical constituents	**Type of test**	**Inference**
Saponins	Frothing	+
Alkaloids	Wagner	+
Flavonoids	Petroleum ether	+
Tannins	Ferric chloride	+
Terpenoids	Liebermann-Burchad's	+
Cardiac glycosides	General test	-
Steriods	Salkowski's test	+
Phenols	Ferric chloride	+

Where: Positive (+) = presence,
Negative (-) = absence

Table 2: Mineral content observed in *Carica papaya* by AAS study and their bioactivity

Elements	**Concentration (mg/kg) Paw paw**	**Bioactivity as regards fertility**
Zinc mg/Kg	26.19	Antioxidant property, prevents premature sperm capacitation, improves reproduction index
Chromium mg/Kg	5.22	No substantial study
Iron mg/Kg	29.17	Lowers risk of ovulatory infertility, negatively affect semen motility
Manganese mg/Kg	7.92	Effect on motility, protein leakage, improve fertility of semen

Table 3a: Bioactive in the methanol fraction *Carica papaya* by GC-MS study

Pk#	RT	Area (ppm)	Name of compound	MW(g/mol)	MF
1	3.329	25900	Propane, 2-fluoro-2-methyl-	76.1	C_4H_9F
2	9.134	69400	4H-Pyran-4-one, 2,3-dihydro-	144.1	$C_6H_8O_4$
3	11.246	24900	Thymol	150.2	$C_{10}H_{40}$
4	19.022	43800	Neophytadiene	278.2	$C_{20}H_{38}$

5	19.715	19500	3,7,11,15-Tetramethyl-2-hexadecan-1-ol	296.5	$C_{20}H_{42}O$
6	20.447	26000	Hexadecanoic acid	256.4	$C_{16}H_{32}O_2$
7	21.100	800	n-Hexadecanoic acid,methyl ester	270.5	$C_{17}H_{34}O_2$
8	23.657	57300	Phytol	296.5	$C_{20}H_{40}O$
9	28.933	24600	Hexadecanoic acid, 2-hydroxy -1- (hydroxymethyl)ethyl ester	330.5	$C_{19}H_{38}O_4$
10	29.248	147700	beta-Sitosterol	414.7	$C_{29}H_{50}O$
11	29.293	95700	beta-Sitosterol	414.7	$C_{29}H_{50}O$
12	30.764	119500	Curan-17-oic acid, 2, 16-didehydro- 20-hydroxy-19-oxo	354.4	$C_{20}H_{22}N_2O_4$
13	31.817	74100	Neryl nitrile	149.2	$C_{10}H_{15}N$
14	32.835	16500	delta-Tocopherol	402.7	$C_{27}H_{46}O_2$
15	33.671	50600	gamma-Tocopherol	416.7	$C_{28}H_{48}O_2$
16	34.374	200000	Vitamin E	430.7	$C_{29}H_{50}O_2$

Where: Pk = Peak MF = Molecular formula MW = Molecular weight

RT = Retention time

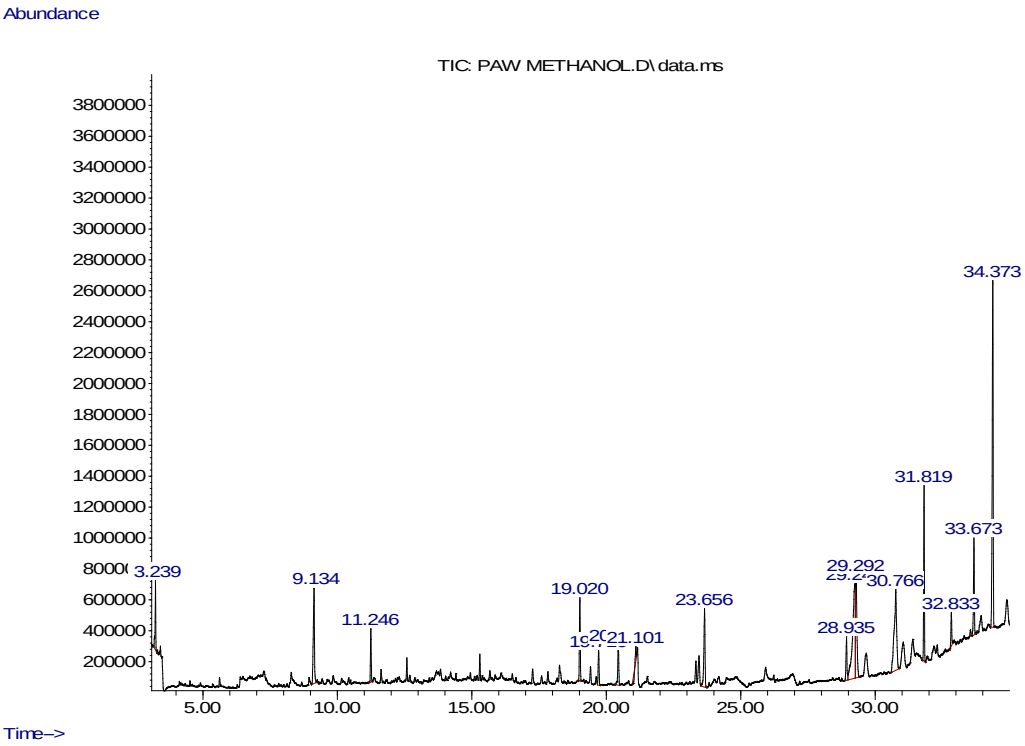

Figure 1: GC spectra for Pawpaw (*Carica papya)* methanol extract

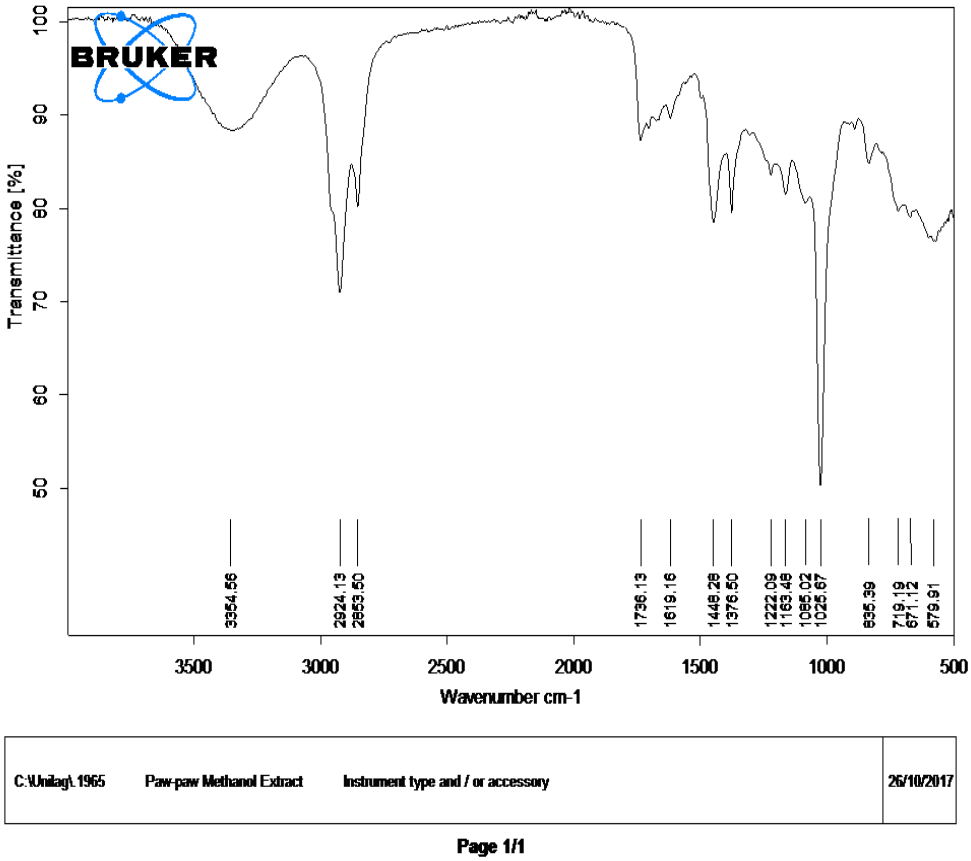

Figure 2: FTIR study of Paw paw (*Carica papaya)* methanolic extract

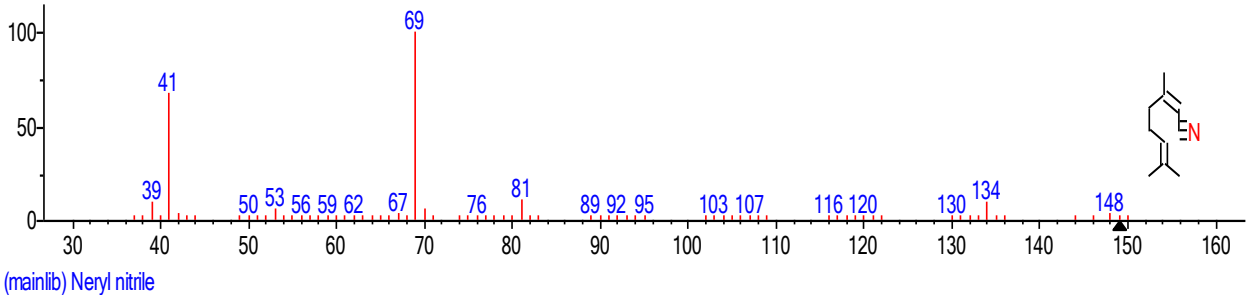

Figure 3: Structural formula of Neryl nitrile

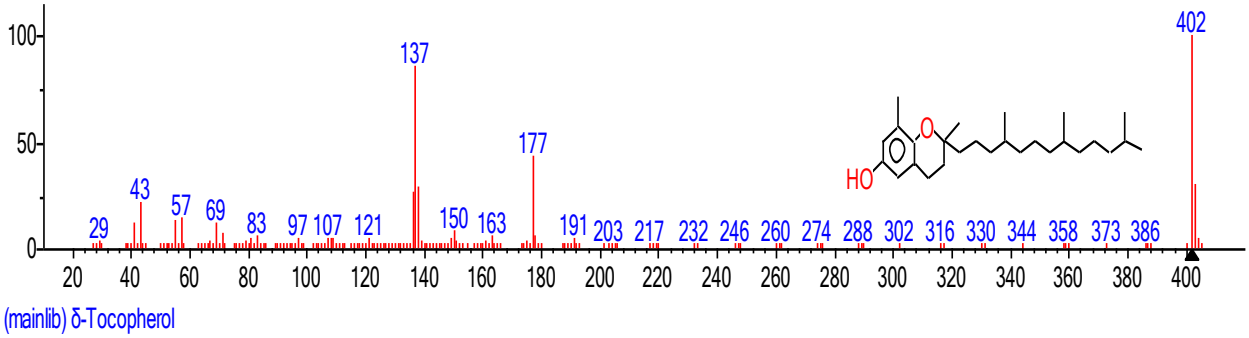

Figure 4: Structure of Δ -Tocopherol

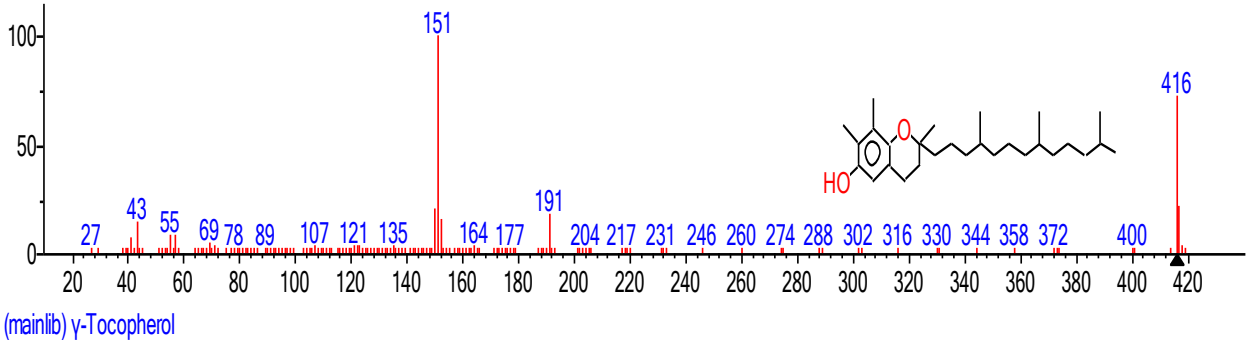

Figure 5: Structure of γ-Tocopherol

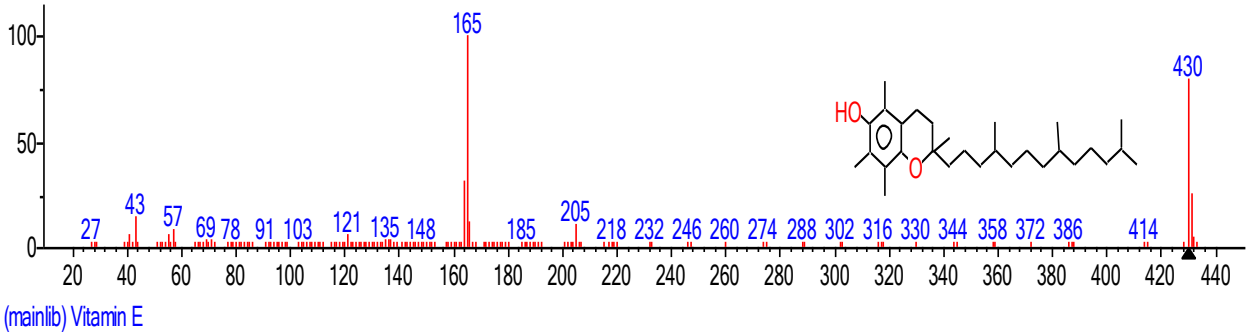

Figure 6: Structure of Vitamin E

Discussion

In this study, methanol was used to extract bioactive from the leaves of *Carica papaya*. The methanol extract of *Carica papaya* leaf showed the presence of flavonoids, alkaloids, phenols, tannins, steroids, saponins and terpenoids, while cardiac glycosides was absent. The finding agreed with the report of [12] Faeji and oladunmoye (2017) who reported a comparative evaluation of phytochemical constituents of some ethno-medicinal plants used in Western Nigeria. AAS (Atomic Absorption Spectrometry) analysis showed in mg/kg the presence of zinc (26.19), chromium (5.22), iron (29.17), and manganese (7.92) for *Carica papaya* which is in agreement with [13] Mahmuda Begum (2014) findings on Phytochemical and pharmacological investigation of *Carica papaya.* As presented in Table 2, the iron content was high followed by the zinc content which makes it useful in the treatment of infertility [14] Fallah *et al.* (2018).

Figure 2:Describing the FTIR study of Paw paw The functional groups identified in the structure of *Carica papaya* in $\bar{\upsilon}$ (cm^{-1}) indicates the presence of: 3354 (O-H stretch); 2924, 2853 (C-H stretch); 1736 (C=O stretch); 1619 (C=C stretch) which is in agreement with the findings of [15] Dyah *et al.*, 2016 who reported the Characterization of fraction of *Carica papaya* leaf.

Figure 1: Showing the GC spectra for Pawpaw (*Carica papya)* methanol extractIn the present study, the GC-MS analysis of methanol leaf extract of *Carica papaya* revealed sixteen compounds which are shown in the figures above . Figure 3: is the structural formula of Neryl nitrile, Figure 4:shows the structure of Δ –Tocopherol, Figure 5: is the structure of γ-Tocopherol and Figure 6: is the structural pattern of Vitamin E

The compounds (vitamin E and β-Sitosterol) were dominant with 200000 ppm and 147700 ppm. The compounds detected using the GC-MS agreed with [13] Mahmuda Begum 2014 findings on Phytochemical and pharmacological investigation of *Carica papaya* and it is in close agreement with [16] Okpe *et al.*, 2017 who reported the chemical constituent and Nutrient composition of *Carica papaya* extract. Many minor components were also identified as shown in Table 3.

CONCLUSION AND RECOMMENDATION

Conclusion

It was discovered and concluded that the methanol extract of *Carica papaya* leaves contains important minerals and pharmacologically important bioactive compounds some of which are flavonoids, tannins and Alkaloids and these are essential for the treatment and management of different ailments.

Recommendation

It can be recommended that the methanol extract of *Carica papaya* is an important and promising natural medicinal plant which contains different bioactive compounds (tannins, flavonoids, alkaloids, saponins, etc.) which could be utilized in several pharmaceutical and medical applications in the nearest future because of its effectiveness, availability and safety. Furthermore, animal studies should be carried out so that we can really affirm its different usage.

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