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Determination of the Proximate Content, Phytochemical Properties and the Antimicrobial Properties of the Seed Extracts of Carica papaya on Some Clinical Isolates

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Abstract: Medicinal plants are the most abundant natural sources of substances used in traditional medicine. The present study was conducted to evaluate the proximate content, the phytochemical and antimicrobial properties of *Carica papaya* seed extracts on clinical isolates. The *Carica papaya* seeds were sourced from fruits sellers from Ekosodin community in Ovia North East Local Government, Benin City, Edo State and was air dried for fourteen days. The extract was obtained by maceration using distilled water and ethanol as solvents. The results of the phytochemical screening for both aqueous and ethanolic extracts showed compounds such as saponins, phenolics, alkaloids and flavonoids. The antimicrobial assay revealed that *Carica papaya* seed extracts had good antimicrobial properties with minimum inhibition of 100 mg/ml observed in *Staphylococcus epidermidis* and *Bacillus subtilis* for aqueous extract, 1.04 mg/ml *Staphylococcus epidermidis* and *Bacillus subtilis* and 2.08 mg/ml *Candida* spp. for ethanol extract. The minimum bactericidal concentration of ethanol extract in this study was 4.16 mg/ml for *Staphylococcus epidermidis* and 8.33 mg/ml for *Bacillus subtilis,* minimum fungicidal concentration was 8.33 mg/ml for *Candida* spp. The results from this study indicate that *Carica papaya* seed possess sufficient nutritional content and phytochemical components, indicating their potential use as supplementary antimicrobial agents and essential nutrients for both humans and animals.

Keywords*: Carica papaya, Phytochemicals, Medicinal plants, Antimicrobial, Clinical isolates*

1. Introduction

The World Health Organization defines any plant that contains substances used to make drugs or has medicinal properties as a medicinal plant. These plants, whether used in their entirety or in specific parts like roots, stems, leaves, stem barks, fruits or seeds, have the potential to be applied for disease control or treatment. The use of medicinal plants has been utilized from ancient times (Sarker, 2007).

Many plants have metabolites that give them pharmacological effects they include primary and secondary metabolites. Nucleic acids, proteins, lipids, carbohydrates and glucose are examples of primary metabolites that are necessary for human development and growth. Conversely, plant-produced secondary metabolites, including alkaloids, saponins, flavonoids, steroids, terpenoids, tannins, glycosides and volatile oils which give them their medicinal qualities and aid in the treatment of a number of illnesses (Chopra and Doiphode, 2002; Dias *et al.,* 2023; Taibi *et al.*, 2024). Terpenoids have antiviral, antibacterial, anthelmintic, antimalarial, anticancer and anti-inflammatory activity, whereas alkaloids have antimalarial, antispasmodic and analgesic activities (Maurya *et al.*, 2008; Haddou *et al.*, 2023). The antioxidative effects of medicinal plants have been evaluated in a variety of animal models, including those for hyperlipidemia, diabetes, autoimmune encephalomyelitis, ischemia-reperfusion, inflammatory bowel disease in rat skeletal muscle or kidney, hepatotoxicity, renal toxicity, radiation injury, and cataract (Rafieian-Kopaie and Baradaran, 2003). *Carica papaya* (Paw paw) is a very popular fruit called Gwada in Hausa, Ojo in Igbo and Ibepe in Yoruba. It is conical or pear shaped with a lot of seeds, it grows up to 4 m-6 m high, the male species do not produce fruits. It has its roots from Mexico and Costa Rica, it is now cultivated widely in China, India, Central Africa, Brazil and even West Africa, especially in Nigeria. Nigeria is the top global producer, according to the Food and Agricultural Organization. The aim of this study was to determine the proximate content, phytochemical properties and the antimicrobial properties of the seed extracts of *Carica papaya* on some clinical isolates.

2. Methodology

2.1 Collection of Plant Materials

The seeds of ripe *Carica papaya* fruits were sourced from fruits sellers from Ekosodin community in Ovia North East Local Government, Benin City, Edo State and was air dried for fourteen days. The dried seeds were grinded into a fine powder with an industrial dry grinding machine (LG-S50B).

2.2 Extraction of Papaya Seeds

800 g of the grinded seeds was added into 2 L of aqueous solvent and 800 g of the grinded seeds was added into 2 L of ethanol, it was allowed to soak for 72 hours. After 72 hours, each extract was then sieved with the double sieve method. The extraction method used is known as the maceration method. The filtrate was then evaporated at 40 °C using a water bath, leaving behind a concentrated extract with a sweet fragrance and a brown hue. The solid powder got from the extract was kept in a tightly sealed glass container which was kept in a refrigerator.

2.3 Proximate Analysis

2.3.1 Ash Content

$$\text{Ash} = 100\ \frac{W_{ash}}{W_o}\ (\%)$$

W_{ash} = content weight after final drying.
W_o = the dried weight of the sample

2.3.2 Moisture Content

An oven was used to dry two grammes (2 g) of the material continuously, which was weighed at 10

minutes intervals, the dried sample was weighed until a constant weight was achieved. The moisture content was determined as a percentage by dividing the weight change from the initial weight by the weight change (A0AC, 2019):

$$\text{The moisture content} = \frac{W_o - W_{dry}(\%)}{W_o}$$

2.3.3 Crude Fiber Determination

The (A0AC, 2019) method was followed in carrying out this. A 250 mL beaker was filled with 4 g of each moisture-free sample, 50 mL of 4 % H_2SO_4, and 200 ml of distilled water. After that, this was brought to a boil over a Bunsen flame and maintained there for precisely thirty minutes, all the while being constantly stirred with a glass rod that had a rubber tip to clear the beads' surfaces of any particles. Hot distilled water was added to maintain a steady volume. When the mixture had boiled for thirty minutes, it was transferred into a Buchner funnel that had been equipped with ash-free man No. 40 filter paper and vacuum-pumped. Beaker was quantitatively transferred using a hot water jet after being repeatedly cleaned with hot distilled water. Washing on the funnel proceeded until litmus paper revealed that the filtrate was free of acid. After quantitatively transferring the residue free of acid from the filter paper into the same beaker, the final traces were eliminated using a 5 % NaOH solution and hot water to fill a 200 ml volume. As previously mentioned, the mixture was brought to a boil for 30 minutes while being constantly stirred, and the water volume was maintained at a consistent temperature. After that, the combination underwent the previously mentioned filtering and washing steps to remove any traces of alkali. Two sections of two milliliters (95% alcohol) were used to cleanse the residue that was left behind. Remaining material on filter paper was moved to a ceramic crucible that had been weighed beforehand. The crucible's contents were cooled in a desiccator before being dried to a consistent weight in an oven kept at 110 °C. Following an 8-hour 550°C muffle furnace ignites, the contents of the crucible were cooled and weighed. For every sample, a triplicate determination was made. The percentage crude fiber was therefore Calculated as:

% Crude Fiber = 100 (y- a) x

x = Weight of sample (g)
y = Weight of insoluble matter (g)
a = Weight of Ash (g)

2.3.4 Crude Fat Determination

The method of (A0AC, 2019) approach was utilized, which was predicated on the idea that non-polar components of samples can be readily extracted into organic solvents. Method: Each sample was weighed out to the nearest three grams (3 g) in moist-free thimbles. These were then placed into Soxhlet extractors with 160 mL of petroleum ether (b.p. 60–80 °C), weighed, and plugged with glass wool. Weighed and attached to the extractors was a dry, clean receiver flask. After that, the extraction unit was put together, and cold water was let to run while the water bath's temperature was kept at 60 °C. Eight hours was spent on extraction. The sample-containing thimble was taken out at the conclusion of this period, and it was dried to constant weight in an oven set to 70 °C for three hours. Next, a typical analytical balance was used to determine the weight of the Thimble and its contents. Calculation: The crude fat was obtained as the difference in weight before and after the exhaustive extraction. Hence, the percentage fat was therefore calculated as:

$$\% \text{ Fat} = \frac{X - Y}{Z}$$

where, x = Weight sample and thimble and oil, Y = Weight of empty thimble, Z = Weight of sample

2.3.5 Crude Protein Determination

Crude protein was determined using a modified version of the micro-Kjeldahl method as reported by (A0AC, 2019). A micro-Kjeldahl digestion flask was pre-weighed with three grammes (3 g) of defatted sample for each sample, along with a small amount of anti-bumping granules. The catalyst combination ($CuSO_4$:Na_2SO_4: SeO_2 5:1:02 w/w) weighed two grammes (2 g) and was added to each flask, followed by the addition of 10 mL of nitrogen-free concentrated H_2SO_4. On a heating mantle in a fume cabinet, the flasks were positioned inclinedly. The temperature was raised to 50 °C for 30 minutes after the fermentation process began at 30 °C until frothing stopped, and then it was heated to 100 °C until a clear solution was achieved. To guarantee full digestion and nitrogen conversion to ammonium sulphate, simmering was maintained below boiling point for an additional half hour. The samples were allowed to cool once digestion was finished, and then they were quantitatively transferred, cleaned, and allowed to cool to room temperature in 100 ml volumetric flasks. With distilled water, volumes were brought up to par. A 25 ml standard flask was filled with 5 ml of the filtrate from the digest, using a 10 ml pipette to help. The solution was adequately mixed by shaking it after adding 2.5 cc of Alkaline Phenate. 2.5 ml of sodium hypochlorite was then added after 1 mg of sodium potassium tartrate had been added and gently shaken. Following that, the solution was produced up to the 25 ml mark using distilled water, and the absorbance of the finished product was determined at 630 nm using a UV/visible spectrophotometer. The sample was handled in the same manner as the nitrogen standards (A0AC, 2019).

$$\% \text{ N} = \frac{\text{Instrument. Reading} \times \text{Slope Reciprocal} \times \text{Colour Vol.} \times \text{Digest Vol.}}{\text{Weight of Sample} \times \text{Aliquot Taken} \times 10000}$$

$$\% \text{ Crude Protein} = \% \text{ Nitrogen} \times 6.25$$

2.3.6 Estimation of Total Carbohydrate

The percentages of crude protein, crude fat, moisture, fibre and ash were subtracted from 100 to get the overall carbohydrate content of the diet samples (A0AC, 2019).

2.4 Phytochemical Screening

The conventional procedures used were used to conduct the phytochemical analyses of the plant extract (A0AC, 2019).

2.4.1 Detection of Alkaloids

To do this, 2.0 ml of the plant extract was first evaporated until it was dry. Following that, 5 ml of HCl (2mol/dm3) was used to dissolve the residues and filter them. Two test tubes were split open to receive the filtrate. Add a few drops of Mayer's reagent to the first test tube; the precipitate that forms become yellow, signifying the presence of alkaloids. Following a few drops of Wagner's reagent treatment in the second test tube, an alkaloid-containing brownish-red precipitate formed (A0AC, 2019).

2.4.2 Detection of Glycoside

This was accomplished by adding an aqueous NaOH solution after 0.5 mg of the extract had been dissolved in roughly 1 ml of water. The development of a yellow hue signifies the existence of glycosides (A0AC, 2019).

2.4.3 Detection of Tannins

1 % sodium chloride-containing gelatin solution was added to 1.0 ml of the extract. Tannins can be detected by the production of a white precipitation (A0AC, 2019).

2.4.4 Detection of Phenols

Four drops of ferric chloride solution were added to 1.0 ml of the plant extract to achieve this. Phenolics are indicated by the creation of a bluish-black colour (A0AC, 2019).

2.4.5 Detection of Saponins

The saponins were found using the foam test and froth test procedures. 0.5 g of the plant extract and 2.0 ml of distilled water were shaken in the foam test procedure. When foam starts to form and stays that way for ten minutes, saponins are present. In the froth test procedure, a 50 ml graduated cylinder was shaken for 15 minutes with 5.0 ml of the extract diluted with distilled water to 20.0 ml. Saponins are present when a layer of foam forms, about 1 centimeter in thickness (A0AC, 2019).

2.4.6 Detection of Flavonoids

Tests with lead acetate and alkaline reagents were used for this. Using a few drops of a 2 mol/dm3 sodium hydroxide solution, the extract was subjected to the alkaline reagent test. A few drops of lead acetate solution were added to the plant component extract in the lead test, which revealed the presence of flavonoids based on the production of a bright yellow hue that turns colourless when diluted hydrochloric acid (2 mol/dm^3) is added. It is evident that flavonoids are present when a yellow-colored precipitate form (A0AC, 2019).

2.4.7 Detection of Eugenols

5 ml of a 5 % KOH solution were combined with around 2 ml of the extract. A filter and separation were applied to the aqueous layer. The filtrate was mixed with a small amount of HCl. Positive results were indicated by a pale-yellow precipitate (A0AC, 2019).

2.4.8 Detection of Steroids

Each extract weighed 0.5 g and 2 ml of H_2SO_4 was added to it. In certain samples, the colour transitioned from violet to blue or green, signifying the presence of steroids (A0AC, 2019).

2.4.9 Detection of Terpenoid

Concentrated H_2SO_4 (3 ml) was carefully added to 0.2 g of the plant sample extract, 2 ml of chloroform ($CHCl_3$), and a layer was formed. Positive results for terpenoids are indicated by the interface's reddish-brown colouring (A0AC, 2019).

2.4.10 Determination of total phenolic contents

Using tannic acid as a standard, the Folin-Ciocalteu reagent was used to quantify the total phenolic content. A test tube was filled with 1.0 ml of the extract solution (250 Ug/ml), to put it briefly. Following a thorough mixing of the contents of the flask, 1.0 ml of Folin-Ciocalteu reagent was added. 15.0 ml of Na_2CO_3 (20 %) was added after 5 minutes, and it was left to stand for two hours. A Jenway

6100 UV-Vis spectrophotometer located in Dunmow, Essex, United Kingdom was used to measure the absorbance at 760 nm. By utilizing an algorithm derived from the standard tannic acid calibration graph, the total phenolic content was calculated as Ug of tannic acid equivalent (TAE) (A0AC, 2019).

2.4.11 Determination of Alkaloids Content

The method of (A0AC, 2019) was used to measure the total alkaloid content. A 250 mL beaker containing 5 g of the extract was filled with 100 mL of 2% acetic acid in ethanol, capped, and let to stand for two hours. This was filtered, and a water bath was used to concentrate the extract to a quarter of its initial volume. Until the precipitation was finished, concentrated ammonium hydroxide was added to the extract drop by drop. After letting the entire mixture settle, the precipitate was filtered out, cleaned with 1% ammonia solution, dried, and weighed. Every sample was examined three times:

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue x } 100}{\text{Weight of sample}}$$

2.4.12 Flavonoid Content Determination

Three duplicate aliquots of the homogeneous 1.5 g cabbage extract were used to measure the flavonoid concentration (A0AC, 2019). Flavonoid determination was performed using thirty-microliter (30 µl) aliquots of the methanolic extract. 90 µL of methanol was used to dilute the samples, followed by 6 µl of 10 % aluminum chloride ($AlCl_3$), 6 µL of 1mol of l sodium acetate (CH_3CO_3Na), and 170 µl of methanol. After 30 minutes, the absorbance at 415 nm was measured. The flavonoid concentration (Ug Qe/g) was calculated using quercetin as the reference.

2.4.13 Estimation of Total Saponins Content

The method reported by Makkar *et al.*, 2014), which was somewhat modified from the vanillin-sulphuric acid colorimetric process, was used to estimate the total saponin concentration. To 250 µL of distilled water, approximately 50 µL of plant extract was added. Next, approximately 250 µL of vanillin reagent (10 mL of 99.5 % ethanol containing 800 mg of vanillin) was added. Next, 2.5 mL of 72 % sulfuric acid was added, and everything was thoroughly mixed. For ten minutes, this solution was maintained at 60 °C in a water bath. It was chilled in ice cold water after 10 minutes, and the absorbance at 570 nm was measured. From the saponin stock solution, standard saponin solutions ranging from 0 to 25 ppm were created. The test samples were handled in the same way as the standard solutions. The values were given in PPM units.

2.4.14 Quantitative Determination of Tannin

A water bath was filled with 20 mL of 50 % methanol and precisely 0.20 mL of the sample. The mixture was then agitated and kept at 77˚–80˚C for one hour. After quantitatively filtering the extract through a double-layered Whatman No. 1 filter paper, 10 mL of 17 % Na_2CO_3 was added, along with 2.5 mL of Folin-Denis reagent and 20 mL of distilled water. The mixture was left to stand for twenty minutes. After colour development, the absorbance of the samples and a series of standard tannic acid solutions produced in methanol were read using a UV/Visible spectrophotometer set to 760 nm. Using the calibration curve, the total tannin concentration was determined (A0AC, 2019).

2.5 Antimicrobial Properties

The agar well diffusion method was utilized to ascertain the antibacterial capabilities. The germs came from the pharmaceutical microbiology lab at the University of Benin's Faculty of Pharmacy in Benin City. The study employed a variety of microorganisms, including *Candida* spp., *Aspergillus* spp.,

Klebsiella spp., *Enterobacter* spp., *Bacillus subtilis*, *Staphylococcus epidermidis* and *Escherichia coli.*

2.6 Standardization of Test Organisms

By mixing 0.5 ml of a 1% w/v solution of barium chloride dihydrate ($BaCl_2.2H_2O$) with 99.5 ml of 1% sulfuric acid (H_2SO_4), the MacFarland 0.5 turbidity standard was produced. Once well combined, this mixture was separated into many test tubes. A loop full of inoculum was taken from a pure culture of the test organism using a sterile wire loop, transferred into sterile normal saline in separate test tubes, and then standardized. The MacFarland turbidity standard was compared to the tube, and the turbidity of the solution was increased to match the standard's level by either adding more organism or sterile saline (Vandapitte *et al*., 2003).

2.7 Agar Well Diffusion Method

For bacteria and fungi, use Muller Hinton agar and Sabouraud dextrose agar, respectively. Following sterilization, the agars were allowed to cool before being divided into 30 ml each and placed into sterile petri plates to solidify. The excess moisture was removed from the surface of the agar plates by drying them in an oven at 50 °C for ten minutes after they had solidified. A homogenous inoculum suspension was swabbed onto the agar plates using the standardized solution; the bacteria inoculum was placed on the Muller Hinton agar plate, the fungi inoculum on the Sabouraud dextrose agar plate, and the inoculum was left to dry The Muller Hilton agar plate base was sealed with Muller Hilton agar, and the Sabouraud dextrose agar plate base was sealed with Sabouraud dextrose agar. A sterile corkborer with a diameter of 8 to 10 mm was used to create a well on the agar plates. Using a calibrated micropipette with a rubber teat, 0.2 ml of the plant extract concentration was added to the well. The plates were then let to stand for 30 minutes to allow for adequate diffusion, and they were then incubated for 24 hours at 37 °C. The control for bacterial organisms was ciprofloxacin (0.5µg/ml), while the control for fungal species was ketoconazole (10µg/ml). Using a ruler and two dividers, the inhibition zone was measured after a 24-hour period.

2.8 Statistics

The research data was subjected to One-way Anova analysis, SPSS was used for various parameter analysis. Further test such as Duncan's multiple range test was carried out to ascertain significance among the parameters.

3.0 Results and Discussion

The proximate analysis result of the *Carica papaya* seed extracts is shown in **Table 1**. The moisture content of the ethanolic aqueous and extract was 12.58 % and 13.43 %, the ash content was 06.17 % and 05.97 %, the amount of crude fiber present was 25.23 % and 23.78 %, the amount of crude fat was 28.63 % and 30.11 %, the amount of crude protein was 31.26 % and 29.85 % and the amount of carbohydrate was 8.71 % and 10.29 %. The qualitative phytochemical analysis shown in **Table 2** revealed that in the aqueous extracts of *Carica papaya* seeds had the following phytochemicals: saponins, phenols, alkaloids and flavonoids, while the ethanolic extract of *Carica papaya* seeds possessed; glycosides, saponins, phenolics, eugenols, terpenoids, steroids, alkaloids, flavonoids and reducing sugar. **Table 3** revealed that quantitative phytochemicals showed that flavonoid was the highest and for aqueous it was 59.75 ± 0.30, while ethanolic extract was 404.90 ± 2.54. For tannis which was the lowest it was 1.40 ± 0.01 for aqueous, while ethanol was 2.00 ± 0.34.

Table 1: Proximate Composition of *Carica papaya* Seeds

S/N	Parameters	Aqueous (%)	Ethanol (%)
1	Moisture Content	12.58	13.43
2	Ash Content	06.17	05.97
3	Crude Fiber	25.23	23.78
4	Crude Fat	28.63	30.11
5	Crude Protein	31.26	29.85
6	Carbohydrate	8.71	10.29

Table 2: Result of the Qualitative Phytochemical Analysis

S/N	Parameter	Aqueous	Ethanol
1	Glycosides	-	+
2	Saponins	+	+
3	Phenols	+	+
4	Eugenols	-	+
5	Terpenoids	-	+
6	Steroids	-	+
7	Alkaloids	+	+
8	Flavonoids	+	+
9	Tannins	-	-
10	Reducing Sugar	-	+

Key: - (Negative), + (Positive)

Table 3: Result of the Quantitative Phytochemical Analysis of Aqueous and Ethanolic Extract

S/N	Parameters	Units	Aqueous	Ethanol
1	Alkaloid	%	4.32 ± 0.11	5.82 ±0.23
2	Total tannins	TAE mg/kg	1.40 ± 0.01	2.00 ± 0.34
3	Total saponin	mg/kg	18.24 ± 1.07	26.24 ± 3.75
4	Total phenolics	TAE mg/kg	36.73 ± 2.66	186.20 ± 3.67
5	Total flavonoid	QE mg/kg	59.75 ± 0.30	404.90 ± 2.54

The result of the antimicrobial properties of the aqueous and ethanolic extract on *Escherichia coli, Staphylococcus epidermidis, Bacillus subtilis, Klebsiella* spp. *Enterobacter* spp. *Candida* spp. *and Aspergillus* spp. is shown in **Table 4**.

Table 4 Result of the Antimicrobial Properties for Aqueous and Ethanol

Organisms	Control (mm)	Aqueous (mm)	Ethanol (mm)
Escherichia coli	NZ	19	NZ
Staphylococcus epidermidis	38	19	20
Bacillus subtilis	38	17	23
Klebsiella spp.	NZ	12	NZ
Enterobacter spp.	18	18	NZ
Candida spp.	25	NZ	20
Aspergillus spp.	23	NZ	12

Key: NZ (No Zone), Control; Bacteria: Ciprofloxacin, Fungi: Ketoconazole

The minimum inhibition concentration of the aqueous and ethanol extracts is shown in **Tables 5 and 6.** The minimum bactericidal concentration and minimum fungicidal concentration of ethanol extract is shown in **Tables 7 and 8**.

Table 5: Result of Minimum Inhibition Concentration of Aqueous Extract

Organisms	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**
Concentration	100	50	25	12.5	6.25	3.12
Escherichia coli	G	G	G	G	G	G
Staphylococcus epidermidis	NG	G	G	G	G	G
Bacillus subtilis	NG	G	G	G	G	G
Klebsiella spp.	G	G	G	G	G	G
Enterobacter spp.	G	G	G	G	G	G

Key: G (Growth), NG (No Growth)

Table 6: Result of Minimum Inhibition Concentration of Ethanol Extract

Organisms	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**
Concentration	16.65	8.3	4.16	2.08	1.04	0.52	0.26
Staphylococcus epidermidis	NG	NG	NG	NG	NG	G	G
Bacillus subtilis	NG	NG	NG	NG	NG	G	G
Candida spp.	NG	NG	NG	NG	G	G	G
Aspergillus spp.	G	G	G	G	G	G	G

Key: G (Growth), NG (No Growth)

Table 7: Minimum Bactericidal Concentration of Bacterial Isolates

Organisms	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**
Concentration	16.65	8.33	4.16	2.08	1.04
Staphylococcus epidermidis	NG	NG	NG	G	G
Bacillus subtills	NG	NG	G	G	G

Key: NG (No growth), G (Growth)

Table 8: Minimum Fungicidal Concentration of Fungal Isolate

Organism	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**	**(mg/ml)**
Concentration	16.65	8.33	4.16	2.08	1.04
Candida spp.	NG	NG	G	G	G

Key: NG (No Growth), G (Growth)

Carica papaya is a plant that has been discovered to have many nutrients, the seed is found to contain a bulk load of nutritional components. In this study **Table 1** revealed high composition of crude protein, crude fibre and crude fat. This shows that *Carica papaya* contain numerous rich nutritional components. It showed that it has high protein content which is essential for body building, the

carbohydrate and fat makes it a good source of energy. Protein serves as a vital dietary element necessary for the survival of both animals and humans. This finding is in agreement with the research of (Azevedo and Campagnol, 2014), (Oche *et al.*, 2016). The crude fibre content in Table 1 showed that it is a good source to remove toxins from the digestive tract and helps in lowering of cholesterol (Wulansari *et al*., 2019). In this study the report of moisture content of aqueous extract was 12.58 % and ethanolic extract was 13.43 %, this was higher than the report of (Kanadi *et al*., 2021) which was 7.40 %. The moisture content of food affects its shelf life as low moisture content can inhibit microbial growth thus reducing spoilage (Kanadi *et al*., 2021). The amount of ash obtained in this study was 6.17 % for the aqueous extract and 5.97 % for the ethanolic extract and was below the value of 8.94 % reported by (Achukwu *et al*., 2022). The crude fiber content of this study was 25.23 % aqueous extract and 23.76 % ethanolic extract. This is close to the value of 20.25 % reported by the research of (Achukwu *et al*., 2022). The research of (Mose and Olanrewaju, 2018) for aqueous extract had the same value of 25.23 % for crude fibre like this study. Crude fiber has been reported by researchers to play a crucial role in digestion as it aids the normal functioning of the large intestine and the elimination of toxins from the digestive tract (Wulansari *et al*., 2019), (Otles and Ozgos, 2014). The carbohydrate content of both the aqueous 8.17 % and ethanol extract 10.29 % in Table 1 was lower compared to 23.34 % in the research of (Kanadi *et al*., 2021). *Carica papaya* seeds are abundant in phytochemicals which play a vital role in combating cellular issues, such as cell growth, protection against cellular damage from oxidation and lowering the likelihood of non-communicable diseases (Ali *et al*., 2018). Phytochemical of *Carica papaya* is shown in Tables 2 and 3. It shows that both aqueous and ethanol extracts of *C. papaya* seeds contain different amounts of phytochemicals like alkaloid, saponin, phenolics, flavonoids depending on the extraction solvent used. Glycosides, eugenols, terpenoids, steroids, and reducing sugar was also found in ethanol extract while tannins was absent in both extracts. Compounds present in this study such as alkaloids are said to be the most important secondary metabolites with the potential to treat various illnesses therapeutically (Ali *et al*., 2018). Alkaloids have an antibacterial and anti-analgestic properties hence its use in preparation of medicinal agents (Okwu and Okwu, 2004). Additional properties of alkaloid that has been documented includes its antimicrobial, amoebicidal and anticancer activities (Kaur and Arora, 2015). Saponin have been observed to display cytotoxic effects like permeating of the small intestine and inhibiting the growth of various types of cells, which may contribute to their documented anti-inflammatory and anti-cancer properties (Yildirims and Kutlu, 2015). Saponin increases the penetration of harmful substances into cells or the leakage of vital cell content when eaten in uncontrolled quantity by animals (Ajiboye and Olawoyin, 2000), (Francis *et al*., 2003). Flavonoids, being lipophilic, can disrupt the formation of cell walls and membranes and inhibit enzymatic activity by forming complexes with the bacterial cell wall (Ali *et al*., 2018). Glycosides contribute to correcting irregular heart rhythms by enhancing the heart's pumping force, which in turn increases the rate of heart contractions through improved sodium-potassium cellular ATPase activity (Jing and Zhang, 2019). The result shown in Table 4 revels that aqueous and ethanolic extracts of *Carica papaya* seeds have antimicrobial activities. The aqueous extract had zone of inhibition of 19 mm for both *Escherichia coli* and *Staphylococcus epidermidis*, 17 mm for *Bacillus subtilis,* 12 mm for *Klebsiella* spp. and 18 mm for *Enterobacter* spp., while the ethanol extract had zone of inhibition of 20 mm for both *Staphylococcus epidermidis* and *Canida* spp., 23 mm for *Bacillus subtills* and 12 mm for *Aspergillus* spp. The result in Table 4 showed that the extracts had similar activities on comparison with the conventional drug used as control. Ethanolic extract showed greater zone of inhibition compared to the aqueous extract, similar result was reported in the research

carried out by (Okoye, 2011). When examining the sensitivity of microbial strains to plant extracts versus synthetic antibiotics, findings indicate that plant extracts could serve as a viable alternative to antibiotics, as they exhibit similar inhibition zones and fewer associated side effects. Unlike antibiotics, these extracts are less prone to resistance issues (Marchese and Shito, 2001), (Poole, 2001), (Kareem *et al*., 2010). The minimum inhibition concentration (MIC) of the aqueous and ethanol extract of *Carica papaya* seed is shown in Tables 5 and Table 6. The antimicrobial activity of plants especially plant seeds using aqueous and ethanol as solvents are proved to be effective in the killing of microbes (Bouslamti *et al*., 2023). This present research showed that aqueous and ethanolic extracts *Carica papaya* seeds had antimicrobial activities just like the research of (Achukwu *et al*., 2022), Kanadi *et al*., 2021). The MIC results from Table 6 and 7 revealed that *Carica papaya* seed extract is dose dependent and has both antibacterial and antifungal activity. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the ethanol extract of *Carica papaya* seed are shown in Tables 7 and 8 respectively. MBC and MFC refers to the lowest concentration of an antimicrobial agent required to kill a specific bacterium or fungus. The result for MIC and MBC of this study agrees with the findings (Obi and Onuoha, 2000), Ogueke *et al.*, 2006) that aqueous and ethanol extracts of plant seeds have active ingredient that has antimicrobial effect on microbes. The research of (Masfufatun *et al.*, 2018) showed similar results to this present study, the research showed the presence of phytochemicals and the potentials of the ethanolic extract of pawpaw seeds. The antimicrobial effect on similar microbes used in the research of (Masfufatun *et al.*, 2018) was similar to the results of pawpaw seeds reported in this study. The research of (Dobo *et al.*, 2021) also reported the antimicrobial activity of papaya seeds on some selected Gram positive and Gram negative bacteria similar to the ones reported in this study, this further proves the antibacterial potential of the extracts of *Carica papaya* seeds. Historical accounts indicate its utilization as a pain reliever, anti-amoebic, antibacterial, cardiotonic, bile flow stimulant, digestive aid, menstrual regulator, fever reducer, blood pressure reducer, laxative, respiratory aid, digestive tonic, worm expeller and as a remedy for jaundice (Anibijuwon and Udeze, 2009).

Conclusion

This study reports that *Carica papaya* seeds contain numerous nutritional and phytochemical compounds crucial for human health, the presence of bioactive substances have been reported to provide plants with resistance against bacteria, fungi and pests, which explains the demonstrated antimicrobial activity observed in *Carica papaya seeds* extract utilized in this research. This present study justifies the traditional uses of *Carica papaya* seeds for therapeutic uses.

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