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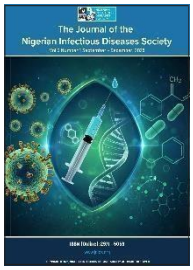

Original Article

The Median Lethal Dose (LD50) and Phytochemical Constituents of Methanol Extract of Caric papaya Leaves

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ABSTRACT

Carica papaya L. which belongs to the family caricaceae, is a plant that was known for its medicinal use for years. This study was carried out to determine the median lethal dose of Carica papaya leaves methanolic extract in albino rats. Fresh leaves of Carica papaya were extracted using methanol as the solvent. Phytochemical analysis was conducted using qualitative procedure. Lorke's method was used to study the acute toxicity using both oral and intraperitoneal routes. The acute toxicity for oral route showed that the LD50 was greater than 5000 mg/kg body weight while, the intraperitoneal route was 2154 mg/kg body weight. The Carica papaya leaf extract revealed the presence of alkaloids, flavonoids, carbohydrates, terpenoids, cardiac glycosides, cardenolides, saponins, and phytosterols, while anthraquinones, phlobatannins and tannins were absent. In conclusion, the leaves of Carica papaya contains bioactive phytochemical constituents that could have clinical potential with safe medicinal properties.

Keywords: Median lethal dose, Phytochemical, Carica papaya leaves, Methanolic extract

INTRODUCTION

Since time immemorial, folkloric use of local plants has been the primary source of medical healthcare by locals. Phytotherapy is the study of herbs and their medicinal potentials. This includes cultivation, collection, and dispensing of edible plants, especially those measured and discovered to possess curative properties. Local herbs are widely available and accessible even in remote areas, and consumers believe and attest to their safety because they are natural (Saalu, 2016). Natural medicinal herbs have been reported to play a vital function in the health care needs of people around the world, especially in developing countries. Traditional medicine is becoming more mainstream as improvements in analysis and quality control, alongside advances in clinical research, show the value of traditional medicine in the treatment of diseases (World Health Organization, 2023). The WHO (2023) estimated that about 80% of people worldwide rely on herbal medicine for some part of their primary health care, and a similar percentage of the Nigerian population was also reported to depend on traditional medicine (Ogunleye et al., 2023).

In Nigeria, lack of quality control, scientific evidence of efficacy, and adverse effects like hepatotoxicity, nephrotoxicity, reproductive, and cardiovascular complications have been associated with herbal remedies. These do not correlate with the increased use of traditional medicine nationwide, especially among the poor.

Recently, phytochemicals present in plants have been recognized for their potential health benefits, with studies indicating that consumption of food and beverages rich in them is associated with significantly reduced risks for a variety of non-communicable diseases (Oluwole and Peter, 2011). The acute toxicity (median lethal dose (LD50)) and phytochemical analyses are experimental techniques used to determine the safety and nature of medicinal plants. Phytochemicals are derived from plants using various extraction solvents depending on the solvent polarity and solubility of the plant's bioactive constituents. There are various toxicity tests used to ascertain the safety of medicinal plants, such as acute toxicity, sub-acute toxicity, and chronic toxicity tests, depending on the duration of the test (Arome and Chinedu, 2013). Acute toxicity testing is conducted to determine the LD50. There are different methods used in determining the LD50 of xenobiotics. These methods are the Graphical method of Miller and Tainter, the Arithmetical method of Reed and Muench, the Arithmetical method of Karbar, and Lorke's method (Abdullahi et al., 2023). The aim of this study is to determine the phytochemical constituents of the methanol extract of Carica papaya leaves and investigate their median lethal dose in Albino rats.

MATERIALS AND METHODS

Collection of Plant Samples and Identification

Fresh leaves of C. papaya were collected in Maiduguri, Borno State, Nigeria. The leaves were authenticated by a botanist from the Department of Biological Science, University of Maiduguri, Nigeria.

Preparation of Plant Extract

The plant sample collected was washed with clean water to rid it of dust particles and shade-dried at room temperature. The dried sample was then pulverized mechanically using a blending machine into dry powder. The dry powder was weighed and added into a conical flask with methanol and kept at room temperature for thirty minutes, shaking after each twenty-four hours for seven days. Finally, the extract was filtered using Whatman filter paper under vacuum and dried at room temperature in a watch glass dish. The weight of each dish was noted prior to drying the extract and after drying. The difference represented the weight of the extract (Ingle et al., 2017).

Phytochemical Analysis

Qualitative phytochemical screening was carried out following the methods of Tiwari et al., 2011; Abdullahi et al., 2023; Ibrahim et al., 2022; Adeleye et al., 2024; Musa et al., 2023; Bello et al., 2022. The extract was analyzed for the presence of tannins, saponins, alkaloids, flavonoids, cardiac glycosides, carbohydrates, steroids, phenols, terpenoids, and anthraquinones.

DETECTION OF ALKALOID

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

Dragendroff's test:

Filtrates were treated with Dragendroff's reagent (solution of potassium Bismuth iodide). Formation of a red precipitate indicates the presence of alkaloids (Tiwari et al., 2011).

Mayer's test:

Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids (Tiwari et al., 2011).

TEST FOR FLAVONOIDS

Lead acetate test:

Extracts were treated with a few drops of lead acetate solution. Formation of a yellow-colored precipitate indicates the presence of flavonoids (Tiwari et al., 2011).

Alkaline reagent test:

Extracts were treated with a few drops of sodium hydroxide solution. Formation of an intense yellow color, which becomes colorless upon addition of dilute acid, indicates the presence of flavonoids (Tiwari et al., 2011).

Shinoda's Test:

Each of the extracts (0.5 g) to be tested was dissolved in ethanol, warmed, and then filtered. Three pieces of magnesium chips were added to the filtrate with a few drops of concentrated HCl. A pink, orange, or red-to-purple coloration indicated the presence of flavonoids (Ibrahim et al., 2022).

Ferric Chloride test:

Each of the extracts was boiled with distilled water and then filtered. To 2 ml of the filtrate, a few drops of 10% ferric chloride solution were added. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group (Ibrahim et al., 2022).

DETECTION OF SAPONINS

Froth test:

0.5 g of extract was shaken with 2 ml of water. Production of persistent foam for ten minutes indicated the presence of saponins (Tiwari et al., 2011).

DETECTION OF PHYTOSTEROLS

Salkowski's test:

Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of concentrated sulfuric acid, shaken, and allowed to stand. The appearance of a golden yellow color indicated the presence of triterpenes (Tiwari et al., 2011).

Libermann Burchard's test:

Extract was treated with chloroform and filtered. The filtrate was treated with a few drops of acetic anhydride, boiled, and cooled. Concentrated sulfuric acid was added. Formation of a brown ring at the junction indicated the presence of phytosterols (Tiwari et al., 2011).

TESTFOR CARDIACGLYCOSIDES AND CARDENOLIDES

Liebermann Burchard test:

To 0.2 g of extract, 2 ml of acetic acid was added; the solution was cooled in ice, followed by the careful addition of concentrated H_2SO_4. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring, i.e., the aglycone portion of cardiac glycosides (Adeleye et al., 2024).

Salkowski's test:

0.5 g of the extract was dissolved in 2 ml of chloroform. Tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. The appearance of a reddish-brown color or yellow at the interface was an indication of the presence of a steroidal ring (Bello et al., 2022).

Keller–Killani test:

5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer (Edeoga et al., 2015).

DETECTION FOR PHLOBATANNINS

Deposition of a red precipitate when an aqueous extract of each plant extract was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (Abdullahi et al., 2023).

DETECTION FOR TERPENOIDS

Salkowski test:

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids (Abdullahi et al., 2023).

DETECTION OF CARBOHYDRATES

Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test:
Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of a violet ring at the junction indicated the presence of carbohydrates (Tiwari et al., 2011).

Benedict's test:
Filtrate was treated with Benedict's reagent and heated gently. An orange-red precipitate indicated the presence of reducing sugars (Tiwari et al., 2011).

Fehling's test:
Filtrate was hydrolyzed with dilute HCl, neutralized with alkali, and heated with Fehling's A and B solutions. Formation of a red precipitate indicated the presence of reducing sugar (Tiwari et al., 2011).

Barfoed's test (General test for monosaccharides):
About 0.5 g of extract was dissolved in distilled water and filtered. A solution of the extract (1 ml) obtained above was mixed with 1 ml of Barfoed's reagent in a test tube and then heated on a water bath for 2 minutes. A red precipitate of cuprous oxide indicated the presence of monosaccharides (Adeleye et al., 2024).

Test for soluble starch:
Two ml of the extract was boiled with 1 ml of 5% potassium hydroxide (KOH), cooled, and acidified with tetraoxosulphate (VI) acid (H_2SO_4). A yellow coloration was taken as the presence of soluble starch (Musa et al., 2023).

Standard test for ketoses (Salivanoff's test):
A few crystals of resorcinol and 2 ml of hydrochloric acid were added to a small quantity of the extract, and the solution was allowed to boil for 5 minutes. A red coloration was an indication of the presence of ketoses (Musa et al., 2023).

DETECTION OF TANNINS
Two grams of the leaves was extracted with 10 ml of 50% alcohol, then filtered, and the filtrate was divided into 2 portions.

Ferric chloride test:
Three drops of a diluted solution of $FeCl_3$ were added to test tube one. Production of a blue or green-black color that changes to olive green as more ferric chloride was added indicated the presence of tannins (Ibrahim et al., 2022).

Lead sub acetate test:
Three drops of lead sub acetate solution were added to the second portion. The occurrence of a colored precipitate indicated the presence of tannins (Ibrahim et al., 2022).

Detection of Anthraquinones
Borntrager's test (for free anthracene derivative):
The leaf powder (0.5 g) was taken in a test tube, and 5 ml of chloroform was added and shaken for 5 minutes. The mixture was filtered, and the filtrate was shaken with an equal volume of 10% ammonia solution. A pink, red, or violet color in the aqueous layer after shaking indicated the presence of free anthraquinones (Ibrahim et al., 2022).

Modified Borntrager's test (for combined anthracene derivatives):
One gram of the leaf powder was boiled with 5 ml of 10% hydrochloric acid for 3 minutes. The hot solution was filtered into a test tube, cooled, and extracted gently with 5 ml of benzene. The upper benzene layer was pipetted off and shaken gently in a test tube with half its volume of 10% ammonium hydroxide solution. A rose-pink to cherry-red color in the ammonia layer indicated the presence of anthraquinones (Ibrahim et al., 2022).

Experimental Animals
Nine (9) albino rats were used for this study. Adult rats of both sexes weighing 72.9 to 240 kg were obtained from the Department of Biochemistry, University of Maiduguri. They were provided adequate ventilation, fed with commercial grower's mash (Livestock Feed® Nigeria LTD), and provided water ad libitum.

Experimental Design for the Acute Toxicity
The acute toxicity test (LD50) was carried out using Umar et al.'s method (2023). The experimental procedure was divided into two phases. Phase 1 comprises nine (9) albino rats divided into three groups of three rats each. Each group of rats was orally gavaged with graded doses of 10, 100, and 1000 mg/kg body weight using methanolic leaf extract of Carica papaya. These animals were physically observed every 2 hours within 24 hours for behavioral signs of toxicity and mortality. In addition, Phase 2 involved three rats, which were distributed into three groups of one rat each. The rats were administered higher doses of 1600, 2900, and 5000 mg/kg body weight with methanolic leaf extract of C. papaya and then observed for signs of toxicity and mortality.

The median lethal dose (LD50) was calculated by the square root of the highest dose of the extract that did not kill the animals multiplied by the lowest dose that caused death.
The LD50 is derived using the formula below:
LD50=(D0×D100)LD50=(D0×D100)
Where D0D0 is the highest dose that gave no mortality, and D100D100 is the lowest dose that produced mortality (Chinedu, 2013).

RESULTS

Extraction and Phytochemical Constituents of Methanolic Extract of Carica papaya Leaves

Pulverized Carica papaya leaves were extracted using 70% methanol as the extracting solvent; 1500 ml of the solvent was used at room temperature to produce 150 g of Carica papaya extract. A dark green semi-solid substance was obtained. The Carica papaya leaf extract revealed the presence of alkaloids, flavonoids, carbohydrates, terpenoids, cardiac glycosides, cardenolides, saponins, and phytosterols, while anthraquinones, phlobatannins, and tannins were absent, as presented in Table 1.

Table 1: Median Lethal Dose of Methanol Extract of *Carica papaya* leaves

Phytochemical Constituents	Type of Test	Results
Alkaloids	Dragendroff's test	+
	Mayer's test	+
Flavonoids	Shinoda's Test	+
	Ferric chloride Test	-
	Lead Acetate Test	-
	Alkaline Reagent test	+
Cardiac Glycosides	Salkowski's Test	-
	Liebermann – Burchard Test	+
Cardenolides	Keller-Killani Test	+
Phytosterols	Salkowski's Test	+
	Liebermann – Burchard Test	-
Carbohydrates	Molisch's Test	+
	Barfoed's Test	-
	Fehling's Test	+
	Benedict's Test	+
	Seliwanoff's Test	-
	Soluble starch	-
Terpenoids	Salkowski's Test	+
Anathraquinones	Free Anthraquinone	-
	Combined Anthraquinone	-
Saponins	Froth Test	+
Tannins	Ferric chloride Test	-
	Lead sub acetate Test	-
Phlobatannins	Phlobatannin Test	-

The median lethal dose of methanol extract of Carica papaya leaves was evaluated by both oral and intraperitoneal routes. Result of the acute toxicity study showed clinical signs of lethargy, droopy eyes, abdominal stretching and weight loss at doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg body weight following intraperitoneal administration. No mortality was recorded following oral administration of all the doses as shown in Table 2.

Table 2: Median lethal dose (LD50) of Methanolic Extract of Carica papaya Leaves by Oral Route (Phases I and II)

Table 2: Median lethal dose (LD_{50}) of Methanolic Extract of *Carica papaya* Leaves by Oral Route (Phases I and II)

Phase	No of Rats	Dose (mg/kg) body weight	Mortality
1	3	10	0/3
1	3	100	0/3
1	3	1000	0/3
2	1	1600	0/1
2	1	2900	0/1
2	1	5000	0/1

Foot note: No mortality was recorded at the highest dose of 5000 mg/kg

However, mortality was recorded at the second phase of extract administration at a dose rate of 2900 and 5000 mg/kg respectively following intraperitoneal administration (I.P). The LD50 was calculated as 2154 mg/kg body weight as shown in Table 3.

Table 3: Median Lethal Dose (LD50) of Carica papaya Methanolic Leaf Extracts by Intraperitoneal Route (Phase I and II)

Table 3: Median Lethal Dose (LD_{50}) of *Carica papaya* Methanolic Leaf Extracts by Intraperitoneal Route (Phase I and II)

Phase	Group	Dose (mg/kg) body weight	Mortality
1	3	10	0/3
1	3	100	0/3
1	3	1000	0/3
2	1	1600	0/1
2	1	2900	1/1
2	1	5000	1/1

$LD50 = \sqrt{(D\_0) \times D\_100)}$
D0 = highest dose that gave no mortality
D100 = Lowest dose that produced mortality
LD 50 = √(1600 x 2900)
LD50 = 2154 mg/kg

Discussion

The phytochemical analysis revealed various bioactive compounds with potential therapeutic effects. The absence of anthraquinones, phlobatannins, and tannins aligns with similar studies (Ayoola and Adeyeye, 2010). The calculated LD50 for the intraperitoneal route was consistent with other toxicity studies, indicating the safety of Carica papaya leaf extract.

Since time immemorial, man has used various parts of plants in the treatment and prevention of many ailments (Chah et al., 2006). Despite the presence of modern pharmaceutical drugs, search still continues for medicinal plants of high therapeutic value to resolve both old and new problems (Patil et al., 2014). The phytochemical analysis of the methanol extract of C. papaya leaves revealed the presence of various bioactive compounds, including alkaloids, flavonoids, carbohydrates, terpenoids, cardiac glycosides, and saponins. These compounds have been associated with potential therapeutic effects in traditional medicine. However, anthraquinones, phlobatannins, and tannins were found to be absent in the extract, this was in agreement with a similar study conducted by Ayoola and Adeyeye (2010) which showed aqueous C. papaya leaves contained saponins, cardiac glycosides and alkaloids. Tannin was absent in both studies.

The calculated LD50 for intraperitoneal administration was 2154 mg/kg, indicating a moderate level of toxicity. It's crucial to acknowledge that the absence of mortality in the oral administration suggests a relatively safer profile when the extract is taken through this route. However, the observed clinical signs at higher doses emphasize the importance of caution and proper dosage in medicinal applications as indicated by Lorke (1983). Any substance with LD50 of 50-500 mg/kg body weight is regarded as highly toxic. Substance having an LD50 of 500-1000 mg/kg are regarded as moderately toxic. Those substances with LD50 above 1000 mg/kg were regarded as being of low toxicity and therefore relatively safe to be applied for treatment (Clarke and Clarke, 1977). Any substance with an intraperitoneal LD50 of above 1000 mg/kg is regarded as safe (Clarke and Clarke, 1975). Any compound with an oral LD50 between 500-5000 mg/kg is considered practically nontoxic (Hodge and Sterner, 2005). According to Hodge and Sterner toxicity scale, Carica papaya is considered safe for use.

The findings contribute valuable information about the safety profile of C. papaya leaf extract, highlighting its potential medicinal benefits. Further studies could explore specific bioactive compounds responsible for observed effects and investigate the extract's therapeutic potential within a controlled and optimized dosage range. Additionally, it is essential to consider these results in the context of traditional medicine practices and explore ways to enhance the safety and efficacy of herbal remedies derived from local plants.

Conclusion

The qualitative phytochemical analysis of C. papaya leaf extract revealed the presence of alkaloids, flavonoids, cardiac glycosides, cardenolides, phytosterols, carbohydrates, terpeniods and saponins. While the elemental analysis of C. papaya leaf extract revealed the presence of potassium, calcium, sodium, magnesium, manganese, iron, copper, chlorine, zinc and lead. The acute toxicity study revealed that
C. papaya leaf extract was found to have low toxicity in albino rats with an intraperitoneal LD50 of 2154 mg/kg body weight. The oral LD50 of Carica papaya leaf extract was greater than 5000 mg/kg thus indicating a wide margin of safety.

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Conflict of Interest

No conflict of interest to declare.

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