Journal of Veterinary Research Advances
ISSN: 2582-774X

Research Article
Open access

Biochemical profile and phytochemical constituents of methanol extract of *Carica papaya* Leaves in albino rats

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Received on: 09/08/2025 Accepted on: 11/12/2025 Published on: 16/12/2025

ABSTRACT

Aim: The main purpose of the study wass to determine the biochemical profile and phytochemical constituents of methanol extract of *Carica papaya* leaves in albino rats.

Method and materials: To evaluate the effects of the extract on some biochemical parameters, using Diacetyl monoxime method for urea, Jaffe's method for creatinine, Cholesterol oxidase method for cholesterol, Jendrassik and Grof method for serum bilirubin, Biuret method for total protein, BCG dye binding method for albumin and Glucose oxidase method for fasting glucose. Elemental analysis using SP-9-single beam atomic absorption spectrophotometer (Philips PyeUnicam LTD, England) at the appropriate wavelength, temperature and lamp current for each of the element under study was determined by a standard calibration curve method.

Results: A significant increase ($p < 0.05$) in urea level was observed in rats treated with 600 mg/kg body weight (28.40 $\pm$1.17) compared to the control. There was a significant ($p < 0.05$) decrease in total protein concentration (5.86 $\pm$ 0.12) and (5.66 $\pm$ 0.12) when extract was used at a respectively dose rate of 400 mg/kg and 800 mg/kg body weight compared to control group. Total bilirubin increased significant ($p < 0.05$) when the extract was used at a dose rate of 800 mg/kg body weight (1.06 $\pm$ 0.13) compared to control group. Fasting blood glucose level decreased significant ($p < 0.05$) in all test groups compared to control.

Conclusion: It was concluded that the leaves of *Carica papaya* contains bioactive phytochemical constituents that could have clinical potential with safe medicinal properties.

Keywords: Biochemical parameter, Carica papaya leaves, Methanolic extract, Phytochemical.

Cite This Article as: Ali ZB, Adawaren EO, Lekko YM, Gwiokura AY, Mahre MB and Ojo NA (2025). Biochemical profile and phytochemical constituents of methanol extract of *Carica papaya* Leaves in albino rats. J. Vet. Res. Adv., 07(02): 42-48.

Introduction

Carica Papaya is a typical and important plant having characteristics of scientific medicine. It belongs to small family *Caricaceae* and is a significant fruit crop cultivated in tropical, sub-tropical and temperate zones such as Australia, Brazil, China, Hawaii, Malaysia, Nigeria, China and India (Nguyen *et al*., 2013; Afzan *et al*., 2012). Use of *Carica Papaya* as medicinal plant is possible because of presence of Phytochemicals in plant which are tannins, steroids, terpenoids, saponins, phenols, flavonoids, ferric reducing antioxidant properties (FRAP), pro-anthocyanidins, alkaloids, anthraquinones and cardiac glycosides (Ghaffarilaleh *et al*., 2019).

The pulp of *Carica Papaya* has some important vitamins: Thiamine, Riboflavin, Niacin, Ascorbic acid and alpha-tocopherol with antioxidant properties, Amino acids: Tryptophan, Methionine, Lysine and minerals: Calcium, magnesium, potassium, copper, zinc, manganese, and iron. *Carica Papaya* also contains the enzyme Chymopapain, Caricain, Glycylendopepetides and papain which increases gut transit time and is also used to ameliorate traumas, allergies, and skin lesions (Amin *et al*., 2019; Sharma *et al*., 2020; Ugbogu *et al*., 2023; Choudhary *et al*., 2025).

Studies have also shown the presence of proteolytic enzymes (chy- mopapain) with antiviral, antifungal, and antibacterial properties (Santana *et al*., 2019). The plant also has Antidiarreheal, and wound healing properties Sharma *et al*. (2020) Antidiabetic Agada *et al*. (2020) antioxidant Yap *et al*. (2020) Anti-inflammatory

Amin *et al*. (2019) Antimalarial Atanu *et al*. (2021) and Anticancer activity (Kyei-Barffour *et al*., 2021).

Dosage forms, side effects and efficacy of most of these medicinal plant preparations has not clearly been defined, despite the common and frequent use for therapy, based on the belief that they are safe because they are natural (Enemali *et al*., 2021). However, the local use of these plants and their product in disease remediation is on the rise particularly in areas where access to modern health services is unavailable. Despite the wide and historical use of *C. papaya* in the traditional management of many diseases, the scientific validation of its use for therapy is lacking (Shubham *et al*., 2019; Enemali *et al*., 2021). In order to boost the confidence of people who solely depend on herbal medicines; this research was aimed at determining the phytochemical constituents and biochemical effects of the leaf extracts on Albino Rats to determine the dosage at which these plant leaves will be safe for usage with minimal toxicological effect on the living system.

Materials and Methods

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*.

Collection of plants 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 of dust particles and shade dried at room temperature. The dried sample was then pulverised mechanically using a blending machine into dry powder. The dry powder was weighed and added into 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 of 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; Edeoga*et al*., 2005; Evans, 2002; Sofowora, 1993; Vishnoi, 1979; Silva *et al*., 1998. The extract was analysed for the presence of tannins, saponins, alkaloids, flavonoids, cardiac glycosides, carbohydrates, steroids, phenols, terpenoids and anthraquinones.

Detection of Alkaloids

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 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-coloured precipitate indicates the presence of alkaloids (Tiwari *et al*., 2011)

Test for Flavonoids

Lead acetate test

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

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids (Tiwari *et al*., 2011).

Shinoda's Test

Each of the extract (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 few drops of concentrated HCl. A pink, orange or red to purple colouration indicated the presence of flavonoids (Evans, 2002).

Ferric Chloride test

Each of extract 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 colouration indicated the presence of a phenolic hydroxyl group (Evans, 2002).

Detection of Saponins

Froth test:

0.5 gm of extract was shaken with 2 ml of water. Production of persistent foam for ten minutes indicated 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 few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes (Tiwari *et al*., 2011).

Libermann Burchard's test

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

Test for Cardiac Glycosides 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 addition of concentrated H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of steroidal ring i.e. aglycone portion of cardiacglycosides (Sofowora, 1993).

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. Appearance of reddish-brown colour or yellow at the interface was an indication of the presence of steroidal ring (Silva *et al*., 1998).

Keller – killani test

Five 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 sulphuric acid. A brown ring at the interface indicated a deoxy sugar characteristics 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 thin layer (Edeoga *et al*., 2005).

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 (Edeoga *et al*., 2005).

Detection for Terpenoids

Salkowski test

5ml 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 colouration of the interface was formed to show positive results for the presence of terpenoids (Edeoga *et al*., 2005).

Detection of Carbohydrates

Extracts were dissolved individually in 5 ml 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 the violet ring at the junction indicated the presence of carbohydrates (Tiwari *et al*., 2011).

Benedict's test

Filtrate was treated with Bendict's reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars (Tiwari *et al*., 2011).

Fehling's test

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

Barfoed's test (General test for monosaccharides)

About 0.5g of extract was dissolved in distilled water and filtered. A solution of 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, red precipitate of cuprous oxide indicated presence of monosaccharides (Sofowora, 1993).

Test for soluble starch

Two ml of extract was boiled with 1 ml of 5% potassium hydroxide (KOH), cooled and acidified with tetraoxosulphate (VI) acid (H_2SO_4). A yellow colouration was taken as presence of soluble starch (Vishnoi, 1979).

Standard test for ketoses (Salivanoff's test)

Few crystals of resorcinol and 2 ml of hydrochloric acid was added to a small quantity of the extract and the solution was allowed to boil for 5 minutes. A red colouration was an indication for the presence of ketoses (Vishnoi, 1979).

Detection of Tannins

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

Ferric chloride test

Three drops of diluted solution of $FeCl_3$ was added to a test tube one, production of a blue or green-black colour that changes to olive green as more ferric chloride was added indicated the presence of tannins (Evans, 2002).

Lead sub acetate test: Three drops of lead sub acetate solution was added to second portion. Occurrence of a coloured precipitate indicated presence of tannins (Evans, 2002).

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 shaken with equal volume of 10% ammonia solution. A pink, red or violet colour in the aqueous layer after shaken indicated the presence of free anthraquinones (Evans, 2002).

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 in a test tube, cooled and extracted gently with 5 ml benzene. The upper benzene layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide solution. A rose pink to cherry red colour in the ammonia layer indicated the presence of anthraquinones (Evans, 2002).

Elemental Analysis

Elemental analysis was carried out for the presence of the following elements: sodium, potassium, calcium, magnesium, manganese, copper, iron, chloride, zinc and lead. The sample (15 g) of the extract was placed in a labelled crucible and heated in a hotspot furnace at 550 °C for 3 hours. The sample was removed and cooled in a dessicator. The ashed sample (0.5 gram) was digested in a 250 ml beaker with 20 ml of 2 M nitric acid and 10 ml of 35% hydrogen peroxide and heated on a hot plate on a fume cupboard until a clear digest was obtained. The content was then filtered after cooling and deionized water was added and made up to 100 ml in a volumetric flask for elemental analysis using SP-9-single beam atomic absorption spectrophotometer (Philips PyeUnicam LTD, England) at the appropriate wavelength, temperature and lamp current for each of the element under study was determined by a standard calibration curve method (Sunderman, 1973; Kolthoff and Elving, 1976).

Experimental protocol for Biochemical Analysis

Biochemical analysis was carried out using the guide lines of Kanagasabapathy and Kumari (2000) for the presence of glucose, creatinine, urea, total bilirubin, total protein, albumin and total cholesterol.

Results and Discussion

Qualitative Phytochemical Constituent of 70% Methanolic Extract of Carica papaya Leaves

Pulverized *Carica papaya* leaves was 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 (Table 1).

Elemental Concentration of 70% Methanolic Extract of Carica papaya Leaves

Elemental analysis revealed the presence of K, Ca, Mg, Fe, Cu, Cl, Zn, and Pb while, Na was absent. Cl had the highest concentration at 11.0 mg/dl as shown (Table 2).

Table 1: Qualitative Phytochemical Constituent of 70% Methanolic 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	-

Table 2: Elemental concentration of 70% methanolic extract of *Carica papaya* leaves

Elements	Concentration (mg/dl)
Potassium (K)	1.70
Calcium (Ca)	0.96
Sodium (Na)	-
Magnesium (Mg)	0.33
Manganese (Mn)	0.01
Iron (Fe)	0.11
Copper (Cu)	0.09
Chlorine (Cl)	11.0
Zinc (Zn)	0.76
Lead (Pb)	0.30

Effect of Methanolic Extract of Carica papaya Leaves on Blood Biochemical Parameters in Albino Rats

The result showed a significant increase ($p < 0.05$) in urea level was observed in rats treated with 600 mg/kg body weight (28.40 ±1.17) compared to the control. There was a significant ($p < 0.05$) decrease in total protein concentration (5.86 ± 0.12) and (5.66 ± 0.12) when the extract was used at a respectively dose rate of 400 mg/kg and 800 mg/kg body weight compared to the control group. Total bilirubin increased significant ($p < 0.05$) when the extract was used at a dose rate of 800 mg/kg body weight (1.06 ± 0.13) compared to the control group. Fasting blood glucose level decreased significant ($p < 0.05$) in all the test groups compared to control.

The presence of alkaloids, flavonoids, carbohydrates, terpenoids, cardiac glycosides, cardenolides, saponins, and phytosterols extracted from Carica *papaya* leaf confirm the report of (Omidiwura, 2017; Ugbogu *et al*., 2023; Agada *et al*.,2020; Agada *et al*.,2021; Atanu *et al*., 2021; Choudhary *et al*., 2025; Enemali *et al*., 2021). Elemental analysis also revealed the presence of K, Ca, Mg, Fe, Cu, Cl, Zn, and Pb as was reported by (Choudhary *et al*., 2025).

Table 3: Effect of methanolic extract of *Carica papaya* leaves on blood biochemical parameters in albino rats

Dose of extract (mg/kg)	Biochemical Parameters						
	Urea	Creatinine	Total protein	Total bilirubin	Albumin	Total cholesterol	Fasting glucose
Control (HUT)	23.40 ± 1.33	1.06 ± 0.11	6.70 ± 0.23	0.64 ± 0.07	3.04 ± 0.29	81.00 ± 12.39	251.60 ± 19.87
400	25.40 ± 1.47	0.86 ± 0.04	5.86 ± 0.12[b]	0.78 ± 0.10	3.00 ± 0.18	53.80 ± 2.73	108.20 ± 2.60[b]
600	28.40 ± 1.17[a]	1.10 ± 0.22	7.14 ± 0.34	0.70 ± 0.18	3.12 ± 0.27	80.40 ± 9.80	93.60 ± 6.72[b]
800	25.60 ± 2.23	0.94 ± 0.21	5.66 ± 0.12[b]	1.06 ± 0.13[a]	2.80 ± 0.16	65.60 ± 8.82	84.60 ± 7.08[b]
1000	23.00 ± 1.41	0.94 ± 0.16	6.76 ± 0.22	0.60 ± 0.05	2.68 ± 0.29	70.00 ± 3.05	82.80 ± 6.86[b]
Insulin treated	26.40 ± 1.29	1.08 ± 0.22	6.88 ± 0.13	0.64 ± 0.14	2.80 ± 0.28	69.60 ± 10.42	79.80 ± 7.20[b]

The presence of alkaloids, flavonoids, carbohydrates, terpenoids, cardiac glycosides, cardenolides, saponins, and phytosterols extracted from Carica *papaya* leaf confirm the report of (Omidiwura, 2017; Ugbogu *et al*., 2023;Agada *et al*.,2020; Agada *et al*.,2021; Atanu *et al*., 2021; Choudhary *et al*., 2025; Enemali *et al*., 2021). Elemental analysis also revealed the presence of K, Ca, Mg, Fe, Cu, Cl, Zn, and Pb as was reported by (Choudhary *et al*., 2025).

The effect of methanol extract of *C. papaya* leaves on blood biochemical parameters in albino rats indicated a significant ($p < 0.05$) increase in urea level at dose rate of 600 mg/kg body weight. Urea and creatinine are waste products excreted from kidney and are indicators for kidney dysfunction (Airaodion *et al*., 2019; Enemali *et al*., 2021). Therefore, the increase in urea level at dose rate of 600 mg/kg body weight is an indication of kidney defect. There was a significant ($p < 0.05$) decrease of total protein at dose rate of 400 mg/kg and 800 mg/kg body weight compared to control. Any change observed in concentration of serum protein is an indication of change in normal liver function (Omonkhua and Onoagbe, 2011). Serum bilirubin significantly ($p < 0.05$) increased at dose rate of 800 mg/kg body weight. Serum bilirubin level is increased when the hepatic biliary system is affected (Airaodion *et al*., 2019; Enemali *et al*., 2021). Increase in serum bilirubin is indicative of bile duct obstruction (Omonkhua and Onoagbe, 2011). Higher total bilirubin levels are less likely to result in hyperglycaemia (Cheriyath *et al*., 2010). Creatinine, albumin and total cholesterol were not significantly different from the control group. The biochemistry of urea, total protein and total bilirubin indicated some changes which were not dose dependent. The fasting glucose level decreased ($p < 0.05$) in all test groups compared to control, this decrease observed indicates that *C. papaya* leaves has an insulin like effect on peripheral tissues (Gray *et al*., 2000). Maintaining pancreatic β cell function is best approach in prevention and treatment of hyperglycaemia (Oh, 2015). Restricted calorie intake of any macronutrients (balanced diet, carbohydrate, protein and lipid diets) does not lead

to glucose intolerance (Airaodion *et al*., 2019; Enemali *et al*., 2021).

Conclusion

It was concluded that 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 LD_{50} of 2154 mg/kg body weight. The oral LD_{50} of *Carica papaya* leaf extract was greater than 5000 mg/kg thus indicating a wide margin of safety.

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