

Phytochemical Screening, Chromatographic Studies and Antibacterial Activity of *Carica Papaya* Leaves Extracts

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Abstract: *The antibacterial activity of Carica papaya leaf extracts on pathogenic bacteria was observed in this study. Papaya leaves were extracted using water, methanol, chloroform and n-hexane as solvents. The C. Papaya leaves extracts were tested against Staphylococcus aureus, Bacillus subtilis, Salmonella typhi and Escherichia coli by agar well diffusion method. The extract demonstrated higher activities against tested bacteria with the highest activity (13 mm zone of inhibition) demonstrated against Escherichia coli. The methanol fraction was fractionated and yielded 7 fractions. The fraction 5 exhibited highest activity against E. coli. Preliminary phytochemical analysis showed that the extracts contain alkaloids, tannins, saponins and phenols. The results justified the use of Carica papaya leaf extracts in the treatment of gastroenteritis, urethritis, typhoid fever and wound infections.*

Keywords: *Carica papaya, antibacterial activity, phytochemicals, methanolic extracts and zone of inhibition.*

1. INTRODUCTION

Human beings have used plants for the treatment of diverse ailments for thousands of years [1]. Plants and their secondary metabolite constituents have a long history of use in modern 'western' medicine and in certain systems of traditional medicine, and are the sources of important drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine. Use of herbal medicines in developed countries has expanded sharply in the latter half of the twentieth century [2].

Column chromatography is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The main advantage of column chromatography is a relatively low expensive and disposability of the stationary phase used in the process [3]. The later prevent cross contaminations and stationary phase degradation due to recycling.

Thin-layer chromatography (TLC) is a highly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction. It also permits the optimization of the solvent system for a given separation problem. In comparison with column chromatography, it only requires small quantity of the compound and is much faster as well. A thin layer chromatography uses a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic. The mobile phase is a suitable liquid solvent or mixture of solvents [4].

Carica papaya, commonly known as papaya or pawpaw belongs to the plant family Caricaceae. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk [5]. It is distributed throughout Asia, Nigeria etc. It is medicinally used in the treatment of smooth upper respiratory tract ailment, psychiatric related illnesses, scorpion bites, hypertension toothache, tuberculosis, liver inflammations, arthritis and rheumatism [5, 6].

In recent years, the world has witnessed an increase in use of medicinal plants to cure infections and diseases. *C. papaya* is one of these plants which are greatly used by the rural communities to cure illnesses in Nigeria. For this reason, study to understand the medicinal value and potential of this plant is been intensified [7].

This study aims at investigating the antibacterial activity of column fractions of methanol, hexane, chloroform and water extracts of *Carica papaya* leaf on clinical isolates of bacteria.

2. MATERIALS AND METHODS

2.1. Collection and Processing of Plant Sample

Plant materials were collected from a farm in Aliero local government area of Kebbi state, Nigeria and were identified and authenticated at the Biological Sciences Department, Kebbi state University of Science and Technology, Aliero, Kebbi State, Nigeria. The fresh leaves were harvested and then rinsed in sterile distilled water. The leaf sample was shed-dried for a week. The dried leaves were pulverized, using sterile laboratory mortar and pestle, to obtain a powdered form. These were stored in air-tight glass containers protected from sunlight until required for analysis.

2.2. Plant Extraction for Phytochemical Assays

100g of the plant sample was dissolved in 400ml of water, methanol, n-hexane and chloroform respectively, and left for 48hrs. The extracts were then decanted and filtered through a Whatman filter paper. The filtered extract was then sterilized using a membrane filter and evaporated to dryness at 45°C. The residues obtained were then stored in the refrigerator at 4°C until used.

2.3. Phytochemical Screening

The extracts were screened for the presence of major phytochemicals using standard qualitative methods as described previously [8, 9, 10]. The plant extracts were screened for the presence of glycosides, phlobatannins, terpenoids, steroids, saponins, tannins, alkaloids, flavanoids, anthraquinones and phenols.

2.4. Collection and Maintenance of Test Organisms

The test organisms that were used were all human pathogenic organisms from clinical origin. These isolates include *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Salmonella typhi*. They were obtained from the Department of Biological Science Laboratory, Kebbi State University of Science and Technology, Aliero. The organisms were collected on sterile agar slants and incubated at 37°C for 24hours. They were then kept as stock cultures in the refrigerator at 4°C.

2.5. Column Chromatography

Column chromatography was used to get the fraction of plant extracts [11]. (2g) of the extract was subjected to column chromatography to separate the extracts into its component fractions. Silica gel (60-120 mesh) was used as the stationary phase, and the solvent system chloroform: methanol as mobile phase. In the setting up of the column chromatography, the lower part of the glass column was stocked with glass wool with the aid of glass rod. The sample was prepared by adsorbing 2.0g of the extract to 10g of silica gel (60-120 mesh) in methanol then allowed to dry, the dry powder was gently layered on top of the column then a glass wool was put on top so as to avoid splashing of the solvent system when pouring it into the column. The elution of the extract was done with solvent system chloroform: methanol 9:1. The eluted fractions were collected in bottles. 50 bottles were collected as eluted fractions.

2.6. Analytical Thin Layer Chromatography (Tlc)

The content of each bottle was spotted on the pre-coated (silica gel) aluminum plates in a chromatographic tank to separate the different fractions based on the relative mobility and colour reactions [4]. The fractions were concentrated; a strip of the pre-coated silica gel was cut out. Using a capillary tube a spot of the sample was applied on the plate about 1.0cm from the edge of the plate, it was allowed to dry, the strip was lowered into the chromatographic tank that contain the solvent system chloroform: methanol 9:1. After the thin layer chromatography was done, the fractions that have the same mobility were joined together. Out of the 50 bottles that were used to run the thin layer chromatography 5 fractions were gotten which were now used for anti-bacteria activity.

2.7. Antibacterial Activity of Column Fractions of *Carica Papaya* Leaves Extract.

This was carried out using agar disc diffusion technique as described by [12]. The Muller Hinton agar medium was allowed to solidify, after which the test organisms were aseptically inoculated on different Petri dishes using sterile swab sticks, with the aid of a syringe the fractions were added to the disc of the inoculated plate methanolic extract of *C. papaya* was used. Ciprofloxacin was used as the positive control and distilled water was used as a negative control was also incorporated in the inoculated plates. The discs were sufficiently spaced out to prevent overlapping of the zones. The

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plates were then allowed for the pre-diffusion time of 15 minutes after which they were incubated for 24 hours at 37°C. Diameters of zones of inhibitions were measured using millimeter rule and the results expressed in millimeter.

3. RESULTS AND DISCUSSION

The result of the qualitative phytochemical screening of *C. papaya* leaves extracts showed the presence of some of the phytochemicals tested as shown in table 1 below. While the result for the antibacterial activity of leave extracts of *C. papaya* is presented on table 2 and antibacterial activity of column fractions (F1 – F7) of crude methanolic extract is shown on table 3.

Table1. Phytochemical components present in leave extracts of *C. Papaya*

Phytochemical	Leaves extracts of <i>C. papaya</i>			
	Aqueous	Methanol	n-hexane	Chloroform
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Tannins	-	+	-	+
Glycosides	+	-	-	+
Phenols	-	+	-	-
Flavonoids	-	+	-	+

Note: +: Detected, -: not detected.

Table2. Zone of inhibition (mm) of the leave extracts (100mg/ml) of *C. papaya* against tested bacteria.

Bacteria	Zones of inhibition (mm)*				
	Methanol	Aqueous	n-Hexane	Chloroform	Cipro.
<i>E. coli</i>	12.03±0.15	9.90±0.30	6.96±0.20	7.03±0.20	13.03±0.20
<i>S. aureus</i>	10.00±0.00	8.03±0.15	6.03±0.15	4.80±0.16	13.52±0.15
<i>S.typhi</i>	9.03±0.05	5.93±0.25	5.96±0.19	5.10±0.13	10.91±0.35
<i>B. subtilis</i>	10.00±0.00	8.00±0.13	5.86±0.23	5.90±0.07	15.73±0.15

Note: Values are presented as mean ± standard deviation of three replicates.

Table3. Antibacterial Activity of Column Fractions (F1 – F7)

Organism	Diameter of zone of inhibition (mm)							Ciprofloxacin
	F1	F2	F3	F4	F5	F6	F7	
<i>S. typhi</i>	0	0	0	3.96±0.20	4.03±0.15	0	0	14.90±0.36
<i>E. coli</i>	0	0	3.00±0.15	1.90±0.20	7.00±0.26	0	0	13.93±0.32
<i>B. subtilis</i>	0	0	0	2.00±0.20	5.00±0.17	0	0	11.98±0.20
<i>S. aureus</i>	0	0	4.03±0.20	2.06±0.20	4.00±0.30	4.02±0.20	0	13.96±0.25

Results are mean ± standard deviation of three determinations

The Phytochemical analysis of the leave extracts of *Carica papaya* is presented on table 1. The result showed that the alkaloids and saponins are found in all the four extracts. Tannins were only found in chloroform and methanolic leave extracts. Glycosides were only found in the aqueous and chloroform extracts. Phenols were only detected in the methanolic leave extract. Lastly, flavonoids were only detected in the chloroform and methanolic leave extracts of *Carica papaya*. The presence of alkaloids, saponins, terpenoids and tannins in the leaves extracts of *Carica papaya* has medicinal implications. These phytochemicals are known to be biologically active. Tannins were found to play a role in antifungal, antibacterial, astringent and antibiotic activities [13, 14]. Tannins were also found to form irreversible complexes with proline-rich proteins leading to the inhibition of the cell protein synthesis.

In addition to antimicrobial activity exhibited by tannins, they also react and form complex with proteins to provide the typical tanning effect. This is important medicinally for the treatment of inflamed or ulcerated tissues [15]. Tannins-containing herbs as their main component are astringent in nature and are used in the treatment of intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. Terpenoids also act as antibiotics to protect plants from pathogenic microorganisms [16].

The results obtained from the antibacterial activity of the four extracts showed that methanol extract has the highest activity of 12.03mm compared to other extracts (table 2). Hence, observation of the

column chromatography of the methanol extract showed seven different fractions and the antibacterial activity of the seven fractions on test organisms showed that fractions 1, 2, and 7 showed no antibacterial activity. While fraction 6 showed activity against *S. aureus* only, fraction 3 showed activity against *E. coli* and *S. aureus*. Fractions 4 and 5 showed activity against all the tested organisms with highest activity exhibited by fraction 5 against *E. coli* (7.0mm) as shown in table 3 below.

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties [17]. Plants are the cheaper and safer alternative sources of antimicrobials [18]. Anibijuwon and Udeze [19] extracted bioactive compounds from leaf and root extracts of *C. papaya* using water and organic solvents, which were investigated for antibacterial activity against some human pathogenic bacteria. Both leaf and root extracts showed pronounced inhibition against gram positive bacteria than the gram negative bacteria tested. This present study however showed highest inhibition against *Esterichia coli*, a gram negative bacterium, than all other organisms tested, using methanol extract.

It has been previously demonstrated that ethanol extracts contributed much more antibacterial activity than the aqueous extract [20]. Our findings is correlated with these results, herein, methanol extracts demonstrated the highest antibacterial activity than the other extracts (table 2 and 3). The highest antibacterial activity brought about by this solvent might be due to better solubility of the active components in the leaves.

Furthermore, column chromatography of the methanol extract showed seven different fractions (FRA 1 to 7). The antibacterial activities of these fractions revealed that fraction 4 showed 4mm, 2mm, 2mm, and 2mm zone of inhibition against *S. typhi*, *E. coli*, *Bacillus subtilis* and *S. aureus* respectively. Fraction 5 shows 4mm, 7mm, 5mm, and 4mm zone of inhibition against *S. typhi*, *E. coli*, *Bacillus* and *S. aureus*. Fractions 3 and 6 had activity against only one bacterium, with 3mm and 4mm zones of inhibition against *E. coli* and *S. aureus* respectively. The rest fractions, 1, 2 and 7 did not show any inhibition, which could be as a result of the absence of phytochemical compounds. Fraction 5 which shows the highest zone of inhibition could be as a result of active ingredients present in the extract.

4. CONCLUSION

The demonstration of antimicrobial activity against tested bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The results of the study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs formulation for the treatment of gastroenteritis, typhoid fever and wound infections. In addition, there is need for the characterization and isolation of the active ingredients.

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