



In-vitro Investigation on the Therapeutic Potential of *Carica papaya* Leaf Extract on Some Pathogenic Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EMA and AUS managed the analyses of the study. Author AUS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Carica papaya is one of the most valuable plants used for various purposes in medicinal field and ethnomedicine. The aim this study was to investigate the therapeutic potential of *C. papaya* leaf extract using cold maceration method with distilled water, methanol and petroleum-ether. Phytochemical screening of the extracts revealed the presence of the following: alkaloids, saponins flavonoids, steroids, cardiac glycosides and tannins. Antimicrobial activities of the extracts were determined by agar well diffusion method by measuring the diameters of zones of inhibition. In aqueous extract, *Klebsiella pneumonia* had the highest zone of inhibition (16 ± 0.00 mm) followed by *Escherichia coli* and *Staphylococcus aureus* at 14 ± 0.00 mm and 12 ± 0.5 mm respectively. In methanol extracts, *E. coli* had the highest zone of inhibition (15 ± 00 mm) followed by *Staphylococcus aureus* (12 ± 0.00 mm) while *K. pneumonia* had no inhibition. With Petroleum-ether, *Staphylococcus aureus* had the highest zone of inhibition (22 ± 0.00 mm) followed by *E. coli* (0.13 ± 0.00 mm) while *K. pneumonia* had no inhibition. It was clear that *C. papaya* had

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antimicrobial activity but varied with organism and extraction solvent. Therefore, for development of drugs, different extraction solvent and bacteria must be used in order to develop effective and tailored made antimicrobials to curb the menace of antibiotics resistance.

Keywords: *Carica papaya*; bacteria; extract; plant and phytochemical.

1. INTRODUCTION

Carica papaya (pawpaw) have phenomenal restorative properties for treatment of various infirmities. The various parts of the *Carica papaya* plant including leaves, seeds, latex and fruits showed to have restorative worth [1]. It is one of the most important plants utilized for different purposes in therapeutic field and ethnomedicine [2]. *Carica papaya* has antimicrobial, antihelmintic and cancer prevention agent properties [3] in the leaf and bark and as well as twig tissues that have high anti-tumor and pesticide properties, as stated by Ali et al [4].

Pawpaw is from *Caricaceae* family; its scientific name is *Carica papaya L.* It is a huge herbaceous plant on the grounds that the stem doesn't have a lot of wood, the stem develops from 5 to 10m tall with all the leaves on the top and are 50-70cm wide [5]. It is broadly found in Indonesia and Nigeria, however the specific territory of origin is obscure, the papaya is accepted local to tropical America, maybe in southern Mexico and neighboring focal America [6].

Plants are significant natural source for items utilized in medication in numerous years [7]. Antimicrobial action in plant extricates is ascribed to the presence of phytochemicals, for example, phenolics, steroids, alkaloids, saponins and terpenoids [8]. The helpful properties of plants have been assessed by numerous examinations everywhere in the world and the vast majority of them have been uncovered to have antimicrobial action as announced by Muhuha et al. [9]. It was also reported that alkaloid, reducing sugar, steroid, terpenoids, phenol, anthraquinone, tannin, saponin and flavonoids in the *Carica papaya* leaf extract [4].

Carica papaya L. contains numerous biochemically dynamic mixtures, two most significant mixtures are chymopapain and papain which should help in absorption [10]. Papain is utilized in the treatment of joint inflammation and the proteolytic catalyst, it has an abundance of industrial applications [11]. As of late, FDA has

cleared chymopapain for intradiscal infusion in patients with reported lumbar intervertebral circles whose signs and indications have not reacted to conventional treatment throughout a sufficient timeframe [10].

Papaya assumes a significant part in the customary treatment of dengue fever and malaria fever by heating up the leaves into tea which helps increment the white platelets and platelets, standardizes coagulating and fixes the liver [1]. The dark seeds of papaya are palatable and utilized as flavour for cooking and furthermore as a substitute for dark pepper [12]. Its underlying foundations can be utilized as medication for renal and urinary bladder issue [13]. The objective of this study was investigate the therapeutic potential of *Carica papaya* leaf extract on some pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 Sample Collection and Authentication

Pawpaw leaves (*Carica papaya L.*) was obtained from Shanu village, Minna, Niger State and transported in a clean polythene bag. They were identified by an ethnobotanist in the department of Plant Biology, Federal University of Technology, Minna, Niger State, Nigeria.

2.2 Preparation of the Plant Samples

The leaves were spread to dry for one week at room temperature. After drying properly, the dried leaves were blended into powdery form. The powder was sieved so as to get fine powder of leaves.

2.3 Extraction of Plant Samples

The extraction of the leaves was carried out as described by Mbakwem-Aniebo et al. [14]. About 100g of the powdered *C. papaya* leaf was weighed in separate beakers and macerated with 500 ml of methanol, distilled water and petroleum-ether, each of these beakers were properly sealed with aluminium foil and was thoroughly hand shaken after 30 minutes for 6 hours, then left for 72 hours. The mixture was

filtered with a muslin cloth, the filtrate obtained was concentrated on a water bath. The concentrated extracts were weighed and kept in a well labelled sterile specimen bottles and refrigerated at 4°C prior to use for antibacterial in- vitro investigations.

2.4 Phytochemical screening of *Carica papaya* extract

The phytochemical components of the plants were detected according to the method of Trease and Evans [15] and Sofowora [16]. The phytochemical screening was done to confirm the presence or absence of certain compounds that are responsible for the anti microbial activities of plants extracts such as alkaloids, saponins, flavonoids, steroids, cardiac glycosides and tannins.

2.5 Reconstitution of Extracts

The stock solution of the extract was prepared by dissolving 0.8g, 1.0g and 1.2g each in 2 ml of DMSO and 3ml of sterile distilled water used in the extraction process to obtain 160 mg/ml, 200 mg/ml and 240 mg/ml respectively. These were dispensed in sterile bottles; this procedure was repeated for each of the solvent used. These were stored at 15°C until further use.

2.6 Test Organisms and Identity Confirmation

The bacteria used for this study were *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*. These organisms were obtained from Microbiology Department, Federal University of Technology, Minna, Niger State, Nigeria. Their identities were confirmed through morphological, biochemical and growth media.

2.7 Standardization of Organism

Approximately 0.1 ml of 1% barium chloride was applied to 9.9 ml of 1% sulphuric acid, which was then reconstituted into 10 ml of sterile distilled water to make a normal solution of 0.5 mL Mcfarland. The broth culture of the research organism was then contrasted to 0.5 percent Mcfarland in terms of turbidity. For the antibacterial assay of the plant extracts, a loop of the uniform culture was used [3].

2.8 Antibacterial Susceptibility Test

The agar well diffusion method was employed to test the antibacterial activities of the plant extracts according to Oyeleke et al. [17]. The standardized suspensions were used to inoculate the surfaces of sterile Mueller Hilton agar plates using sterile cotton swabs. Wells were punched (6 mm in diameter) using sterile cork borer in duplicate agar plates each well was filled with 1 ml of 240 mg/ml. The plates were allowed to stand for about 1-2 hours at room temperature for the extracts to diffuse into the agar, the agar plates were incubated at 37°C for 24 hours. The antibacterial activities of the plant extracts were assessed by measuring the diameter zone of inhibitions.

2.9 Data Analysis

The data obtained from diameter zones of inhibition produced by the isolates against the extracts used were analyzed using One- Way ANOVAs (IBM SPSS Statistic version 23). All data were expressed as Mean \pm Standard Error Mean of duplicate determinations.

3. RESULTS

The qualitative phytochemical screening of *C. papaya* leaf extracts revealed the presence of Alkaloids, Saponins Flavonoids Steroids, Cardiac glycosides and Tannins. However, different solvents revealed different bio constituents depending on their penetrating capacity (Table 1). All the phytochemical screened were found in aqueous solvent except flavonoids. Saponins and steroids were absent in methanol. Petroleum-ether also lack saponin and tannins.

The results of antibacterial activity are given in the Table 2, which clearly show that all the extracts have shown antibacterial activity but not as high as that of standard drug against the entire tested organisms. Petroleum-ether extract was more effective against *Staphylococcus aureus* than both aqueous and methanol extracts. Methanol extracts was more effective *E. coli*. than aqueous extract and Petroleum ether. Aqueous extract was the only one that was effective against *K. pneumonia* while methanol and petroleum ether extract were not effective against it.

The identity of the three isolates were confirmed to be: *Staphylococcus aureus*, *Escherichia coli* and *Klebsilla pneumonia* in Table 3.

Table 1. Qualitative phytochemical screen of *C. papaya* leaves extract

| Phytochemical | Aqueous | Methanol | Petroleum-ether |
|-------------------|---------|----------|-----------------|
| Alkaloids | + | + | + |
| Saponins | + | - | - |
| Flavonoids | - | + | + |
| Steroids | + | - | + |
| Cardiacglycosides | + | + | + |
| Tannins | + | + | - |

Keys: '+' presence of compound, '-' absence of compound

Table 2. Susceptibility of *C. papaya* leave extract at 240mg/ml of all the solvents

| Organisms | Aqueous 240 mg/ml | Methanol 240 mg/ml | Petroleum-ether 240 mg/ml | Standard drug (ciprofloxacin) |
|-------------------------|----------------------|-----------------------|------------------------------|----------------------------------|
| <i>Staph. aureus</i> | 12±0.5 mm | 12±0.00 mm | 22±0.00 mm | 36±0.00 mm |
| <i>Escherichia coli</i> | 14±0.00 mm | 15±00 mm | 0.13±0.00 mm | 36±0.00 mm |
| <i>K. pneumonia</i> | 16±0.00 mm | NA | NA | 35±0.00 mm |

Keys: NA = No zone of inhibition

4. DISCUSSION

In this research, the existence of alkaloids, saponins, flavonoids, steroids, cardiac glycosides and tannins in the *Carica papaya* leaf extract confirms the validity of the plant's long-standing usage for the treatment of various human ailments [18]. Muhuha et al. [8]. reported that particular phytochemicals, concentration, bioactive concepts, antagonistic and synergistic behavior depend on the antimicrobial properties of medicinal plants. In related studies, Ali et al. [4] found alkaloids, saponins, flavonoids and steroids in *C. papaya* leaf extract, with the exception of cardiac glycosides and tannins. Tannins in *C. papaya* leaf extracts were discovered by Ajiboye and Olawoyin [3]. Due to the environmental condition, nature of solvents and method of extraction, plant phytoconstituents are not always the same as stated by Elijah et al. [19].

Ajiboye and Olawoyin [3] reported that tannin forms irreversible protein-rich proline complexes that result in cell protein synthesis inhibition. Tannins are known to react with protein to provide the typical tanning effect, which is important for the treatment of inflamed or ulcerated tissues, according to Parekh and Chanda [20]. As revealed by Ajiboye and Olawoyin [3], it is also used to treat conditions such as diarrhea.

Alkaloids, the most effective therapeutically important plant material, were another recognizable bioactive factor [21]. According to the report of Dwivedi et al. [22], alkaloids are a

class of nitrogenous compounds known to be generated by plants. Due to their analgesic, antispasmodic and bacterial properties [21], pure alkaloids and synthetic derivatives are used as basic medicinal agents.

In several plants, the bioactive compound, flavonoids are known to protect plants from stress [22], as well as being anti-allergic, anticancer, hepatoprotective, anti-diabetic, antibacterial, anti-inflammatory and anti-viral in nature [23,22]. Ali et al. [4] reported that flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity or microbial membranes at low concentrations. Flavonoids are also reported for their antiviral, antimicrobial and spasmolytic properties [21].

Saponins are a wide group of foam-forming glycosides with detergent properties known in nature to be antimicrobial, anti-malarial, anti-allergic, anti-diabetic, insecticidal, and anti-inflammatory [9,22]. Okigbo et al. [24] suggested that the existence of saponins supports the fact that *C. papaya* leaf has cytotoxic effects as saponins are cytotoxic, such as intestinal permeabilization.

Cardiac glycosides is effective in treatment of cancer [25] and congestive heart failure, inability to pump enough blood to take care of body needs [26]. In addition, Morsy [26] also reported that some cardiac glycosides display an inhibitory activity against rhinovirus.

Table 3. The result of confirmatory test of isolates

| Gram reaction | Shape | Catalase | Coagulase | Indole | Urease | TSIA | | | MSA | Hoekten agar | Citrate | Confirmed Bacteria |
|---------------|-------|----------|-----------|--------|--------|-------|------------------|------|-----|--------------|---------|---------------------|
| | | | | | | Slope | H ₂ S | Butt | | | | |
| - | Rods | + | - | - | + | R | + | - | - | + | + | <i>K. pneumonia</i> |
| + | Cocci | + | + | - | - | R | - | - | + | - | + | <i>S. aureus</i> |
| - | Rods | + | - | + | - | Y | - | + | - | - | - | <i>E. coli</i> |

Keys: - = Negative, + = Positive, Y = Yellow, R = Red, TSIA = Triple Sugar Iron Agar and MSA = Mannitol Salt Agar

Steroids form a group of secondary metabolites whose structure and biological functions are diverse. In pursuit of these secondary metabolites as a possible lead in drug design/discovery, these natural products with many therapeutic uses and research is still ongoing [25].

The antimicrobial activities revealed from empirical measuring of diameter zone of inhibition varied. Petroleum ether had the highest zone of inhibition (22±0.00 mm) with *Staphylococcus aureus* followed by *Klebsiella Pneumonia* (16±0.00 mm) *Escherichia coli* (15±00 mm) in aqueous extracts and methanol extract respectively. The different might be as result of different phytoconstituents present in each of the extract even at same concentration [21]. This study was in agreement with Ajiboye and Olawoyin [3] who recorded different diameters zones of inhibition for different bacteria and extraction solvents at same concentration. petroleum ether extract and methanol extracts did not have activity on *Klebsiella Pneumonia*. This might be as result key bioactive active components that were lacked in the extraction. However, Baskaran et al. [21] in similar study recorded no activity With petroleum -ether for all the bacteria used.

5. CONCLUSIONS

From this investigative study, it become crystal clear that all the reports of the medicinal values of *Carica papaya* for both traditional uses and scientific researches were true owing to the presence of some key bioactive components found and its antimicrobial effect on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella Pneumonia*. It was also noted that different solvent had different behaviour in respect to the phytochemistry of the plant and antimicrobial activity.

Therefore, since there is no parallel antibacterial activity, further study is recommended for different extraction solvents and methods and different bacteria test in order to develop effective and tailored made antimicrobial agents to curb the menace of antibiotics resistance.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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