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Preliminary Study of the Phytochemical Constituents and Antimicrobial Activity of the Ethanolic Extract of Papaya (*Carica Papaya*) Seed

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Abstract

This study undertook a comprehensive examination of the phytochemical constituents and antimicrobial activities of *Carica papaya* seed. The seeds were subjected to ethanol extraction, and the resulting extract was meticulously screened for the presence of phytochemicals. Subsequent testing assessed the extract efficacy against two bacterial strains: *Escherichia coli* and *Staphylococcus aureus*. The results of the study revealed that the extract of *Carica papaya* seeds is replete with an array of phytochemicals, including alkaloids, flavonoids, tannins, saponins, and terpenoids. Notably, the extract exhibited pronounced antimicrobial activity against both bacterial strains. The minimum inhibitory concentration (MIC) of the extract was determined to be 6.25 mg/mL against *S. aureus* and 12.50 mg/mL against *E. coli.* The findings of this study conclusively demonstrate that *Carica papaya* seed possess significant antimicrobial properties, rendering them a promising natural remedy for the treatment of diseases caused by bacteria. Furthermore, this study underscores the importance of exploring the potential medicinal applications of *Carica papaya* seed and their constituents.

Keywords: Antimicrobial Activity, Bacterial strains, *Carica papaya* seeds, Medicinal applications, Phytochemicals

Introduction

Papaya (*Carica papaya*) is a tropical fruit widely cultivated in many parts of the world, known for its rich nutritional value and various culinary applications. While the flesh and juice of the papaya fruit are commonly consumed, the seeds have long been recognized in traditional and folk medicine practices for their potential medicinal properties [1]. In recent years, there has been a growing interest in exploring natural plant-based remedies as alternative or complementary therapies, leading to increased scientific investigations into the therapeutic potential of various plant materials, including papaya seeds [2].

Numerous studies have reported the presence of various bioactive compounds in papaya seeds, such as alkaloids, flavonoids, phenolic acids, and

enzymes like papain, which may contribute to their potential medicinal effects [3]. According to Nguyen *et al.* [4], the seeds of papaya have been traditionally used for treating a range of ailments, including intestinal worm infestations, digestive disorders, and even certain types of cancers. Scientific research has also suggested that papaya seeds may possess antimicrobial, antioxidant, anti-inflammatory, and other pharmacological properties, making them a subject of interest for further investigation [5].

Papaya (*Carica papaya*) is a tropical fruit tree native to southern Mexico and Central America, but now cultivated in many tropical and subtropical regions around the world [6]. In Ayurvedic medicine, a traditional system of medicine originating in India, papaya seeds have been employed for their carminative, anthelmintic, and digestive properties [7].

Similarly, traditional Chinese medicine has recognized the therapeutic potential of papaya seeds, utilizing them for treating intestinal worms, facilitating digestion, and managing various inflammatory conditions. Indigenous communities in South America and the Caribbean have also incorporated papaya seeds into their traditional remedies for a range of ailments, including bacterial and fungal infections and fever [8].

This widespread traditional use of papaya seeds across diverse cultures and geographic regions has sparked scientific interest in investigating the potential medicinal properties and active compounds present in these seeds. Numerous studies have reported the presence of various phytochemicals in papaya seeds, such as alkaloids, phenolic compounds, flavonoids, and enzymes like papain [9].

These bioactive constituents are believed to contribute to the medicinal important of the seeds.

The growing interest in exploring natural plant-based remedies as alternative or complementary therapies has further driven research efforts to evaluate the therapeutic potential of papaya seeds. With the increasing prevalence of antibiotic resistance and concerns over the side effects of synthetic drugs, there is a growing demand for safe and effective natural alternatives [10].

Papaya seeds, with their diverse phytochemical profile and traditional medicinal applications, have emerged as a promising product/drug for further investigation [11]. Additionally, some findings from previous studies may be contradictory or inconclusive, highlighting the need for further research to clarify the medicinal properties and safety profiles of papaya seeds [12].

This research work aimed to address this gap by conducting a study of the phytochemical constituent and antimicrobial activity of the ethanolic extract of papaya (*Carica papaya*) seed.

Materials and Methods

Plant Material

The plant material used in this study was the ripe fruits of *Carica papaya*. (Papaya), belonging the Family *Caricaceae*. The Papaya fruits were obtained from local organic farms located in the Mallum, Ardo-kola Local Government Taraba State, Nigeria in December, 2024. The fruits were selected based on uniform ripeness, determined by parameters such as color (yellow-orange), texture (slightly soft) and absence of physical damage or insect infection [13].

Solvents and Apparatus

Ethanol (500ml), Mortar and Pestle, Weighing Balance, Beakers, Round bottom flask, Measuring Cylinder, Funnel, Linen (Muslim cloth), Oven, Light Source, Knife.

Phytochemical Reagents

Dragendorff's reagent

Mayer's reagent

Sodium hydroxide

Ferric chloride

Gelatin solution

Distilled water

Salkowski reagent

Sample Preparation

The harvested papaya fruits were transported to the Laboratory in Sterile polyethylene bags to prevent contamination. Upon arrival, the Papaya seeds were manually extracted from the fruit pulp using a clean stainless-steel knife. The extracted seeds were washed thoroughly under running tap water to remove any adherent fruit pulp or other contaminants.

The washed papaya seeds were then spread out on clean, dry paper towels and air-dried at room temperature (25 ± 2°C) for a period of 7 days as per the method outlined by [14]. The dried seeds were stored in airtight containers at 4°C centile further analysis to prevent any deterioration or microbial contamination.

Extraction

The dried Papaya seeds were ground into a prime powder using a laboratory grinder. The powdered seeds were then subjected to sequential extraction using ethanol as solvent. A 40 g portion of the powdered Papaya seeds were packed into a reagent bottle and extracted with 200ml of the solvent for 72 hours. The extracts were concentrated under reduced pressure using a rotary evaporator (Buch: R-210, Switzerland) at a temperature not exceeding 50°C. The concentrated extracts were then stored in airtight containers at 4°C until further analysis.

Preparations of culture media

The microbiological culture media used in this study were Mueller-Hinton agar and nutrients broth, obtained from the laboratories, as recommended by the clinical and laboratory standards institute (CLSI, 2018) for antimicrobial (susceptibility).

Test organisms

The bacterial strains used in this study were: Gram-positive bacterium: *Staphylococcus aureus* (ATCC 25923) and Gram-negative bacterium: *Escherichia coli* (ATCC 25922).

Antimicrobial activity

The antimicrobial activity of the Papaya seed extract was evaluated against Staphylococcus aureus (ATCC 25923) and *Escherichia coli* (ATCC25922) bacteria strains using the disk diffusion methods as described in the clinical and laboratory standards institute (CLSI, 2018) guidelines.

The bacteria cultures were grown in nutrient broth at 37°c for 18-24 hours and adjusted to a turbidity equivalent to 0.5 McFarland standards, which corresponds to a cell density of approximately $1.5x10^{-8}$ CFU/ML.

Aliquots of the standardized bacterial suspensions were spread into Mueller- Hinton agar plates. Using sterile cotton swaps. Sterile filter paper discs (6mm diameter) were then impregnated with 20 µL of the respective papaya seeds extracts at all concentration and placed on the inoculated agar plates. Antibiotic discs containing standard antimicrobial agents were used as positive control.

Results and Discussions

Table 1: Phytochemical constituents of ethanol extract of *Carica Papaya* seed

Bioactive compounds	Results
Alkaloids	
Dragendroff's test	+
Flavonoids	
Sulfuric acid	+
Alkaline reagents test	+
Tannins	
Lead acetate solution test	+
Dilute iodine solution test	+
Saponins	
Frothing test	+
Terpenoids	
Salkowski test	+
Liebermann-Burckhardt test	+

Keys +: present -: Not detected.

Table 2: Antimicrobial activities of ethanol extract of *Carica papaya* seed against test isolate

Test organism	Zone of inhibition (mm)			Control
	5.0mg/ml	10.0mg/ml	20.0mg/ml	Ciprofloxacin tab
E. coli	12.25 ± 0.30	15.50 ±0.00	18.65 ± 0.30	25.00 ± 0.00
S. aureus	13.50 ± 0.00	16.25 ± 0.71	20.75 ± 1.06	22.00 ± 0.00

Values are presented as mean ± standard deviation $p \leq 0.05$

Table 3: Minimum Inhibitory Concentration (MIC) of ethanol extract of *Carica Papaya* seed.

Organisms	MIC (mg/ml)
E. coli	12.50
S. aureus	6.25

Discussions

The results of the phytochemical compounds, antimicrobial activity and minimum inhibitory concentration (MIC) were presented in Table 1- 3, the phytochemical studies show that the extract contains bioactive constituents such as alkaloids, flavonoid, saponins and terpenoids which are responsible for the biological properties of the plants seed. This result corresponds with the finding of [15]. All these facts could be some of the reasons why *C. papaya* seeds are widely used for the treatment of many ailments among many tribes in Nigeria [16].

The antimicrobial activities of the ethanol seed extract of *C. papaya* seeds could be due to the presence of active chemical constituents detected in the extracts. These may be associated with the high polarity of the solvents which allows it to penetrate well into the plant tissues and extracts more active compounds that are responsible for the inhibition observed [17]. Previous studies show that the extracts of *C. papaya* seeds possess phytochemicals responsible for antibacterial activity against *S. aureus* and *E. coli* [18] and this fining also confirmed it.

The test organisms showed a degree of susceptibility, the order of sensitivity being *S. aureus* which is more susceptive to extract than *E. coli* the deference's in antibacterial could be due to the mode of actions and structural properties of the organism. The cell walls of Gram positive bacteria are more sensitive to antimicrobial chemical compounds. The present of lipopolysaccharides layer and periplasmic space in Gram negative

bacteria are the reason of relative resistance of gram negative bacteria [19].

The lower MIC exhibited by *C. papaya* seed extract against the bacteria isolates was attributed to the effectiveness of the seed extracts, showing the bacteriostatic character of the extracts [20]. The facts that extract of *C. Papaya* seeds inhibited commonly encountered microorganism explain the popularity of the *C. Papaya* seed among the local folks in the extracts to some infections [21]. In addition, the ability of the extracts to inhibit the growth of test organisms explains the rationale for the use of the seed-drug as against intestinal parasites like tapeworms and roundworms and also aid in wound healing, due to their antimicrobial properties [22]. It is a common clinical experience that many microorganisms are acquiring resistance to the routine antimicrobial agents with broader therapeutic spectrum potency. Thus, there is a need to search for new antibiotics. Plants continue to prove rich sources of therapeutic agents and the results of this study reveal the potential value of *Carica Papaya* seeds and has added yet new potential antimicrobial agents [23].

Conclusion

The phytochemicals present in the crude extract of *Carica Papaya* seeds are alkaloids, flavonoids, tannins, saponins and terpenoids. The extract showed significant inhibitory effect against isolates of *E. coli* and *S. aureus* The extract was found to be more effective against *S. aureus* However, *Carica Papaya* seeds extract can be used in the treatment of diseases caused by bacteria and could be more effective against Gram positive bacteria. The phytochemicals detected in the seeds extract of *Carica Papaya* could be responsible for the observed antimicrobial activities.

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