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# ANTIBACTERIAL ACTIVITY OF *CARICA PAPAYA* LEAF EXTRACTS ON SOME BACTERIA ISOLATED FROM WOUNDS OF PATIENTS ATTENDING THE JOS UNIVERSITY TEACHING HOSPITAL (JUTH)

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## ABSTRACT

The antibacterial activity of the aqueous and methanolic leaf extracts of *Carica papaya* (pawpaw) was investigated using the agar diffusion method. The test organisms used were *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. Gentamycin at concentration of 4 mg/ml was used as a standard drug (positive control) whereas distilled water was used as the negative control. The aqueous extract did not show antibacterial activity against any of the test organisms. The methanolic extract had antibacterial activity against *Proteus mirabilis* only, at concentrations of 700 mg/ml, 350 mg/ml and 175 mg/ml. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values recorded were 87.5 mg/ml and 350 mg/ml respectively. Phytochemical screening showed the presence of alkaloids, tannins and steroids in the methanolic extract, whereas only carbohydrates and steroids were detected in the aqueous extract. Despite the insignificant activity of the extracts, these results suggest that *Carica papaya* leaves could still be used in the treatment of wound infections caused be *Proteus mirabilis*. It might be that the constituents of the leaves were greatly affected by the extraction procedures employed, hence the need for more investigations using other extraction methods.

**KEYWORDS:** Antibacterial activity, *Carica papaya*, leaf extracts, bacteria, wounds, patients.

## INTRODUCTION

*Carica papaya* Linn belongs to the family of Caricaceae, and several species of Caricaceae have been used as a remedy against a variety of diseases.[1] *Carica papaya* Linn is commonly known for its food and nutritional values throughout the world.[2] *Carica papaya* L. is commonly called Pawpaw (English), Gwanda (Hausa), Ibebe (Yoruba), or Okoegbo (Igbo).[3] It is also known as Papaya, Papayer, Pepol, Tinti, Chich put, Fan kua, Wan shou kuo, Kavunagaci, Kepaya, e.t.c.[2]

The searches for new compounds with antimicrobial from plants have been the subject for intense research in recent years.[4] This is due to the fact that plants are widely used in folk medicine to combat various diseases in human caused by pathogenic organisms. For this reason, many researchers are aiming to scientifically prove the use of plant extracts as an effective means of controlling infections and body malfunctions.[5] The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine.[2] Each Part of papaya tree possesses economic value when it is grown on a commercial scale.[6] Even though the active components are normally extracted from all parts of the plant, the concentration of these compounds vary from structure to structure. However, parts known to contain the highest concentration of the principles are preferred for the therapeutic purposes, and it can either be the leaves, stem, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits, and the seeds.[7]

Multidrug resistance among pathogens has become a global problem for the treatment and cure of bacterial infections.[8] There is need to explore new and alternative avenues for antimicrobials that are less susceptible to microbial resistance. The aim of this study is therefore, to determine the antibacterial activity of *Carica papaya* leaves extracts on some bacteria isolated from wounds of patients attending the Jos University Teaching Hospital (JUTH), Plateau State, Nigeria.

## MATERIALS AND METHODS

### Collection of *Carica papaya* Leaves

Fresh, young, disease free, and green leaves were collected from the *Carica papaya* plants, [2] at Federal College of forestry Jos, Plateau State, Nigeria. The plant

was identified and confirmed by a staff that is a horticulturalist in the institution.

**Preparation of the Leaves for Extraction**

The leaves were washed thoroughly 3-10 times in sterile distilled water as described by. [2] Then, they were air-dried under shade at room temperature for 14 days and pulverized to finely powdered form using sterilized pestle and mortar under laboratory conditions as described by.[9]

**Extraction of the Leaves and Preparation of the Extracts**

The *Carica papaya* leaves powder were extracted and prepared using the methods of. [10][11] One hundred grams (100g) of powdered leaves was extracted with 500ml each of distilled water and methanol at 100$^{o}$C for 72 hours in a Soxhlet extractor. The extracts were slowly evaporated to dryness using a rotary evaporator at 45$^{o}$C to yield 18% w/w and 20% w/w of dry weight residue of the aqueous and the methanolic extracts respectively. The extracts were dissolved each in sterile distilled water, and the solutions were further diluted with diluents to the desired concentrations of 700mg/ml, 350mg/ml, and 175 mg/ml.

**Collection and Preparation of Test Organisms**

The test organisms that were used are some bacteria isolated from wound swabs and pus of human patients attending the Jos University Teaching Hospital (JUTH). They were collected from the bacteriology laboratory of the hospital. The bacterial isolates collected were *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Each isolate after collection, was preserved by refrigeration on sterile nutrient agar slant and incubated at 37$^{o}$C for 48 hours.[12] They were then kept as stock cultures in the refrigerator set at 4$^{o}$C. Each isolate was subcultured into sterile nutrient broth and incubated at 37$^{o}$C for 24 hours after which the various desired tests were carried out.

**Phytochemical Screening of the Extracts**

They extracts were subjected to various phytochemical tests to determine the phytochemical constituents present using standard methods of. [13][14] Phytochemical screening was carried out to tests for carbohydrates, tannins, cardiac glycosides, saponins, flavonoids, alkaloids and steroids.

**Tests for Antibacterial Activity**

Agar well diffusion methods of[2][12][15] were adopted to determine or assess the antibacterial activity of the aqueous and methanolic extracts of the leaf of *Carica papaya* with some slight modifications. 1 ml of the broth culture of each of the test organism was introduced separately and thoroughly mixed with 30 ml of molten nutrient agar each in a sterile petri dish and allowed to set or solidify for at least 30 minutes then labeled with the name of the organisms for easy identification. Duplicate inoculation was done for each test organism. Five (5) wells were aseptically made using a sterile stainless 6.0 mm in diameter cork borer on each well labeled inoculated agar plate. 0.3 ml each of the different concentrations of each leaf extracts (700 mg/ml, 350 mg/ml and 175 mg/ml) were aseptically dispensed into each of the wells while the 4$^{th}$ and the 5$^{th}$ wells in each were for Gentamycin (4 mg/ml) and distilled water which served as positive and negative controls respectively. The plates were then kept at room temperature for 1 hour to facilitate adequate diffusion of the extracts, after which they were incubated at 37$^{o}$C for 24 hours. After incubation, antibacterial activities were indicated by the development of zones of growth inhibition around the wells. The diameter of the zones of inhibition around each well were measured to the nearest millimeters along two axis i.e. 90$^{o}$ to each other and the mean value of the two readings were then calculated and recorded.

**Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Extracts**

Broth dilution method was used to determine the minimum inhibitory concentration (MIC) of the extracts.[16][17] 7 sterile tests tubes labeled 1 to 7 were selected for each extract. The first tube contained 5ml of double strength nutrient broth while the remaining tubes contained 5 ml of single strength of the nutrient broth. 5 ml of 700 mg/ml concentration of each of the crude extract in each case was introduced into tube 1 and was mixed thoroughly by shaking, and 5 ml of this content was transferred into tube 2 and was also mixed thoroughly. The procedure was continued for the remaining tubes up to tube 6. 5 ml from the tube 6 was then taken and discarded with tube 7 containing no extract which served as a control. To all the test-tubes, 0.1 ml of 24 hours broth culture of the test organisms was added or inoculated and was incubated at 37$^{o}$C for 24 hours. The tubes were then observed for growth. Minimum inhibitory concentrations (MICs) were recorded as the lowest concentration of the extracts showing no visible growth in the broth after 24 hours of incubation at 37$^{o}$C.

The minimum bactericidal concentration (MBC) of the extracts was determined using the method of. [18] Tubes that showed no growth in the MIC were selected and subcultured into fresh and sterile nutrient broth which contained no extract. [16] One loopful from each of these tubes was subcultured onto the surface of sterile, dry and extracts free nutrient agar plate and was incubated for 24 hours at 37$^{o}$C. The lowest extract concentration from which the organism did not recover and grow on the agar or the lowest concentration of extracts showing no bacterial growth was noted and recorded as the minimum bactericidal concentration (MBC).

## RESULTS

### Phytochemsical Screening of the Extracts

Phytochemical screening of the methanolic extract revealed the presence of tannins, alkaloids and steroids whereas carbohydrates, cardiac glycosides, saponins and flavonoids were absent (Table 1). Also in table 1, the screening revealed the presence of only carbohydrates and steroids in the aqueous extract while other constituents tested for were absent.

**Table 1: Phytochemical Constituents of *Carica papaya* Leaf Extracts.**

| Constituents | Methanolic extract | Aqueous extract |
|---|---|---|
| Carbohydrates | - | + |
| Tannins | + | - |
| Cardiac glycosides | - | - |
| Saponins | - | - |
| Flavonoids | - | - |
| Alkaloids | + | - |
| Steroids | + | + |

+ Constituent present - Constituent absent

### Antibacterial Activity of the extracts

None of the test organisms was sensitive to any of the aqueous extract concentrations prepared (Table 2). That is, all organisms developed complete resistance to the extract. Only *Proteus mirabilis* was sensitive to the methanolic extract at concentration of 700 mg/ml, 350 mg/ml and 175 mg/ml with zones of inhibitions of 18 mm, 15 mm and 11 mm respectively (Table 3), while other organisms tested were completely resistant.

**Table 2: Antibacterial activity of the aqueous extract of *Carica papaya* leaves and means zones of inhibition (mm).**

| Test organisms | Extract concentrations | | | Gentamycin (control) |
|---|---|---|---|---|
| | 700 mg/ml | 350 mg/ml | 175 mg/ml | 4 mg/ml |
| *Escherichia coli* | - | - | - | 32 |
| *Proteus mirabilis* | - | - | - | 23 |
| *Pseudomonas aeruginosa* | - | - | - | 14 |
| *Staphylococcus aureus* | - | - | - | 21 |
| *Streptococcus pyogenes* | - | - | - | 37 |

- No zone of growth inhibition

**Table 3: Antibacterial activity of the methanolic extract of *Carica papaya* leaves and means zones of inhibition (mm).**

| Test organisms | Extract concentrations | | | Gentamycin (control) |
|---|---|---|---|---|
| | 700 mg/ml | 350 mg/ml | 175 mg/ml | 4 mg/ml |
| *Escherichia coli* | - | - | - | 32 |
| *Proteus mirabilis* | 18 | 15 | 11 | 24 |
| *Pseudomonas aeruginosa* | - | - | - | 13 |
| *Staphylococcus aureus* | - | - | - | 21 |
| *Streptococcus pyogenes* | - | - | - | 36 |

- No zone of growth inhibition

### Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Methanolic Extract of *Carica papaya* Leaves

The minimum inhibitory concentration (MIC) for the methanolic extract on *Proteus mirabilis* revealed that the organism was inhibited at concentrations of 350 mg/ml, 175 mg/ml and 87.5 mg/ml, but the lowest concentration of the extract that inhibited the growth of the organism was 87.5 mg/ml, hence the MIC (Table 4). Consequently, the minimum bactericidal concentration (MBC) for the methanolic extract on *Proteus mirabilis* revealed that the extract was bactericidal at the minimum concentration of 350 mg/ml (Table 5). This was the lowest concentration of the extract that the bacterium did not recover and grow on the sterile and extract free nutrient agar.

**Table 4: Minimum inhibitory concentration (MIC) of the methanolic extract of *Carica papaya* leaves.**

| Test organisms | Extract concentrations (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 350 | 175 | 87.5 | 43.75 | 21.88 | 10.94 |
| *Escherichia coli* | * | * | * | * | * | * |
| *Proteus mirabilis* | - | - | - | + | + | + |
| *Pseudomonas aeruginosa* | * | * | * | * | * | * |
| *Staphylococcus aureus* | * | * | * | * | * | * |
| *Streptococcus pyogenes* | * | * | * | * | * | * |

- Growth absent + Growth present * MIC not determined

**Table 5: Minimum bactericidal concentration (MBC) of the methanolic extract of *Carica papaya* leaves.**

| Test organisms | Extract concentrations (mg/ml) | | |
|---|---|---|---|
| | 350 | 175 | 87.5 |
| *Escherichia coli* | * | * | * |
| *Proteus mirabilis* | - | + | + |
| *Pseudomonas aeruginosa* | * | * | * |
| *Staphylococcus aureus* | * | * | * |
| *Streptococcus pyogenes* | * | * | * |

- Bactericidal + Growth present * MBC not determined

## DISCUSSION OF RESULTS

The result of the phytochemical screening of the leaves of *Carica papaya* showed that it contain some phytochemical constituents which exhibit good antibacterial properties on some test organisms used in this study. The phytochemical screening of the leaves of this plant showed the presence of carbohydrates, tannins, alkaloids and steroids. This finding almost corroborates with the results of[19] who detected tannin, alkaloids, reducing sugar, among others in the leaves and stem bark of *Carica papaya*.

The aqueous extract of the plant leaves did not exhibit any antibacterial activity against any of the test organisms in this study (Table 2). This result disagrees with the finding of[2] that discovered a high antibacterial activity of water extract of the leaves against most of the bacteria tested in their research. The antibacterial activity of the methanolic extract from the findings of this present study exhibited a good antibacterial activity against *Proteus mirabilis* which is a gram negative bacterium (Table 3). This result seemingly agrees with the findings of[2] who also established an antibacterial activity of the methanolic extract of the leaves of *Carica papaya* on *Proteus mirabilis*. The antibacterial activity displayed by the methanolic extract against *Proteus mirabilis* in this study as against the aqueous extract might be due to better solubility of the active constituents of the leaves in the organic solvent.

From the results of this study, the minimum inhibitory concentration (MIC) showed a concentration of 87.5 mg/ml (Table 4) in which the organic solvent extract used can inhibit the growth of the organism tested. This result is in conformity with the minimum concentration range of 25-200 mg/ml of the leaf extract of the plant established by[19] in their research.

## CONCLUSION

Despite the insignificant activity of the leaf extracts as revealed by the present study, it can be concluded that *Carica papaya* leaves could still be used in the treatment of wound infections caused by *Proteus mirabilis*, owing to the fact that the methanolic extract exhibited a significant antibacterial activity against the organism.

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