



IN-VITRO SENSITIVITY PATTERN OF SOME URINARY TRACT ISOLATES TO CARICA PAPAYA EXTRACTS

*¹Yusha'u, M., ¹Onuorah, F. C. and ²Murtala, Y.

¹Department of Biological Sciences, Bayero University, P. M. B. 3011, Kano

²Department of Science Laboratory Technology, Hussaini Adamu Federal Polytechnic, Kazaure

*Correspondence author e-mail: mryushau@gmail.com

ABSTRACT

Powdered leaves of *Carica papaya* (L.) were extracted with ethanol and partitioned in chloroform and distilled water. The extract and fractions were tested for antibacterial activity against clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas* species using disc diffusion and microbroth dilution technique. The extract and fractions were further subjected to phytochemical tests for the presence of secondary metabolites using standard procedures. Results of sensitivity test results showed that ethanol extract of the leaf was active against *E. coli* and *K. pneumoniae* (7mm each) at 1000µg/disc concentration while chloroform and water fractions of the leaf were active against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* at 1000µg/disc concentration with zone diameter of 7mm each. Both the extract and fractions were inactive against *P. aeruginosa* at all concentrations used in this study. The results of phytochemical screening indicated the presence of alkaloids, flavonoids, steroids and tannins in either ethanol extract, fraction(s) or both. This indicates that the *Carica papaya* has the potential for the production of drugs against organisms causing urinary tract infections.

Keywords: Sensitivity, Clinical isolates, Urinary tract, *Carica papaya*, Extract, Fractions

INTRODUCTION

Medicinal plants are cheap and renewable sources of pharmacologically-active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al.*, 1999).

Carica papaya is an evergreen shrub or small tree that grows best in full sun to light shade. The plant likes lots of water and fertilizer in warm weather. Flowers are similar in shape to the flowers of *Plumeria* but are much smaller, wax-like and appear on the axils of the leaves, maturing into fruit, which ripens when it feels soft and its skin has attained an amber or orange colour. It has been used locally in the treatment of urinary tract infections (Aliyu, 2006).

The milky juice of *Carica papaya* when extracted and dried, is used as chewing gum, medication for digestion problems, toothpaste and meat tenderizers. It has been used to treat digestive problems and intestinal worms as well as warts, sinusitis, eczema, cutaneous tubercles and hardness of the skin. Green fruits are used to treat high blood pressure, roundworm infection, dyspepsia, constipation, amenorrhoea, skin disease, general debility and genito-urinary disorders (Burkhill, 1985). The traditional use of *C. papaya* leaves in the treatment of various ailments including urinary tract infections informed the need for this study, which was aimed at evaluating the antibacterial activity of the leaves against some agents of urinary tract infection.

MATERIALS AND METHODS

(a) Collection and Identification of plant materials

The leaves of *Carica papaya* were handpicked at Bayero University staff quarters in Gwale Local

Government Area of Kano state, identified and authenticated by Dr. B. S. Aliyu of Biological Sciences Department, Bayero University, Kano. They were washed, air-dried and ground into fine powder using mortar and pestle in the laboratory as described by Mukhtar and Tukur (1999).

(b) Extraction

Eighty grams of the powdered plant leaves was dispensed in 800ml of ethanol, kept for two weeks with shaking at regular intervals after which the content was filtered and the filtrate was evaporated at 30°C. Four grams of the crude extract was partitioned in a mixture of 20ml chloroform and 20ml water (1:1). The mixture was shaken properly, placed in a separating funnel and allowed to separate before collection in separate beakers. Both the water and chloroform extracts were allowed to evaporate at room temperature (Fatope *et al.*, 1993).

(c) Phytochemical screening

(i) Test for alkaloids

To 0.1ml of the extract and fractions in a test tube, 2 – 3 drops of Dragendoff's reagent was added. An orange red precipitate with turbidity denoted the presence of alkaloids (Ciulci, 1994).

(ii) Test for flavonoids

To 4mg/ml of the extracts and fractions a piece of magnesium ribbon was added followed by drop-wise addition of concentrated HCl. A colour change from orange to red indicated the presence of flavones; red to crimson indicated the presence of flavonoids (Sofowora, 1993).

(iii) Test for glycosides

Ten milliliters of 50% H₂SO₄ was added to 1ml of the filtrate in separate test tubes and the mixtures heated for 15mins followed by addition of 10ml of Fehling's solution and boiled. A brick red precipitate indicated presence of glycosides (Sofowora, 1993).

(iv) Test for reducing sugars

To 1ml of extract and fractions in separate test tubes, 2.0mls of distilled water were added followed by addition of Fehling's solution (A + B) and the mixtures were warmed at 40°C. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar (Brain and Turner, 1975).

(v) Test for saponins

Half gram of the powdered leaf was dispensed in a test-tube and 5.0ml of distilled water was added and shaken vigorously. A persistent froth that lasted for about 15 minutes indicated the presence of saponins (Brain and Turner, 1975)

(vi) Test for steroids

Two milliliters of the extracts were evaporated to dryness in separate test tubes and the residues dissolved in acetic anhydride followed by addition of chloroform. Concentrated sulphuric acid was added by means of a pipette via the side of the test tubes. Formation of brown ring at the interface of the two liquids and violet colour in the supernatant layer denoted the presence of steroids (Ciulci, 1994).

(vii) Test for tannins

Two milliliters of the extract/fraction was diluted with distilled water in separate test tubes, 2 – 3 drop of 5% ferric chloride (FeCl₃) solution was added. A green – black or blue colouration indicated tannin (Ciulci, 1994).

(d) Bioassay studies

(i) Disc preparation

Sensitivity discs were punched from Whatman No. 1 filter paper, sterilized in Bijou bottles by autoclaving at 121°C for 15mins. Sensitivity discs were prepared by weighing the appropriate amount of the extract or fraction and serial doubling dilution in Dimethyl-sulfoxide (DMSO) followed by placing the improvised paper discs in the solution such that each disc absorbed 0.01ml to make the disc potency of 500µg, 1000µg, 2000µg and 4000µg ((Akinyemi *et al.*, 2005; Vallekobia *et al.*, 2001)).

(ii) Test isolates

Respiratory tract isolates were collected from the microbiology laboratory of Aminu Kano Teaching Hospital (AKTH) and maintained on nutrient agar slants in the refrigerator (4°C) prior to use.

(iii) Inoculum standardization

A loopful of the test isolate was picked using a sterile wire loop and emulsified in 3 – 4mls of sterile

physiological saline. The turbidity of the suspension was matched with that of 0.5 McFarland Standard (Cheesebrough, 2000).

(i) Sensitivity testing

Using sterile swab stick, standardized inocula of each isolate was swabbed onto the surface of Mueller Hinton Agar in separate Petri dishes. Discs of the extracts and standard antibiotic (Augmentin 30µg) were placed onto the surface of the inoculated media. The plates were inverted and allowed to stand for 30mins for the extract to diffuse into the agar after which the plates were incubated aerobically at 35°C for 18 hours. This was followed by measurement of zone of inhibition formed by the test organisms around each of the extract and standard antibiotic discs (NCCLS, 1999).

(e) Micro-broth dilution technique

(i) Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the extract and fractions were prepared by serial doubling dilution using distilled water to obtain concentrations of 4000µg/ml, 2000µg/ml and 1000µg/ml. Equal volume (2mls) of extract and Mueller – Hinton broth were mixed. Specifically 0.1ml of standardized inocula (3.3 x 10⁶ CFU/ml) was added to each of the test tubes above. The tubes were incubated aerobically at 35°C for 24 hours. Tubes containing broth and leaf extracts without inocula which served as positive control while tubes containing broth and inocula served as negative control. The tubes were observed after 24 hours of incubation to determine minimum inhibitory concentration. That is the lowest concentration that showed no evidence of growth (Akinyemi *et al.*, 2005; Vallekobia *et al.*, 2001).

Minimum Bactericidal Concentration (MBC)

Sterile Mueller-Hinton agar plates were separately inoculated with sample from each of the test tubes that showed no evidence of growth. The plates were further incubated at 35°C for 24 hours and observed. The highest dilution that yielded no bacterial growth was regarded as MBC (Akinyemi *et al.*, 2005; Vallekobia *et al.*, 2001).

RESULTS AND DISCUSSION

High yield of ethanol extract was obtained at the end of extraction, with red colour and hard texture. The high yield of ethanolic extracts in both plants may be due to the stronger extraction capacity of ethanol as indicated by Tschehe (1971).

Both chloroform and water fractions had same colour but different texture with chloroform fraction being hard and water fraction being gummy, which may be as a result of the difference in polarity of the different solvents.

Table 1: Some physical properties of *Carica papaya* Extract/frcions

| Extracts | Yield / (%) | Colour | Texture |
|------------|-------------|--------|---------|
| Ethanol | 5.0(06.25) | Red | Hard |
| Chloroform | 2.0(50.00) | Green | Hard |
| Water | 1.9(47.50) | Green | Gummy |

NB: Figures in parenthesis are percentages

The results of phytochemical screening of ethanol, chloroform and water extracts and fractions of *C. papaya* revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins (Table 2). These metabolites have been reported to possess

antimicrobial activity (Cowan, 1999). In particular the flavonoids were reported to be responsible for antimicrobial activity associated with some ethnomedicinal plants (Singh and Bhat, 2003).

Table 2: Phytochemical constituents of *Carica papaya* leaf Extract/fractions

| Extracts | Alkaloids | Flavonoids | Glycosides | Reducing sugar | Saponins | Steroids | Tannins |
|------------|-----------|------------|------------|----------------|----------|----------|---------|
| Ethanol | + | + | - | - | - | + | + |
| Chloroform | + | + | - | - | - | + | + |
| Water | - | - | - | - | + | - | + |

Key: EE = Ethanolic Extract, CF = Chloroform Extract, WF = Water Extract, + = Present, - = Absent

Table 3 and 4 next page

Sensitivity of the test isolates to *Carica papaya* leaf extracts and fractions was indicated by observation and measurement of inhibition zones formed around discs prepared from various concentrations of the extracts and fractions. Absence of turbidity in tube cultures indicates the activity of the extract or fraction using micro-broth dilution technique, the least concentration amongst the tubes without evidence of turbidity was considered the minimum inhibitory concentration (MIC). Microbroth dilution technique employed in this research is important in determining whether the extract and fractions are capable of inhibiting the growth or completely killing the test isolates.

The results of sensitivity tests using both procedures indicated that chloroform and water extracts of the leaf powder were more active than ethanol extract on the isolates tested. The activity exhibited by the extracts may be related to the presence of tannins that are well documented for

antimicrobial activity (Tschehe, 1971) in addition to alkaloids and flavonoids, which were reported to be responsible for antimicrobial properties of some ethnomedicinal plants (Singh and Bhat, 2003).

Conclusion

From the results of this work, it can be concluded that *Carica papaya* has the potential for the production of drug for the treatment of urinary tract infections.

Recommendations

- In view of the results obtained in this work, it is recommended that scientists should;
- a) Isolate and identify the active compound(s) present in the ethanol extract and fractions.
- b) Determine the toxicity level of both crude extract and the active compound(s).
- c) Screen more plants view of finding alternative treatments to microbial infections.

REFERENCES

Akiyemi, K.O., Oluwa, O.K. and Omomigbehin, E.O. (2005). Antimicrobial activity of crude extracts of three medicinal plants in south-west Nigeria folk medicine on some food borne bacterial pathogens. Department of MCB/Department of BOT Lagos State. University Pp. 1-2.

Aliyu, B. S. (2006): *Some ethnomedicinal plants of the Savannah Regions of West Africa Description and phytochemicals*. Triumph publishing company. 1: 135-152.

Basile, A., Giordano, S., Lopez-saez, J. A. and Cobianchi, R. C. (1999): Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry*. **52**:1479- 1482.

Brain, K.R. and Turner, T.D. (1975): *The practical evaluation of phytochemicals*. Wright Scientechina, Bristol: Pp.57 – 58.

Burkill, H. M.(1985): *The useful plants of West Tropical Africa*. Royal Botanical Garden Kew (7)1: 200-219.

Cheesbrough, M. (2000): *District Laboratory practice in tropical countries*. Press syndicate Publishers, University of Cambridge, Edinburgh, Cambridge United Kingdom. Pp. 194 – 201.

Ciulci, I. (1994): *Methodology for the analysis of vegetable drugs*. Chemical industries branch, Division of industrial operations. UNIDO, Romania: Pp.24 - 67.

Cowan, M. M. (1999): Plant products as antimicrobial agents. *Clinical Microbiology Review* **12(4)**: 564-582.

Fatope, A. O., Ibrahim, H. and Takeda, Y. (1993): Screening of higher plants reputed as pesticides using brine shrimp lethality bioassay. *International Journal of Pharmacognosy* **31**: 250-256.

Mukhtar, M. D. and Tukur, A. (1999): In-vitro screening for activity of *Pistia stratiotes* extracts. *NISEB Journal* **1(1)**:51 – 60.

National Committee for Clinical Laboratory Standards (1999): Performance standard for antimicrobial susceptibility testing. NCCL approved standard M100-59.

Singh B. and Bhat T. K. (2003): Potential therapeutic applications of some antinutritional plant secondary metabolites. *Journ. Agric. Food Chem.* **51**:5579-5597.

Sofowora, A. (1993): *Medicinal plants and Traditional Medicines in Africa*. Chichester John Wiley & Sons New York. Pp. 34-36.

Tschehe, R. (1971): Advances in the chemistry of antibiotic substances from higher plants: Pharmacognosy and phytochemistry. *In proceeding of 1st International Congress, Munich, 1970*: 274-289.

Vallekobia, A., Kostalova, D. and Sochorova, R. (2001): Isoquinolone alkaloid from *Mahonia aquifolium* stem bark is active against *Malassezia species*. *Folia Microbiology* **46**: 107-111.

Table 3: Inhibition Zones (mm) formed by isolates in response to *Carica papaya* leafextracts and standard Septrin disc

| Isolates | EE (µg/disc) | | | | CF (µg/disc) | | | | WF (µg/disc) | | | | Sxt (µg/disc) |
|-------------------------------|--------------|------|------|------|--------------|------|------|------|--------------|------|------|------|---------------|
| | 500 | 1000 | 2000 | 4000 | 500 | 1000 | 2000 | 4000 | 500 | 1000 | 2000 | 4000 | 30 |
| <i>Escherichia coli</i> | 0 | 7 | 8 | 9 | 0 | 7 | 8 | 9 | 0 | 7 | 8 | 9 | 10 |
| <i>Klebsiella pneumoniae</i> | 0 | 7 | 7 | 8 | 0 | 7 | 7 | 8 | 0 | 7 | 7 | 8 | 10 |
| <i>Proteus mirabilis</i> | 0 | 0 | 7 | 9 | 0 | 7 | 7 | 9 | 0 | 7 | 8 | 9 | 10 |
| <i>Pseudomonas aeruginosa</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 8 | 15 |

Key: EE = Ethanolic Extract, CF = Chloroform fraction, WF = Water fraction, Sxt = Septrin

Table 4: MIC and MBC of *Carica papaya* leaf extracts

| Isolates | Ethanol Extract (µg/ml) | | Chloroform Extract (µg/ml) | |
|----------------------------|-------------------------|-----|----------------------------|-----|
| | MIC | MBC | MIC | MBC |
| <i>E. coli</i> | 4000 | ** | 4000 | ** |
| <i>K. pneumoniae</i> | ** | ** | ** | ** |
| <i>P. mirabilis</i> | 4000 | ** | 4000 | * |
| <i>Pseudomonas species</i> | ** | ** | ** | ** |

Key: MIC – Minimum Inhibitory Concentration, MBC - Minimum Bactericidal Concentration, ** - MIC or MBC value greater than 4000µg/ml