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Phytochemical Constituents and Antimicrobial Activity of *Carica papaya* Leaf Extract on Some Clinical Isolates

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Abstract

Herbal medicine has gained increasing usage globally quite recently, as a result of increasing drug resistance to conventional drugs. This has led to an increase in the search for new and effective sources of antimicrobials. This study assessed the antimicrobial efficacy of Carica papaya extract on some clinical bacteria and fungi isolates and the phytochemical composition of the leaves. The clinical isolates used for this study were 5 bacterial isolates (Klebsiella pneumonia, Escherichia coli. Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa) and 3 fungal isolates (Aspergillus niger, Candida albicans, and Aspergillus flavus) The leaf of Carica papaya was extracted using ethanol, and water. The antimicrobial activity was determined using the agar well diffusion technique. The extracts were prepared at a concentration of 200mg/ml and were used on both the bacteria and fungi isolates. The MIC was assessed using broth serial doubling dilution at different concentrations. Both extracts are active to the isolates at varying degrees. The MIC and MMC range between 50 -200mg/ml. The phytochemical screening showed that Carica papaya leaves contain essential secondary metabolites that aid in the antimicrobial activity. This study reasonably supports the use of Carica papaya leaves in the treatment of infections as used by traditional herbal practitioners.

Keywords: *Carica papaya*; Antimicrobial; Clinical isolate; Phytochemical; MIC; MMC

Introduction

Plant extracts of various plant parts have been used as natural antimicrobials and antioxidants as a result of the presence of bioactive substances reported to be in them that confer resistance against bacteria, fungi, viruses and pests (Okunola *et al.,* 2012) There is a quest for search of natural antimicrobials for use in health care and food industry. Recently, there has been an increase in the rate of antimicrobial resistance that has made the treatment of man and animal a difficult task and costly which has led to the search for newer drugs that could be used as an alternative source of antimicrobial activity. Such natural antimicrobials can readily be available at low cost and with less toxicity (Aruljothi *et al.,* 2014). About 95% of local drug preparations globally are from plant sources (Gidey, 2001).

Carica papaya belongs to the cariacae family, popularly referred to as pawpaw in English, okwuru bekee or okwuruezi, in Igbo, Gwanda in Hausa and Ibebe in Yoruba. It is commonly known for its nutritional value. The various parts have been used in traditional medicine for the treatment of various health conditions with varying degrees of secondary metabolites present. It is effective in the treatment of malaria, indigestion bacterial infection, ulcer, inflammatory and also serves as an antioxidant (Aruljothi *et al.,* 2014). It is used in the treatment of wound infections to facilitate healing (Murthy *et al.,* 2012). The emergence of resistance strains of most clinical pathogenic organisms has imposed a serious health challenge, especially on antibiotics (Aruljothi *et al.,* 2014). The aim of this study was to evaluate the antimicrobial activity of *Carica papaya* leaf extract on some

clinical isolates as an alternative source for antimicrobial

Materials and methods

Ethical permission

Ethical permission to carry out this work was obtained from the Ethics Committee College of Medicine University of Nigeria.

Plant material collection

Uninfected, fresh, young green leaves of C*arica papaya* were collected from a family garden in the University of Nigeria Enugu Campus Senior Staff Quarters. The plant was identified and authenticated by Mr Onyeukwu (an expert Botanist) and voucher specimen UNH no 111 was deposited for future reference at Herbarium in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. The leaves were transported to the laboratory and thoroughly washed several times with clean distilled water and spread under shade at room temperature to dry. The dried leaves were ground to fine powder using clean laboratory mortar and were placed in a sterile air-tight container till ready for use.

Preparation of plant for extraction

Solvent – solvent extraction method was used to remove the extract of the leaves of *Carica papaya.* 100g of powder was extracted in 500ml of 95% Ethanol and Water. The solvent was evaporated using a rotary vacuum evaporator. The extracts were tested for sterility by culturing them on sterile Mueller Hinton Agar for 24 hours. The extracts were stored in sterile bottles at 4^{o}C in the refrigerator.

Percentage Yield Determination

The percentage yield (%, w/w) from all the dried crude ethanol and water extracts was calculated using the formula:

$$\text{Percentage (\%) yield} = \frac{\text{Weight of extract}}{\text{Weight of dried plant extract}} 100$$

Phytochemical Screening

The extracts were subjected to various phytochemical tests to determine the phytochemical constituents present in the leaves and this was done using standard methods as described by (Rahman *et al.,* 2017; Rondón *et al.,* 2018).

Isolation of Test Organism

The isolates used for this study were bacteria (Klebsiella pneumonia, Escherichia coli. Staphylococcus aureus Streptococcus pyogenes and Pseudomonas aeruginosa) and fungi (Aspergillus niger, Candida albicans, and Aspergillus flavus) obtained from pure stock culture bottles at Department of Medical Microbiology University of Nigeria. The microorganisms were isolated on Mueller-Hinton agar (MHA) and Sabouraud's dextrose agar (SDA) and were sub-cultured before use.

Media preparation

Mueller-Hinton agar (MHA) and Sabouraud's Dextrose agar were prepared and treated according to the manufacturer's specifications. About 20ml of the sterilized medium cooled to about 50^{0}C was aseptically poured into 90 mm diameter sterilized Petri dishes and allowed to set. The sterility of the prepared media was assessed by incubation of randomly selected 3 plates each at 37°C for 24hours and 48 hours for bacterial and fungal contaminants respectively.

Preparation of extract concentrations

The stock (200mg/ml) was prepared by reconstituting 4g of each of the extracts in 20ml of Dimethyl sulphuroxide (DMSO).

Determination of Antimicrobial properties of all extract

The antimicrobial properties of water and ethanol extracts of the leaves were determined by the agar-well diffusion method (Emencheta *et al.,* 2019). Sterile swab sticks were dipped into the already standardized cell suspension, the swab was pressed firmly against the inside wall of the test tube to remove excess fluid. It was evenly streaked over the entire surface of MHA and SDA agar plates by rotating the plate to ensure an even distribution of the inoculum on the agar plate and allowed to dry for 10 minutes. A Sterile 6mm cork borer was used to make two wells in the MHA and SDA. The wells were filled with 0.1ml of each extract at 200mg/ml concentrations and allowed to stand for 30

minutes at room temperature to allow the extract to diffuse into the agar. Ciprofloxacin (5 µg) and miconazole (50 µg/ml) were used to serve as the positive controls for the bacteria and fungi isolates respectively while Dimethyl Sulphuroxide served as the negative control. The cultures were incubated at 37ºC for 18 - 24 hours for the bacteria plates and 28ºC for 3 – 14 days for the fungal plates. Each extract was tested against all the bacterial and fungal isolates in three replicates against each organism. After incubation, the diameter of the zone of inhibition around each well (excluding the diameter of the well) was measured to the nearest millimeter and the mean of the three readings was then calculated.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbiocidal Concentration MMC

The minimum inhibitory concentrations were determined by adopting the technique used by Anumudu *et al. (*2019). For each of the extracts, 5 ml of Muller Hinton and Sabouraud's dextrose agar broth was placed in test tubes. One milliliter of each extract was introduced into the first tube and serial dilution of the extract was undertaken to reduce the concentration of the extract in the broth serially (200, 100, 50, 25mg, 12.5, 6.25). A standard inoculum (0.1 ml) of the test organism (adjusted to the McFarland standard) was introduced into each of the test tubes. All the tubes were incubated overnight at 37°C, while the tube for fungi were incubated for 48hours at 28 ± 2°C. The MIC is the broth containing the lowest concentration of the extract, which showed no turbidity (evidence of no growth) in the tube. The tubes without turbidity were cultured on MHA and SDA and incubated. The Minimum microbiocidal (bactericidal and fungicidal) concentration is the lowest concentration of the extract that did not show any visible growth on the media.

Statistical Analysis

The experiment results were expressed as the mean of three replicates. Data obtained were statistically analyzed using one-way Analysis of Variance (ANOVA), a tool in Statistical Packages for Social Sciences (SPSS 17.0).

Results

Table 1: Percentage yield of *Carica papaya* leaves Extract Using Ethanol And Water

Solvent Used	% Yield
Ethanol	68.50
Water	55.00

Table 2: Phytochemical content of *Carica papaya* leaf extract using Ethanol and water

Phytochemical	Flavonoid	Phenol	Saponin	Alkaloid	Tannin	Glycosides	Quinines	Steroid
Ethanol extract	+	+	+	+	+	+	+	+
Aqueous extract	+	+	+	+	+	-	-	-

Key: += Present - = Absent

Table 3: Mean Zone of inhibition of the leaves extracts of *Carica Papaya* against some clinical isolates at 200mg/ml concentration

Clinical isolates	Ethanol Extract (mm)	Aqueous Extract (mm)
Bacteria isolates		
E. Coli	33.00	27.00
S. pyogenes	27.00	27.00
P. aur eginosa	30.00	26.00
K. pneumonia	32.00	27.00
S. Aureus	33.00	29.00
Fungal isolates		
Aspergillus niger	26.00	23.00
Candida albicans	28.00	25.00
Aspergillus flavus	25.00	23.00

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal and Fungicidal Concentration (MBC and MFC) of the leaves extract of *Carica papaya* against some clinical isolates.

Clinical Isolates	Ethanol Extract (mg/ml)		Aqueous Extract (mg/ml)	
Bacteria isolates	**MIC**	**MMC**	**MIC**	**MMC**
E. coli	12.5	25	50	100
S. pyogenes	50	50	25	50
P. aureginosa	50	100	50	100
K. pneumonia	50	100	50	100
S. Aureus	25	50	50	100
Fungal isolates				
Aspergillus niger	50	100	100	200
Candida albicans	50	50	100	100
Aspergillus flavus	50	100	100	200

Discussion

The ethanol crude extract gave a higher yield of 68.5% than the aqueous extract which gave a yield of 55%. As presented in Table 1. Proper choice of solvents with different polarity during extraction plays a significant role in the extraction ability. The phytochemical content of the leaf extracts showed that both ethanol and water extracts contain (Table 2) tannin, saponin, alkaloids and flavonoids which are known to possess antimicrobial properties (Dagne *et al.,* 2021). Alkaloids are among the major phytochemicals that play a vital role in the treatment of infectious diseases (Auwa *et al:*. 2018). It possesses an anti-analgesic and an anti-bacteria and properties thus its use in the production of drugs (Okwu and Okwu, 2004). Flavonoids, a lipophilic agent, use their enzymatic activity to cause inhibition of bacteria cell membrane and cell wall formation by binding with the bacteria cell wall (Auwal *et al.,* 2018). Phenol serves as a scavenging molecule that aids in protecting the cellular environment from harm against activities of oxidative and inflammatory agents (Olcum *et al.,* 2020). Saponin produces a cytotoxic effect (Okwu and Okwu, 2004). And induce the entry of toxic material into the cell (Ajiboye and Olawoyin, 2020).

The antimicrobial activity of *Carica papaya* ethanol and aqueous extract at 200mg concentration tested against some clinical isolates (bacteria and fungi) is presented in Table 3. The extracts were active against the isolates with different zones of inhibition recorded. The highest zone of inhibition recorded was ethanol extract against *Staphylococcus aureus and E. coli 33mm while the least was 30.00mm for P. aeruginosa for bacteria isolates. The highest zone of inhibition for ethanol extract against fungal isolates was 29.00mm for Candida albicans and the least A. flavus was 25.00mm. The aqueous extract for bacteria isolates* highest zone of inhibitions recorded was for *Staphylococcus aureus 29.00mm and the least for P. aeruginosa 26mm. The* highest zone of inhibition recorded for the fungal isolates was 25.00mm against *Candida albicans while the least was 23.00mm for A. flavus and A. niger. The extracts were found to be effective against all the clinical isolates tested and this is in line with the report of Yahaya et al., (2017)* whose report shows that ethanol extract of *Carica papaya* leaves against *Staphylococcus aureus., Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi,* and report of Aruljothi *et al.* (2014) with Qumqum (2018) who reported effective inhibition of ethanol extracts on *Aspergillus niger* and *Aspergillus flavus.* The report of this study is also in line with that of Chávez-Quintal *et al*. (2011) who reported antifungal activity of ethanol extract of *Carica papaya* leaves.

The MIC and MMC tested at different concentrations (6.25, 12.5, 25, 50 100 and 200 mg/ml) of each extract show that ethanol extract has a higher bactericidal and fungicidal activity at lower concentrations than the water extract. The MIC ranges between 25 – 100mg/ml of both extracts on all the clinical isolates while the MMC ranges between 50 -200mg/ml. The MIC result showed that increasing concentration has an increasing efficiency in inhibiting the organisms tested. Since the MIC values indicated the definite nature of the antimicrobial activities of this plant, the inhibition zone values, only, indicated the extent of effectiveness of the extract with increasing concentration.

Conclusion

This study showed that ethanol extracts had higher antimicrobial activity than the aqueous extracts on the clinical isolates tested. The better efficacy of the ethanol extract as against the aqueous extract may be because different solvents have different polarities, hence different degrees of solubility for the various phytochemicals that are responsible for the antimicrobial activity of plant extracts.

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