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Original Research Article

Current Assessment of the Antimicrobial Activities of *Carica Papaya* extracts on selected Bacterial Pathogens isolated from Urogenital Specimens in Calabar, Nigeria

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ABSTRACT

Urinary tract infections (UTIs) have posed numerous challenges to human health and account for a significant part of the workload in clinical microbiology laboratories. One global challenge with UTIs is the increasing resistance rate to available antimicrobials. This study assessed the antimicrobial activity of *Carica papaya* stem, root, and leaf on Escherichia coli and *Staphylococcus aureus* isolates from genitourinary tract specimens. Clinical isolates of *S. aureus* and E. coli were obtained and confirmed microbiologically. Plant extracts were prepared and diluted into 500mg/ml,250mg/ml,125mg/ml, and 62.5mg/ml concentrations, respectively. The agar well diffusion method was used to determine the antimicrobial activity. This study observed promising activities with papaya roots and stems. The ethanol extract of the stem showed maximum zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* at 500mg/ml with mean diameter of 17.6±1.0mm and 14.1±1.4mm, while the lowest activity was reported with the 62.5mg/ml concentration. The aqueous extract also showed the highest activity with increasing concentration. Both ethanol and aqueous root extracts showed the highest activity at 500mg/ml concentration against *S aureus* and E coli isolates, with 30.5±0.6mm and 21.5±1.4mm mean diameters for ethanol and 23.2±1.6mm and 8.40±1.58mm for aqueous extracts, respectively. However, the activity of both ethanol and aqueous extract of papaya leaf were lower than the activities of papaya root and stem, respectively. The findings in this study show that extracts of pawpaw stem, root, and leaf have different levels of antibacterial activity against the urinary tract pathogens tested with increasing concentrations.

Keywords: Antimicrobial activity, *Carica Papaya*, *Staphylococcus aureus*, *Escherichia coli*,

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Introduction

Infectious diseases caused by bacterial pathogens continue to pose significant threats to global public health, particularly in developing regions like Nigeria.[1] The rise of antibiotic resistance and limited access to effective antibiotics has further compounded the challenges in managing bacterial infections.[2] As a result, there is a growing interest in exploring alternative antimicrobial agents derived from natural sources, such as medicinal plants, to combat bacterial pathogens. [3]

Carica papaya, commonly known as papaya, is a tropical fruit that has been traditionally used for its medicinal properties in various cultures around the world.[4] The plant possesses a rich repository of bioactive compounds, including alkaloids, flavonoids, tannins, and phenolic compounds, which have shown promising antimicrobial activities against various bacterial strains.[5]

The urogenital tract is particularly susceptible to bacterial infections due to its proximity to the external environment and its unique physiological characteristics.[6] Bacterial pathogens commonly associated with urogenital infections include Escherichia coli, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus agalactiae*.[7]

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These organisms can cause a wide range of infections, such as urinary tract infections (UTIs) and sexually transmitted infections (STIs), leading to significant morbidity and economic burden in affected individuals.[8]

The city of Calabar, located in Nigeria, experiences a high incidence of urogenital infections, imposing a considerable burden on the local healthcare system.[9] Addressing this public health concern requires innovative and sustainable approaches, and exploring the antimicrobial potential of *Carica papaya* extracts represents a promising avenue for potential therapeutic interventions.[10]

However, due to the increasing search for plants-based therapeutic agents for human bacterial infections, this current study was designed to assess and evaluate the antibacterial activities of aqueous and ethanol extracts of papaya root, stem, and leaf on selected bacterial pathogens isolated from genitourinary tract specimens in Calabar, Nigeria.

Materials and Methods

The leaves, stem (bark) and root of authenticated *Moringa oleifera* tree were collected in September 2022 and deposited in the herbarium with voucher number UHAE 2022012. The leaves were air dried while the stem and root were sun dried. They were then ground and a part of each were extracted with 70% methanol of analytical grade. The extract and residue were then dried. The dried extract, dried residue and unextracted sample (ground but not extracted) were analysed using a GCMS. Hexane and acetone (twice the amount of sample) were added to the sample. This was ultrasonicated at 27^0C for 20 minutes. They were then filtered and concentrated for the GC-MS analysis.

Phytochemical levels were determined by operating MSD scan mode. An Agilent 7820A gas chromatograph which is coupled to 5975C inert mass spectrometer that has triple axis detector with electron-impact source (Agilent Technologies) was used. 5% Phenyl Methyl Siloxane was used to coat HP-5 capillary column for the stationary phase. The carrier gas Helium was at an initial pressure of 1.4902 psi, a constant

flow (1.4871 mL/min) and average velocity of 44.22 cm/sec. The injection mode was splitless at temperature of 300^0C for 1µL of samples. The mass spectrometer was used in electron-impact ionization mode at 70 eV, with the ion source set to 230 °C, the quadrupole at 150 °C, and the transfer line at 280 °C. The different peaks detected were matched with National Institute of Standards and Technology's library (NIST14.LIB). The library matches with not less than 80% quality or the first three in the case of more than three matches with more than 80% quality were considered.[18–20]

Study area
The study was carried out in the Medical Microbiology and Parasitology Laboratory of the University of Calabar Teaching Hospital, Calabar, Nigeria. The identification of the plant was done by a plant taxonomist in the Department of Plant Science and Biotechnology while the phytochemical screening was conducted at the Department of Biochemistry both in the University of Cross River State, Nigeria.

Sampling technique
The purposive sampling technique also known as judgmental, selective or subjective sampling was employed to collect a total of ten (10) bacterial isolates, each of Escherichia coli, and *Staphylococcus aureus*, respectively. All test organisms were confirmed to be isolates from genitourinary tract specimens through the Laboratory Bench Registers of the UCTH Laboratory, respectively.

Study design
An experimental study design was used. Ten (10) bacterial isolates each of *E. coli* and *Staphylococcus aureus* were used for the assessment of the antimicrobial activities of the papaya plant. Papaya extracts were prepared in different strengths (concentrations) of 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml, respectively. Each isolate was subjected to different concentrations of both aqueous and ethanol extracts of *Carica papaya* root, leaf, and stem. The susceptibility pattern of isolates to each plant extract concentration was assessed and their various zones of inhibition were compared.

Preparation of plant extracts
The papaya plant was harvested and taken to a plant taxonomist for identification at the Department of Plant Science and Biotechnology, University of Cross River State, Calabar in January 2023 with voucher number: HoUCR 008. Various parts of the plant such as root, stem, and leaf were collected and prepared into aqueous and ethanol extracts using cold maceration and Soxhlet extraction methods, respectively.

Harvesting of plants
Disease-free, fresh, young, and green papaya leaves, stems, and roots were collected from the papaya plants. The plant's parts were chopped into smaller particles, washed thoroughly in sterile distilled water air-dried at room temperature for days, and made into finely powdered form using a mortar and pestle.

Drying and extraction proper
Extracts from papaya were prepared according to the procedures described by Alabi et al. [11] The aqueous extract was prepared by suspending grams of each powdered plant part in distilled water, at a proportion of 1 in 4 parts of powder to distilled water. This mixture was allowed for 48 hrs. The solution was filtered through a Whatman filter paper. The filtrates were concentrated while the extracting solvent (water) was allowed to evaporate at 75ºC in a water bath. The ethanol extract was prepared by suspending grams of powdered plant parts in absolute ethanol contained in 500-milliliter capacity Soxhlet equipment and heated for complete extraction of the organic components of the plant.

Phytochemical Screening
Phytochemical screening of the different extracts was carried out to check for the presence of tannins, saponins, alkaloids, flavonoids, glycosides, steroids, and anthraquinones as indicated by the formation of precipitates and color changes.[12-15]

Test for Tannins: To 2ml of extracts in a test tube, 10ml of distilled water was added and stirred, then heated in a water bath for 30 minutes. Finally, 10% FeCl3 was added. Blue-black or blue-green precipitate indicated the presence of tannins.

Alkaloids: To 2ml of extracts in a test tube, I added 5ml of 1% aqueous HCL, then heat in a water bath. A few drops of Mayer's reagent were then added. The presence of a green color or white precipitate indicates the presence of alkaloids.

Flavonoids (Alkaline Reagent Test): To 2ml of extract, 2ml of 2% NaOH was added. The presence of yellow color which becomes colorless upon the addition of 2 drops of dilute acid indicates the presence of flavonoids.

Polyphenols (Potassium ferrocyanide test): There was an addition of 5ml of distilled water to 2ml of extract and heat for 30 minutes in a water bath. Then 1 ml of 1% Fecl3 followed by 1% potassium ferrocyanide solution was added. The formation of green-blue coloration indicates the presence of polyphenols.

Test for Saponins: To 2ml of extract, 10ml of distilled water was and heated in a water bath, allowed to cool, and agitated vigorously. Stable foam indicates the presence of saponins.

Test for Cardiac Glycosides: 2ml of extract was added to 2ml H2SO4 which immediately formed a lower layer. A reddish-brown coloration at the inter-phase indicates the presence of glycosides.
Phenols: To 1ml of extract, I added 2ml of distilled water followed by 10% FeCl3. The formation of blue or green color indicates the presence of phenols.

Terpenoids: To 2ml of chloroform, 5ml of the extract was added and heated in a water bath. Then boil with 3ml H2SO4 (concentrated). A grey color indicates the presence of terpenoids.

Test for Anthraquinones: Five grams (5g) of powdered sample of plant material was added to 10ml benzene, filtered, and ammonia solution was added. A pink, red, or violet coloration in the ammoniacal phase indicated the presence of anthraquinones.

Test for Steroid: To about 2.0 ml of extract, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. A red color produced in the chloroform layers shows the presence of steroids.

Quinones: To 1ml of extract, 1ml of conc. H2SO4 was added. The formation of red color indicates the presence of quinones.

Coumarines: To 1ml of extract, a few drops of 10% NaOH were added. The formation of yellow color indicates the presence of coumarines.

Phlobatanins: To 2ml of extract, a few drops of 2% HCL were added and the appearance of red precipitates indicates the presence of phlobatanins.

Concentration of Extracts
Serial dilutions of the extracts were made to obtain four concentrations of each plant extract in the order of 1000mg/ml (stock), 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml solutions, respectively. A stock

solution of each extract was made in a concentration of 1000mg/ml by dissolving 1g of the crude extracts in 1 ml of sterile distilled water. A 500mg/ml concentration was later prepared by pipetting 1 ml of stock into 1 ml of distilled water. One milliliter of tube No. 2 containing 500mg/ml extract was transferred into another 1ml of distilled water in tube 3 to make a concentration of 250mg/ml solutions. The 125mg/ml concentration was made by pipetting 1 ml of the 250mg/ml solution into another test tube containing 1 ml of sterile distilled water. Finally, the 62.5mg/ml concentration was prepared by transferring 1 ml of 125mg/ml solution into a fresh tube containing 1 ml of sterile distilled water.

Collection, Confirmation, and Standardization of Inoculum
Test organisms (*E. coli* and *Staphylococcus aureus*) isolated from genitor-urinary tract specimens from the University of Calabar Teaching Hospital Microbiology Laboratory were collected and confirmed via culture and biochemical testing. These isolates were then prepared to obtain an inoculum size of 0.5 McFarland standard.

Confirmation of test organisms
Following the procurement of the isolates, the organisms were confirmed by sub-culturing on Cysteine lactose electrolytes deficient (CLED) and incubated at 370C for 18 to 24 hours. Isolates were then confirmed using colonial, gram, and biochemical characteristics. The following biochemical tests were carried out for confirmation of isolates: Coagulase, catalase, indole, motility, and oxidase. Confirmed isolates were used for antimicrobial assessment of plant extracts.

Standardization of Inoculum
About 0.1ml of 1% Barium chloride was added to 9.9ml of 1% sulphuric acid which was later reconstituted into 10ml of sterile distilled water to make 0.5ml McFarland standard solution. The broth culture of the test organism was then compared in turbidity with the turbidity of the prepared 0.5% McFarland. A loopful of the standardized culture was used for the antibacterial assay. [12]

Antimicrobial testing
The different strengths of the crude extracts were tested against the bacterial isolates using the agar well diffusion technique on Muller-Hinton agar plates. Following the preparation of the Muller-Hinton medium, a sterile cotton swab stick was used to make a bacterial lawn on the medium, and four wells of about 6mm were made on the solidified and inoculated Muller-Hinton agar plates using agar borer.

Agar well diffusion method
The agar well diffusion method was adopted to assess the antibacterial activity of papaya extracts against test pathogens as described by Srinivasan et al.[16] Following preparation of the sensitivity medium (Muller-Hinton agar) on the petri-dish and inoculation of test organisms on the plates, the four agar wells were filled with about 0.1ml of the different concentrations of the extracts using a micropipette. This setup was incubated at 370c for 18-24hrs and observed for antibacterial activities and measurement of zones of inhibition.

Measurement of antibacterial activities
After incubation for 24 hrs, the antibacterial efficiency of the extracts was determined by measuring the zones of inhibition formed around the well and discs. The zones of inhibitions from both well and disc diffusion methods were recorded for the different extracts and the conventional antibiotics.

Determination of Minimum Inhibitory Concentration (MIC)
The estimation of MIC of plant extract was carried out using the method described by Ajiboye et al and Kowalska-Krochmal et al in 2020 and 2021, respectively.[12, 17] Different concentrations ranging from 62.5-500 mg/ml of the extracts was prepared and introduced into each test tube containing 9ml of the Muller-Hinton broth. About 1ml of the 18-hour standardized organism was also introduced into test tubes containing sensitivity broth and extract. A control test tube was also set up. All the test tubes were incubated for 24 hours at 37oc. The lowest concentration of the extract that did not permit any visible growth in the broth was taken as the minimum inhibitory concentration.

Determination of Minimum Bactericidal Concentration (MBC)
The MBC of the plant extracts was determined by the method of Rodríguez-Melcón in 2021.[18] About 1 ml of broth taken from the tubes with no visible growth in the MIC assay was sub-cultured on freshly prepared Muller-Hinton agar plates and incubated at 37oc for 48 hours. The MBC was taken as the concentration of the extract that did not show any growth on fresh sensitivity plates.

Data analysis
Data obtained from the antimicrobial activities of the plant extracts on test organisms were analyzed descriptively using Statistical Package for Social Science (SPSS) and presented in Tables as percentages as well as mean ± standard error of the mean (SEM).

Results and Discussion

Antibacterial activity of commercially prepared antibiotic discs on S. aureus and E. coli.
The increasing quest for other sources of antibiotics is a global call, especially among the pharmaceutical industries and research institutions due to the high rate of resistance by pathogenic organisms to the available antimicrobials.[19] This study evaluated the antibacterial activity of five (5) commercially prepared antibiotics as well as ethanolic and aqueous stem, root and leaf extracts of Carica papaya.
Table 1 below shows the antibacterial activity of commercially prepared antibiotic discs on the test organisms. All test isolates were relatively sensitive to the commercially prepared antibiotics used. However, Ceftriaxone (CRO) recorded the highest activity with a mean zone of 25.0±1.44, followed by 23.10±1.31 with Meropenem (MEM) and 21.80±1.08 with Ceftazidime (CAZ), while gentamicin (CN) showed the lowest activity with 18.50±0.96 mean zone diameters on S. aureus isolates, respectively. For the E. coli isolates tested, the highest activity was also recorded with (CRO) 23.7±1.14, followed by 22.40±0.75 (CAZ) and 22.0±1.08 (MEM) while the lowest activity (20.20±1.22) was also reported with gentamicin, respectively. Following the Clinical and Laboratory Standard Institute (CLSI) guidelines, all isolates were sensitive to the tested commercially prepared antibiotics.

Table 1: Antimicrobial effect of commercially prepared antibiotic on bacterial isolates

	Mean ±SEM diameter of Zones of Inhibition (mm)	
Antibiotic/Conc.	*S. aureus*	*E. coli*
(mg/ml)	(N=10)	(N=10)
CN10ug	18.5±0.96	20.2±1.22
MEM10ug	23.1±1.31	22±1.08
CIP5ug	21.3±1.04	21.3±1.71
CAZ30ug	21.8±1.08	22.4±0.75
CRO30ug	25±1.44	23.7±1.14

Values are mean of ten isolates ±SEM of zone of inhibition of the drugs against isolates tested.

Antibacterial Activities of Ethanol and Aqueous Extracts of Carica papaya Stem on S. aureus and E. coli isolates.
Table 2 shows the antibacterial activities of the different concentrations of the ethanol and aqueous extracts of *Carica papaya* stem on *S. aureus* and *E. coli* isolates. In this result, the dilution of the ethanol extract with the highest antibacterial activities was 500mg/ml concentration with a mean zone of (17.6±0.99) and (14.1±1.43) for *S. aureus* and *E. coli*, respectively. This was followed by 250mg with mean zones of

13.8±1.04 for *S. aureus* and 10.0±1.57 for *E. coli* isolates, respectively. The lowest activities were recorded with the 62.5mg/ml extract concentration with mean zone diameters of 4.90±1.15 for *S. aureus* and 3.20±1.40 for *E. coli* isolates, respectively.

Like the ethanol stem extract, the 500mg/ml concentration of the aqueous stem extract showed the highest activity on both bacterial genera with a mean zone diameter of 8.30±1.19 for *S. aureus* and 7.60±1.47 *for E.* coli isolates tested (table 2). This was followed by 250mg/ml concentration with 5.00±1.27 and 4.60±1.37 mean zone diameters for *S. aureus* and *E. coli* isolates, respectively. However, in a similar pattern with the ethanol concentrations, the 62.5mg/ml aqueous stem extract concentration had the lowest antibacterial activity with 0.90±0.60 mean zone diameter for *S. aureus* and 1.00±1.68 for *E. coli* isolates, respectively.

Antibacterial Activities of Ethanol and Aqueous Extracts of Carica papaya Root on S. aureus and E. coli isolates

Table 3 and Figures 1 – 4 show the antibacterial activity of the ethanol and aqueous extracts of *Carica papaya* roots on *S. aureus* and *E. coli* isolates. On both organisms, the ethanol extract with the lowest dilution (500mg/ml) had the highest antibacterial activities with 30.50±0.56 mean zone diameter for *S. aureus* isolates and 21.50±1.38 for *E. coli* isolates, respectively (table 3 and figure 1 & 2). This was followed by 250mg/ml concentration with mean zone diameters of 27.10±0.87 and 16.10±1.40 mean diameters *for S. aureus* and *E. coli* isolates, respectively. The least inhibition activities were reported with the 62.5mg/ml ethanol extract concentration with mean zone diameters of 12.40±1.59 for *S. aureus* and 4.20±1.30 for *E. coli* isolates, respectively.

Also, the activity of the aqueous root extract on both pathogens (*Staphylococcus aureus* and *Escherichia coli*) in this current study was concentration-dependent (table 3 and Figures 3 & 4). On these *S. aureus* and *E. coli* isolates, the 500mg/ml concentration recorded the highest activities against the isolates with mean diameters of 23.20±1.59 for *S. aureus* and 8.40±1.58 for the *E. coli* isolates tested. This was followed by 250mg/ml concentration with 18.90±1.52 and 4.90±1.44 mean zone diameters for *S. aureus* and *E. coli* isolates, respectively. However, the 62.5mg/ml concentration was less active against the isolates tested with mean zone diameters of 8.4±1.22mm and 0.8±0.44mm for *S. aureus* and *E. coli*, respectively.

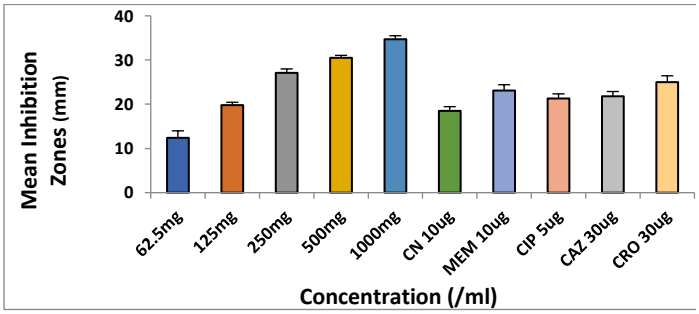

Figure 1: Antibacterial activity of Ethanol extracts of *Carica papaya* root on *S. aureus*

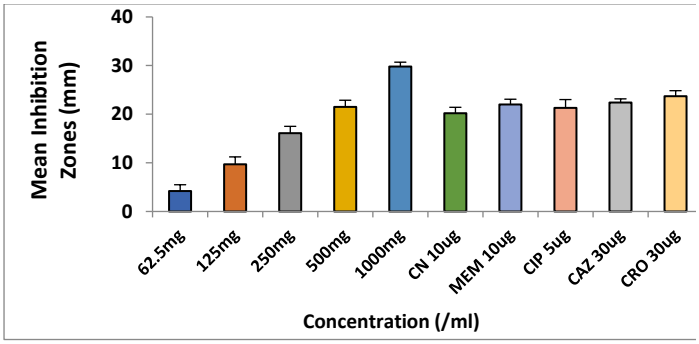

Figure 2: Antibacterial activity of Ethanol extracts of *Carica papaya* root on *E. coli*

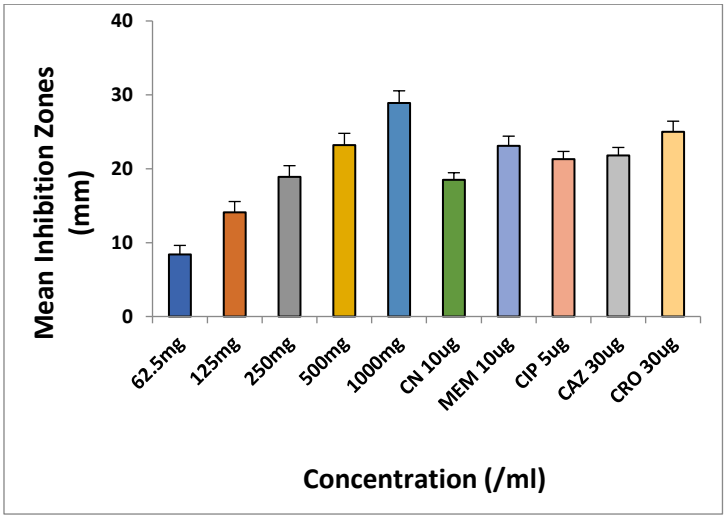

Figure 3: Antibacterial activity of aqueous extracts of *Carica papaya* root on *S. aureus*

Antibacterial Activities of Ethanol and Aqueous Extracts of Carica papaya Leaf on S. aureus and E. coli isolates.

Table 4 shows the antibacterial activity of the ethanol and aqueous extracts of *C. papaya* leaf on *S. aureus* and *E. coli* isolates. Compared to the effect of the pawpaw stem and root in this study, the activity of *C. papaya* leaf recorded in this study was lower. However, the highest concentration of the ethanol extract (500mg/ml) showed the highest activity with 8.90±1.09mm and 6.80±1.21mm for *S. aureus* and E. coli isolates. This is followed by 250mg/ml with 5.70±1.20 and4.00±1.26 for *S. aureus* and *E. coli*, respectively. The least activity was however reported with the 62.5mg/ml concentration with 1.50±0.86 and 1.00±0.68 for *S. aureus* and *E. coli*.

The activity of aqueous extract of *Carica papaya* leaf on *S. aureus* and *E. coli* was also highest with 500mg/ml concentration with of 3.10±0.72 and 2.7±0.60 mean diameters for *S. aureus* and *E. coli* (table 4). However, the lowest dilution with visible antibacterial activity on the *S. aureus* isolates was 250mg/ml with a mean of 1.30±0.47, while the least concentration with visible inhibitory activity on the *E. coli* isolates was 125mg/ml concentration with a mean of 0.30±0.30.

Both ethanol and aqueous extracts of *Carica papaya* stem, root, and leave exert inhibitory effects of varying extents against *Staphylococcus aureus* and *Escherichia coli* isolates from genito-urinary tract specimens. However, the aqueous exhibited lower antibacterial activity compared to its ethanol counterpart. All extracts (ethanol and aqueous) of the papaya parts used for this study exhibited an increasing zone of inhibition on *Staphylococcus aureus* and *Escherichia coli* according to their increasing concentrations. This study has found a higher inhibitory action with the various papaya parts against *Staphylococcus aureus* and *Escherichia coli* only with the ethanol extracts than the aqueous extracts. This however implies that the solvent for extraction is a major factor in the dissolution and solubility of the bioactive, organic ingredients present in the plant and will determine the antimicrobial potency of organic extracts. This is in tandem with the report of Ajiboye and Olawoyin in 2020 who reported that the solvent for extraction influences the antibacterial ability of plant extracts on test organisms. [12] This study has also revealed a higher antibacterial activity with the papaya root (Table 2), followed by the stem, while the lowest activity was reported with the leaf extracts, respectively. The results of the antibacterial activities of the papaya parts observed in their ethanol and aqueous extracts may support an assertion that pawpaw root and stem may have better antimicrobial effects than the leaf since their activities (root and stem) were higher than the activities of the papaya leaf.

The inhibitory activities of the papaya root extract were greater in activity compared to the activities of the commercially prepared antibiotic discs. However, the activities of the standard antibiotics disc in this study were higher than those of the papaya stem and leaf,

suggesting that papaya root has better antimicrobial action than its stem and leaf. The findings in this study have shown that the papaya plant has promising abilities to serve as an alternative sources of antimicrobials.

Minimum inhibitory concentration (MIC) of Carica papaya Extracts (mg/ml) on S. aureus and E. coli isolates.

Table 5 shows the minimum inhibitory concentrations of both aqueous and ethanol extracts of papaya stem, root, and leaf on *S. aureus* and *E. coli* isolates. In this study, the mean MIC of the aqueous leaf extract on *S. aureus* was higher (900±100) compared to its ethanol group (550±81.65) and the MIC of the aqueous leaves on the *E. coli* isolates was also higher compared to the ethanol counterpart (800±133.33 vs 750±105.41).

The minimum inhibitory concentrations of the stem extracts were also higher with the aqueous groups than their ethanol counterparts on both *S. aureus* and *E. coli* isolates,450±33.33 vs 375±41.67 and 550±81.65 vs 400±40.82, respectively.

On the MIC of the root extract on *S. aureus* and *E. coli*, the aqueous extract recorded a higher mean MIC on both organisms compared to their ethanol counterparts, 475±25.00 vs 200±20.41 for *S. aureus* and 600±66.07 vs 337.50±45.83 for the *E. coli* isolates, respectively. The ethanol extracts of all papaya parts used in this study (root, stem, and leaf) had lower MIC values on the test isolates compared to their aqueous counterparts (Table 5). This report is in line with other reports where the antibacterial activity of acetone extracts of *Carica papaya* parts as well as extracts from other organic solvents were more active than their aqueous counterparts when used against bacterial isolates. [20, 21] The minimum inhibitory concentration (MIC) is a key factor that determines that an agent has an antimicrobial potential. In this study, the MIC values of the ethanol extracts were found to be much lower than their aqueous counterpart. These findings are in agreement with the findings of Ajiboye and Olawoyin in 2020, who reported a lower MIC value with acetone extract of pawpaw than its aqueous group. [12] However, the MIC concentration of papaya extracts in this study ranged between 200-900mg/ml for ethanol and aqueous extracts respectively.

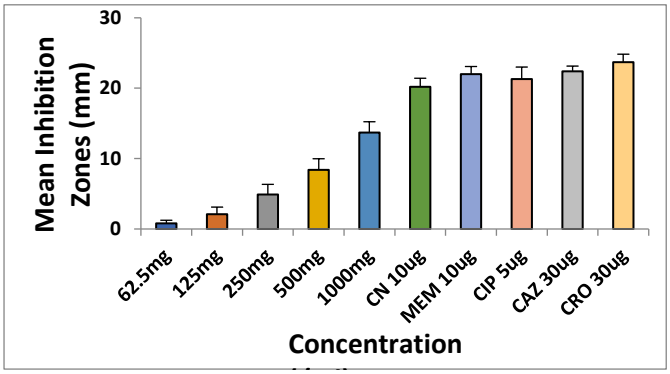

Figure 4: Antibacterial activity of aqueous extracts of *Carica papaya* root on *E. coli*

Minimum Bactericidal concentrations (MICs) of Carica papaya extracts in (mg/ml) on S. aureus and E. coli isolates

The minimum bactericidal concentrations of both aqueous and ethanol extracts of the different papaya plant parts on *S. aureus* and *E. coli* isolates are given in Table 6 below.

In this study, the aqueous extracts of the papaya leaves recorded 0.00 MBCs for both *S. aureus* and *E. coli*. Whereas, the MBCs for the ethanol groups of the leaves were 700±133.33 for *S. aureus* and 300±133.33 for *E. coli* isolates, respectively.

The MBC study of the stem extracts showed a higher mean MBC of 900±66.67 with the aqueous group compared to the ethanol group (750±83.33) against the *S. aureus* isolates but a lower MBC on the *E. coli* isolates with the aqueous group compared to their ethanol counterpart (700±133.33 vs 800±81.65).

The root extracts however, had higher MBCs with the aqueous groups than their ethanol groups for both *S. aureus* and *E. coli* isolates (1000±0.0 vs 575±75.0) and (800±133.33 vs 700±81.65), respectively.

The minimum bactericidal concentration of the different papaya parts and their extracting solvents ranges between 300-1000mg/ml for ethanol and aqueous extracts. However, the aqueous extract of the leaf did not indicate any MBC value. This may imply that the aqueous extract of papaya leaf may not give a bactericidal effect if used against *Staphylococcus aureus* and *Escherichia coli* pathogens.

Table 7 represents the qualitative analysis of different phytochemicals detected in the plant extracts. Alkaloids, polyphenols, flavonoids, cardiac glycosides, quinine, cumarine, and phlobatanin were present in all the plant extracts. However, terpenoid was not detected in any of the extracts, and tannin was found in the leaf extracts (ethanolic and aqueous) only. Other than the ethanolic extract of the root, saponin was detected in all extracts with little amount detected in the ethanol leaves, aqueous leaves, and aqueous stem extracts, respectively.

According to Popoola et al, in 2007, plant chemicals like alkaloids, tannins, saponins, glycosides, oleic acid, and stearic acids are naturally present in plants and have been suggested to possess antimicrobial effects. [22] The qualitative and quantitative studies of these metabolites in plants are important considerations when sourcing for plant-based antimicrobials. Tannin has been found to form irreversible complexes with proline-rich protein which results in the inhibition of cell protein synthesis.[23] The tannin reacts with protein to provide the tanning effect which is important for the treatment of ulcerated tissues. [24] Therefore, the presence of these phytochemicals in a considerable and variable amount in the different plant parts (Table 7) may be responsible for their varying antibacterial activities observed in this study. The occurrence of some bioactive compounds in both aqueous and ethanol extracts and the absence of anthraquinones in both papaya leaves, stem and root used for this study is in agreement with the work of Ajiboye and Olawoyin in 2020.[12] However, this contradicts the report of Omidiwura (2017) that detected the presence of anthraquinones in *Carica papaya* leaf.[25] Perhaps, this could be due to differences in soil composition and location where these plants were harvested or probably, genetic variations in the papaya plants used for these studies.

Finally, the observations in this study may support the use of *Carica papaya* stem, root, or leaf in treating illnesses resulting from *Staphylococcus aureus* and *Escherichia coli*.

Conclusion

The findings in this study have confirmed that Carica papaya stems, roots, and leaves have the following plant chemicals: glycosides, saponin, tannins, steroids, alkaloids, etc, and are responsible for their antibacterial actions. The ethanol and aqueous extracts of the papaya root, stem, and leaf have antimicrobial activities according to their increasing extract concentrations against S. aureus and E. coli isolates tested. These findings however justify and support the traditional application of these plant parts for therapeutic reasons. The demonstration of antibacterial activity against S. aureus and E. coli isolates by papaya root, stem, and leaf is an indication that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of urinary tract, gastrointestinal tract, and wound infections as well as other ailments associated with these test organisms.

Conflict of Interest

The authors declare that there was no conflict of interest.

Authors' Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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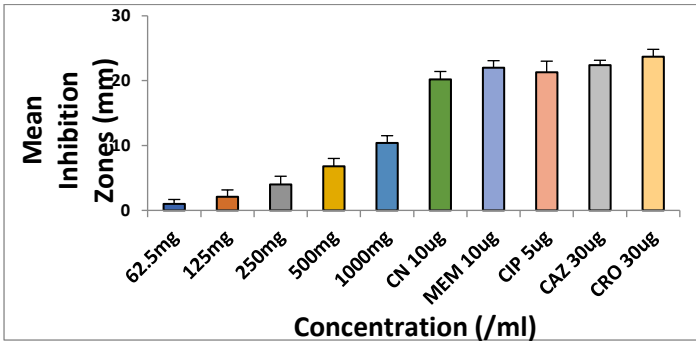

Figure 6: Antibacterial activity of Ethanol extracts of *Carica papaya* Leaf on *E. coli*

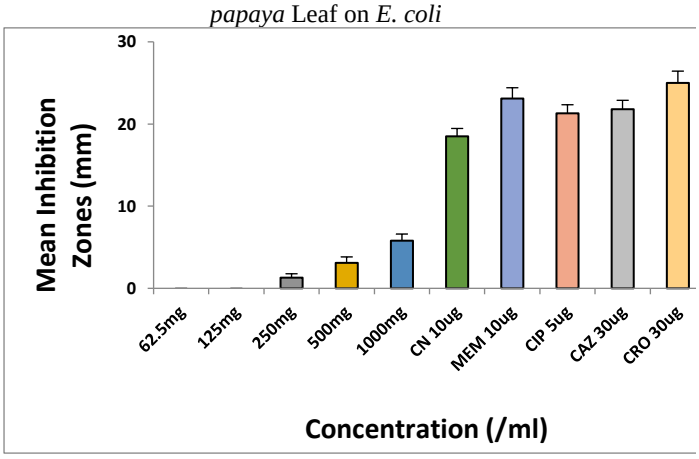

Figure 7: Antibacterial activity of aqueous extracts of *Carica papaya* Leaf on *S. aureus*

Table 2: Antimicrobial effect of ethanol and aqueous extract of *Carica papaya* stem on bacterial isolates

Concentration of Extract (mg/ml)	Mean ±SEM diameter of Zones of Inhibition (mm)			
	Ethanol Extract		Aqueous Extract	
	S. aureus (N=10)	*E. coli* (N=10)	*S. aureus* (N=10)	*E. coli* (N=10)
62.5	4.9±1.15	3.2±1.40	0.9±0.60	1±1.68
125	9.5±1.36	6.2±1.67	2.5±1.03	2.1±1.05
250	13.8±1.04	10±1.57	5±1.27	4.6±1.37
500	17.6±0.99	14.1±1.43	8.3±1.19	7.6±1.47

Note: Values are the mean of ten isolates ±SEM of the zone of inhibition of ethanol extract of *Carica papaya* against test isolates.

Table 3: Antimicrobial effect of ethanol and aqueous extract of *Carica papaya* root on bacterial isolates

Concentration of Extract	Mean ±SEM diameter of Zones of Inhibition (mm)			
	Ethanol Extract		Aqueous Extract	
(mg/ml)	*S. aureus* (N=10)	*E. coli* (N=10)	*S. aureus* (N=10)	*E. coli* (N=10)
62.5	12.4±1.59	4.2±1.30	8.4±1.22	0.8±0.44
125	19.8±0.65	9.7±1.52	14.1±1.46	2.1±1.02
250	27.1±0.87	16.1±1.40	18.9±1.52	4.9±1.44
500	30.5±0.56	21.5±1.38	23.2±1.59	8.4±1.58

Note: Values are the mean of ten isolates ±SEM of zone of inhibition of ethanol extract of *Carica papaya* root against test isolates

Table 4: Antimicrobial effect of ethanol and aqueous extract of *Carica papaya* leaf on bacterial isolates

Concentration of Extract (mg/ml)	Mean ±SEM diameter of Zones of Inhibition (mm)			
	Ethanol Extract		Aqueous Extract	
	S. aureus (N=10)	*E. coli* (N=10)	*S. aureus* (N=10)	*E. coli* (N=10)
62.5	1.5±0.86	1±0.68	00±00	00±00
125	3.5±1.11	2.1±1.05	00±00	0.3±0.3
250	5.7±1.20	4±1.26	1.3±0.47	0.9±0.53
500	8.9±1.09	6.8±1.21	3.1±0.72	2.7±0.60

Note: Values are the mean of ten isolates ±SEM of the zone of inhibition of ethanol extract of *Carica papaya* leaf against test isolates.

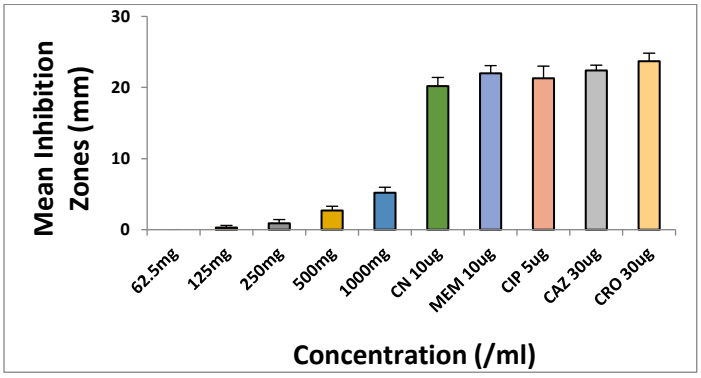

Figure 8: Antibacterial activity of aqueous extracts of *Carica papaya* Leaf on *E. coli*

Table 5: Minimum inhibitory concentration of *Carica papaya* Extracts (mg/ml)

	Leaf		Stem		Root	
Type of isolates	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
***S. aureus* (N=10)**	900±100	550±81.65	450±33.33	375±41.67	475±25	200±20.41
***E. coli* (N=10)**	800±133.33	750±105.41	550±81.65	400±40.82	600±66.67	337.5±45.83

Table 3.6: Minimum Bactericidal concentration of *Carica papaya* Extracts (mg/ml)

	Leaves		Stem		Root	
Type of isolates	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
S. aureus **(N=10)**	00±00	700±133.33	900±66.67	750±83.33	1000±0	575±75
E. coli **(N=10)**	00±00	300±133.33	700±133.33	800±81.65	800±133.33	700±81.65

Table 2.7: Qualitative Phytochemical Screening of *Carica papaya* Extract

Phytochemicals	E. Leaf	E. Root	E. stem	Aq. Leaf	Aq. Root	Aq. Stem
Alkaloid	+	+	+	+	+	+
Tannin	+	ND	ND	+	ND	ND
Saponin	+	ND	+	+	+	+
Polyphenol	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+
Terpenoid	ND	ND	ND	ND	ND	ND
Glycoside	+	+	+	+	+	+
Phenol	+	ND	ND	+	ND	ND
Quinone	+	+	+	+	+	+
Cumarine	+	+	+	+	+	+
Steroids	ND	+	+	+	ND	+
Phlobatanin	+	+	+	+	+	+

\+ Present
ND Not detected

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