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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL EFFECTS OF *CARICA PAPAYA* LEAF EXTRACTS ON URINARY TRACT INFECTIONS.

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ABSTRACT

The herbal medicine is getting more attention due to its efficacy, cheap and avalabilty. This study was carried out to evaluate the phytochemical and antimicrobial activities of *Carica papaya* leaf extracts on three urinary tract infection (UTI) pathogens: *Escherichia coli, Staphilococus aureus* and *Candida albicans* in comparison with corresponding starndard microbial strains as controls. Phytochemical analysis of the leaf extracts revealed presence of bioactive compounds including tannins, alkaloids, flavonoids, saponins, glycosides, steroids, and phenolics. Minimum inhibitory concentration (MIC), Minimum Bacteriostatic Concentration and Minimum fungustatic Concentration (MBC and MFC) of the extracts were determined. MIC, MBC and MFC values of the extracts against tested uropathogens ranged from 62.5 to 125 mg/ml including the fractionated extracts. The aqueous extracts showed weak antimicrobial activity with 3.5±0.5mm as the highest zone of inhibition at a concentration of 500mg/ml and 1.4±0.0mm as the lowest zone of inhibition at a concentration of 31.25mg/ml while the ethanolic extracts exhibited strong activity against *E.coli, S. aureus* and *C. albicans* with maximum zones of inhibitions of 23.5±1.5, 23.6±0.6 and 18.25±1.3mm at a concentration of 500mg/ml showing a dose dependednt inhibition. 16 fractions were eluted from *C.papaya* leaf extract. Fractions 5-15 were mainly saponins and phenol for *C.papaya* leaf extract and these fractions have the highest antimicrobial activity against the 3 uropathogens. Ethanolic extacts of *Carica papaya* contain good antimicrobial activity in-vitro and could be encouraged for use in the treatment of patients suffering from urinary tract infections, especially by organisms resistant to currently available antibiotics.

Keywords: *Carica papaya*, Urinary Tract Infection, Antimicrobial, Drug Resistant Bacteria, Herbs, Alternative Medicine

1.1 INTRODUCTION

Medicinal plant can be defined as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products (WHO, 2003). *Carica papaya* has been mentioned among the herbs, vegetables, plants and fruits readily available, cheap and effective as medicinal plants are of great importance to the health of individuals and communities especially in the management of difficult to treat diseases, re-emerging infections and antimicrobial resistant organisms (Obeta etal., 2021). Interestingly, the use of alternative, herbal or complementary medicine has been encouraged and advocated (Obeta etal., 2020; Ikeagwulonu etal., 2020).

Urinary tract infections (UTIs) has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and they are the second most common cause of bacteremia in hospitalized patients (Samm and Norby, 2001). Untreated UTI may lead to several serious complications like intrauterine growth restriction, preeclampsia and preterm deliveries and caesarean deliveries (Mazor *et al*., 2009). It is also noted that the asymptomatic bacteriuria can lead to cystitis and pyelonephritis were it can lead to acute respiratory distress, transient renal failure, sepsis and shock during pregnancy (Gilstrap *et al*., 2011). Unfortunately, UTI is re-emerges after treatment among many surferers and there is a need to find an alternative to ensure total treatment.

The use of of medicinal plants as traditional medicines is well known in rural areas of many developing countries (Sandhu *and* Henrich, 2005). *Carica papaya* leaves are held out like natural gift of life, whose every part serves as potent medicine for variety of human ailments, among these indigenes of the tropics particularly in Nigeria.

Majority of the rural populance in developing nations who are at high risk of infection with pathogens due to unsanitary conditions cannot afford the high cost of chemotherapeutic agents and so are in urgent need of affordable treatment. Thus, the utilization of medicinal plants in traditional and complementary medicine is very important to people in developing countries particularly the rural population. Poverty is so high in Nigeria, and less privileged people cannot afford hospital bill and high cost of antibiotics, screening of medicinal plants is targeted at getting some cheaper, safer, and more effective antimicrobial agents that will be used to cure UTIs. Medicinal plants are cheap and renewable sources of pharmacologically-active substances and are known to produce certain chemicals that are naturally toxic to microorganisms but non-toxic to humans (Basile *et al*., 2000).

Dhanalakshmi *et al*. (2013) investigated the antibacterial activity of some medicinal plants used against UTI causing pathogens including *Carica papaya* and recommended it as antibiotics. Other researchers added voice to the use of *Carica papaya* as antibiotics especially in the aspect of resistant and urinary tract infections (Hajera *et al.* 2013; Priyadharsini *et al*. 2014). Ankur *et al*., (2011) demonstrated the antibacterial activitity

of *Carica papaya* in multi drug resistant *Pseudomonas aeruginosa* causing urinary tract infections. (Bazzaz etal., 2021; Floress-Mireles etal., 2015).

In addition to its medicinal value, *Carica papaya* (Pawpaw) is regarded as an excellent source of vitamin C (ascorbic acid); a good source of carotene, riboflavin and a fair source of iron, calcium, thiamin, niacin, pantothenic acid, vitamin B-6 and vitamin K (Obeta *et al.*, 2021; Adetuyi *et al*., 2008).

The aim of this research was to evaluate the efficacy for the use of *Carica papaya* leaf extracts in the treatment of urinary tract infections (UTIs) with specific objectives to carry out the *Carica papaya* leaves extraction , determine the *in vitro* antimicrobial activities of the extracts on *E .coli, S. aureus and C. albicans* through antimicrobial susceptibility assay, determine the Minimum inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of the plant extracts and possibly identify the bioactive compounds in the extracts in the separated fraction in order to ascertain which fraction has the highest antimicrobial activity against the UTI isolates using thin layer chromatography and column chromatography.

MATERIALS AND METHODS

Collection of *Carica papaya*

The *Carica papaya* leaves as shown in Figure 1 were obtained from Attahiru Drive, Rantiya of Jos South LGA, Jos Plateau State and used for this study.

Identification of *Carica papaya*

The *Carica papaya* plant leaves was identified in Habarium of Plant Science Department in University of Jos with voucher number UJH16000271.

Preparation of plant *Carica papaya* for extraction

The *Carica papaya* leaves were separated from the stalk and washed under running tap water. It was allowed to dry and then shredded. The leaves were cut into thiny pieces to aid drying due to its large size, they were air dried for 3 days as a result of rainy season, then later dried for 5 minutes in hot air oven at 40°C to constant weight. The dried *Carica papaya* leaves were made into fine powder in Chemistry Laboratory University of Jos using mechanical blender according to Nweze *et al.*, (2004). The blended *Carica papaya* leaves powder were sieved, labeled, weighed into sterile dry containers and stored at room temperature for use.

Solvents used for extraction

The two solvents *Carica papaya* leaves extraction were:

(i) Water,

(ii) Alcohol.

Figure 1: ***Carica papaya*** **leaf**

Aqueous Extraction

Aqueous extraction was done using sterile distilled water as a solvent by cold maceration method (*Ncube et al.,* 2008). Initially 300g of air dried powder of *Carica papaya* leaves were seperately mixed well in 600 ml of distilled water in a sterile flask. The solutions were kept at room temperature for 3 days swirled to ensure effective mixing and stoppered to avoid loss of volatile liquid at ambient temperature (28°C). It was then filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15minutes. The supernatant was again filtered using Whattmans Filter No. 1 under strict aseptic conditions. The extract was evaporated to dryness by steaming in a hot water bath at 40°C until the water evaporated (*Ncube et al.,* 2008).

Ethanolic Extraction

The process used for the ethanolic extractions was the method described by Harbone (1994).

Percentage yield of extracts

The percentage yield was obtained using this formula:

$$\frac{W2 - W1}{W0} \times 100$$

Where:

W2 is the weight of the extract and the container,

W1 is the weight of the container alone and

W0 is the weight of the initial dried sample.

Phytochemical Analysis of Extract

Phytochemical analysis of each extracts was carried out by using the description of Kolkate *et al.*, (2003). By this analysis, the presence of several phytochemicals like phenols, alkaloids, flavonoids, tannins, saponins, steroids and glycosides was tested. The following tests were carried

out to determine the phytochemical identity of *Carica papaya* extract:

Detection of alkaloids:

About 0.5g of each extract was dissolved in dilute hydrochloric acid and filtered.

The filterate were treated with Mayer's reagent (potassium mercuric iodide) brown creamy precipitate was observed within 5 seconds indicating the presence of alkaloids (Evans, 2009).

Detection of glycosides:

About 0.5g of each extract was hydrolysed with 2ml of dil. Hcl, and then subjected to test for glycosides. Legal's Test was carried out by using 0.5g of *Carica papaya* extracts and treated with sodium nitropruside in pyridine and sodium hydroxide for 30 seconds. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins:

Foam Test method was used. About 0.5 gm of each extract was shaken with 5ml of water for 30 seconds. Persistent of foam produced for ten minutes was taken as the presence of saponins (Evans, 2009).

Detection of phytosterols:

Salkowski's Test was conducted on *Carica papaya* powder. About 0.5g of the extract was treated with chloroform and filtered. The filtrates were treated with few drops of Conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour was taken as the presence of triterpenes.

Detection of phenols:

Ferric chloride test on *Carica papaya* was carried out. About 0.5ml of each extract was dissolved in 10ml of water and filtered. Four drops of ferric chloride was added to the filterate. Formation of bluish black colour was taken within 5seconds was taken as the presence of phenols.

Detection of tannins:

This was done using Gelatin Test technique. To 0.5g of the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins (Evans, 2009).

Detection of flavonoids:

Lead acetate Test on *Carica papaya* powder. About 0.5g of the extract was treated with 3 drops of lead acetate solution for 30 seconds. Formation of yellow colour precipitate indicates the presence of flavonoids (Evans, 2009).

Detection of diterpenes:

Copper acetate Test on *Carica papaya* extracted powder was performed. About 0.5g of the extracted powder was dissolved in water and treated with 3-4 drops of 1% copper acetate solution for 30 seconds. Formation of emerald green colour indicates the presence of diterpenes (Roopashree *et al*., 2008)

Detection of carbohydrates:

About 0.5g of each extract was dissolved seperately in 5 ml of sterile distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Using Benedict'sTest, filtrate was treated with 2 drops of Benedict's reagent and heated gently for 15 seconds. Orange-red precipitate indicates the presence of reducing sugars.

Preparation of the Thin-Layer Chromatography Plates

Eight silica gel TLC plates of size 16x5cm were cut into rectangle glass pieces using a diamond tipped glass cutter and following a template. Before scoring the glass, a ruler and a pencil was used to lightly mark baselines on the silica side of the plate. Once the entire plate was

scored, the glass was then cut into individual pieces.

250g of silica gel (60F254 Merck) was used for this research. The plates were prepared by mixing the adsorbent (4.0g silica gel (ground) with inert binder 1.0g $CaSO_4^{2-}$• H_2O, pH of 6.8. This mixture was spread as thick slurry on an unreactive, greese free carrier sheet of glass. The thickness of the absorbent layer was 0.25 mm. The resultant plates were dried and activated by heating in an oven for thirty minutes at 110 °C. Precoated plates and standards were used to compare the coated ones. Plate marking was made with soft pencil. Glass capillary tubes were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 2 tracks for aqueous and ethanol extracts, respectively. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plates, hexane, ethyl acetate and water (4:2:1) (solvent mixture known as the mobile phase) was drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation was achieved. After the run, plates were dried and sprayed, freshly prepared iodine reagents was used to detect the bands on the TLC plates. The plates were equally viewed under UV light to visualize the spots.

Choice of Solvent System (hexane, ethyl acetate and water (4:2:1)

Solvent system was selected according to polarities of the two plants extracts starting from non- polar to polar. Different compounds travel different distances up the plate depending on the solvent system. First of all, hexane, ethyl acetate (3:1) were used for non-polar solvents most polar compounds did not move, while non-polar compounds traveled some distance up the plate. Also hexane, chloroform and water (4:2:1), chloroform, methanol and water (7:4:1), ethyl acetate chloroform, chloroform and water (15:2:2:1) were tried for good separation of both polar and non polar mixtures of the plant extracts. At the end of the many trials, hexane, ethyl acetate and water (4:2:1) was the best solvent system that moved most components of the mixtures in the plant extracts off the baseline.

Preparation of stock extracts to be spotted.

10ul of 10mg/ml stock solutions were prepared and dissolved in their respective solvents. Ethanol extract was dissolved in ethanol while aqueous extract was dissolved in water.

Development of Thin layer chromatography (TLC)

i. TLC chamber was filled with 10 ml of the solvent system.
ii. A piece of cut filter paper was placed in the chamber to saturate the chamber.
iii. The extracts were spotted on the baseline of the TLC plate using sterile capillary tubes then allowed to dry.
iv. The TLC was runned by letting the solvent go about 90% of the way up the plate.
v. The plate was removed from the chamber and the solvent front was marked immediately with a pencil.
vi. The solvent was allowed to dry off of the plate.

Detection of Bands

The TLC was visualized 526nm using non-destructive technique(s) like iodine crystals, and UV lamp. The plates were placed under the UV lamp and UV active spots were circled with pencil.

The TLC plate was labeled and their retardation factor (Rf) was calculated.

Rf = Distance traveled by the solute / Distance traveled by the solvent front TLC plate

Procedure for Column Chromatography

i. The column was washed w ith sterile distilled water and sterilized in hot air oven and placed in a retort stand with clamp in a vertical position.

ii. A plug of sterile glass wool was soaked in the solvent system, pushed down to cover the bottom of the column.

iii. 50g of silica gel powder (mesh 200-600 dimension) was weighed, and 100ml of solvent was used to make slurry which was poured gently into the column.

iv. The stop cock was opened to allow some solvent to drain out.

v. In order to develop the chromatogram, 1g of each extracts to be separated was dissolved in 5ml of ethanol and was placed on the top of packed stationary phase with second cotton in between.

vi. The mobile phase (100ml) was poured into the column over the sample. A collecting beaker was placed at the bottom of column near the end to collect the eluent.

vii. The mobile phase percolates through the entire stationary phase and reaches the bottom of the column from there; it was eluted out and gets collected in the bijou bottles placed below.

viii. Care was taken to make sure that the layer of the absorbent was always covered by the solvent to avoid cracks development in the column.

ix. This elution was drop by drop and the process took hours, although the length of elution depends on the sample size, length of the column, mobile phase used and the packing material used.

x. The different fractions were collected in their specific sterile containers (Bijou bottles) and allowed to evaporate.

xi. Rf of each bands for the two extracts were calculated.

xii. Phytochemical tests were run on each of the fractions to determine what fraction is which phytochemical. Fractions were identified by spraying:-Dragendorffs reagent, Ammonia solution, Conc. Hcl and $FeCl_3$ as a reagent and colour change were recorded. Those of the same phytochemical were pooled together.

Antimicrobial activitiy of the fractions

About 20ul of each fraction was used to test each isolate to determine which of the fractions has the highest antimicrobial activity. Five holes were dug on the surface of each dried sterile agar plates, using 5mm cork borer, each plate containing the 5 fractions of each plant and this was done for the 3 different organisms used. The zones of inhibitions were measured and recorded.

Collection of Standard Strain

Standard strains were collected from Microbiology laboratory in central diagnosis of National Veternary Research Institute (NVRI) Vom.The control microbial species: -*E.coli* (ATCC 11775), *S. aureus* (ATCC 6538) and *Candida albicans* (ATCC 10231) were used. The *S. aureus* strains was cultured on Blood agar plates at 37°C and maintained on nutrient agar slants while the *Candida albicans* was grown on Sabroud dextrose agar (SDA) plate and maintained on SDA slant at 4^0C.

Collection and culturing of urine samples

Urine samples of patients suffering from urinary tract infection that was ready for analysis were collected from the Laboratory of Bingham University

Teaching Hospital Jos Plateau State, from which the three UTI pathogens (*E.coli, Staph aureus, and C.albicans*) from where isolated. Macconkey and blood agar plates were used to culture *E.coli* and *S. aureus* while Sabroud dextrose agar was used for *Candida albicans.* All these media were prepared according to manufacturer's instruction.

The agar plates were brought to room temperature and incoculated with 2 loopful (1ul calibrated loop) of uncentrifuged urine sample. The plates were incubated aerobically for 24h at 37^0C. The resultant colonies were subjected to biochemical tests for identification.

Identification of organisms.

Colonies from the cultured plates were selected and characterized on the basis of morphological, cultural, physiological and biochemical characteristics. Biochemical identification was performed by Gram staining reaction, indole test, catalase production, Coagulase test, and *Candida albicans* was identified by germ tube test.

Standardization of Test Organisms

Standardization of test organisms was done in Microbiology laboratory in University of Jos. The suspension was incubated at 37^oC for 24hours and the size was adjusted by diluting each of them with sterile broth to the 0.5 MacFarland standard turbidity, approximately 1.5×10^6 CFU/ml.

Antimicrobial susceptibility testing

The antimicrobial activities of the plant extracts were tested on the test isolates using the agar-well diffusion method. Mueller-Hinton agar plates were used for determining the antibacterial activity. 10 grams of each extract was measured into a sterile test tube and 10 ml of 20% dimethyl sulfoxide (DMSO) was added to dissolve it.This gave 500 mg/ml concentration of the extract and this was diluted in two-folds to obtain four different dilutions of the extract: 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml, in addition to the 500 mg/ml concentration.

Mueller-Hinton agar plates were lawn with overnight broth culture of microbial suspension (equivalent to 0.5 McFarland standards) with the help of sterile swabs. Wells of 5mm diameter were made in each plate using a sterile borer. Plant extract (20μl) was poured in the wells using micropipette. Sterile distilled water was used as negative control, whereas, antimicrobial agents Nitrofurantoin, Streptomycin and Fluconazole were used as positive control for *E.coli, S. aureus* and *Candida albicans,* respectively while sterile distilled water was used as negative control. The plates were kept upright for 1-2hours until the solution diffused into the medium and then incubated aerobically at 37°C for 24 hours. Later, the zone of inhibitions were measured and recorded. All the experiments were performed in duplicate.

Assessment of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using standard two-fold dilution broth methodology (NCCLS, USA, 1998). A stock solution of each active extract was serially diluted in six tubes with Mueller Hinton broth to obtain a concentration of 500mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml, concentration. A standardized inoculum for each bacterial strain was prepared so as to give an inoculum size of approximately 5 x 105CFU/ml in each

tube. The tubes were then kept at 37°C for an overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain. Positive and negative cultures were also prepared. The lowest concentration of the extract that did not show any visible bacterial growth was considered MIC of the extract for that microbial species. All the MIC experiments were performed in duplicate and the mean diameter of zones of inhibition were recorded.

Determination of Minimum Bacteriocidal Concentration and Minimum Fungucidal Concentration (MBC and MFC)

A portion of liquid (5ul) from *Carica papaya* tube that exhibited no growth was taken and then incubated at 37°C for 24h. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC and MFC. Positive and negative cultures were also prepared. Freshly prepared sterile nutrient agar was poured separately into sterile petridishes and allowed to set firmly, a loopful of mixture in the tube not showing growth was transferred to the agar in the plates and incubated for 24 hr at 37°Cand observed for growth. The plate not showing growth was recorded as MBC and MFC against the test organisms, respectively.

Statistical Analysis

One way analysis of variance (ANOVA) was used to statistically analyze all the data obtained from antimicrobial susceptibility testing using Statistical Package for Social Sciences (SPSS) Software version 22.

RESULTS

The Yields of *Carica papaya* Leaf Extracts

300g of *Carica papaya* dry leaves were used for extraction with ethanol and water as solvents. Aqueous extract of *Carica papaya* yeilded 168.6g, ethanol extract yielded 134.1g.

Phytochemical Analysis

Phytochemical tests were carried out on *Carica papaya* leaf extracts. The phytochemical analysis of *Carica papaya* extracts revealed that these extracts contain Tannins, Alkaloids, Flavonoids, Saponins, Glycosides, Steroids, Phenolics, and carbohydrates (**Table** 1).

The results were interpreted according to intensity of the colour observed and was qualified with Highly present (+++), Moderately present (++), Slightly present (+) and Absent (-). Most of the (+++) were seen in ethanolic extracts while aqueous extracts have (++) for example, flavonoids and phenols were more in ethanolic extracts than in aqueous extracts.

Table 1: Results of the Phytochemical Composition of Aqueous and Ethanolic Extracts of *Carica Papaya* Leaves

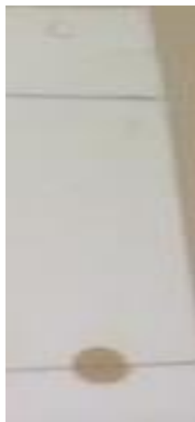
Extraction Solvents	AQUEOUS	ETHANOL
Phytochemicals		
Tannins	+	+
Alkaloids	+	+++
Flavonoids	+	+++
Saponins	+++	+++
Glycosides	-	+
Steroids	-	+
Phenolics	-	+++
Carbohydrates	-	-

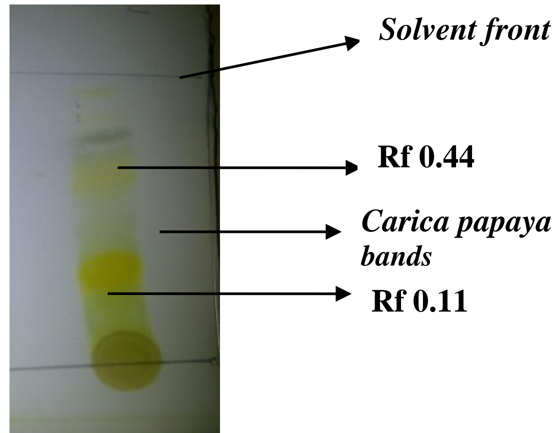
Thin Layer Chromatography

Thin-layer chromatographic study revealed that the ethanolic extracts of *Carica papaya* have some bioactive compounds in them while aqueous extract showed no band after many traits of different solvent system as seen in Figure 2. After observing the plates under UV light (254nm), *Carica papaya* leaf extract has many bands with solvent front of 9.0cm and Rf of between 0.11-0.44.

The chromatogram of *Carica papaya* leaf extracts showed varying coloures of bands which represent each bioactive compound. The Yellow and the Greenish colours predominates the other colours on the plate. It was equally noticed that hexane, ethyl acetate and water (4:2:1) was the best solvent system that moved most of the mixtures in the plant extracts off the baseline.

The ethanolic extract gave better separation than the aqueous extracts. The bands were seen clearly in Figure 2b for the ethanolic extract while none was seen in Figure 2a for the aqueous extract.





a. Aqueous extract **b. Ethanolic extract**

Figure 2: TLC bands of Aqueous and Ethanolic *Carica papaya* leaf extracts

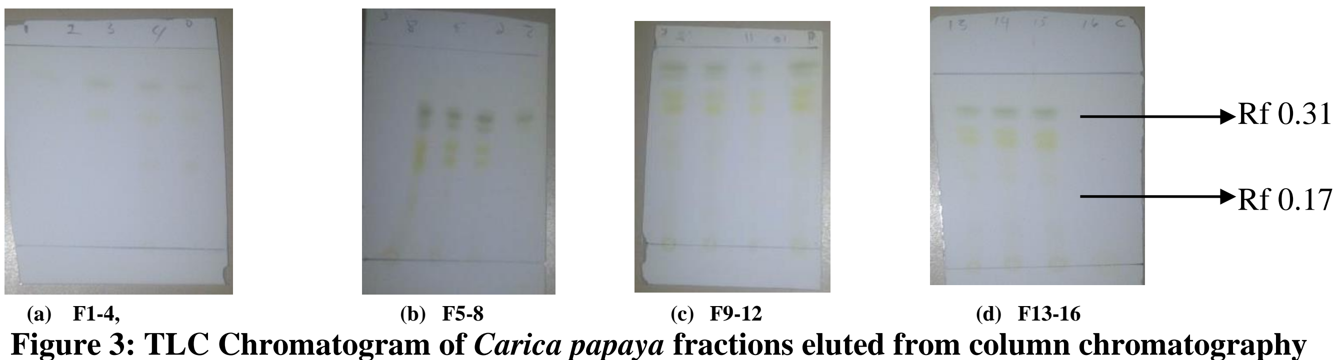
Fractions of *Carica Papaya* Leaf Extract Eluted from Column Chromatography

During column chromatography, 16 fractions were eluted from *Carica papaya* leaf extract. Initial solvent system (Hexane, Ethyl acetate and Water 4:2:1) was used until the higher Rf compounds have come off the column. The distance moved by solvent front was 7.0cm with Rf values of between 0.14- 0.43 as shown in Table 2 and figure 3.

Table 2 shows the fractions of *C.papaya* leaf extract. F1 has Rf values of 0.06 with orange colour and was identified with Dragendorffs reagent and the resultant colour was still orange it was classified as alkaloid. F2-3 has Rf values of 0.13 with light yellow colour and was identified with ammonia solution reagent and the resultant colour was yellow, it was classified as flavonoid.The treatment was also given to F4 which was light green and and was also identified as flavonoid. Saponin was detected in F5-15 which was dark grren in colour, after treament with concentrated hydrogen chloride, it turned dark brown. F16 was light grey and was treated with intense ferric chloride, red colour was observed and was classified as phenol (Figure 4).

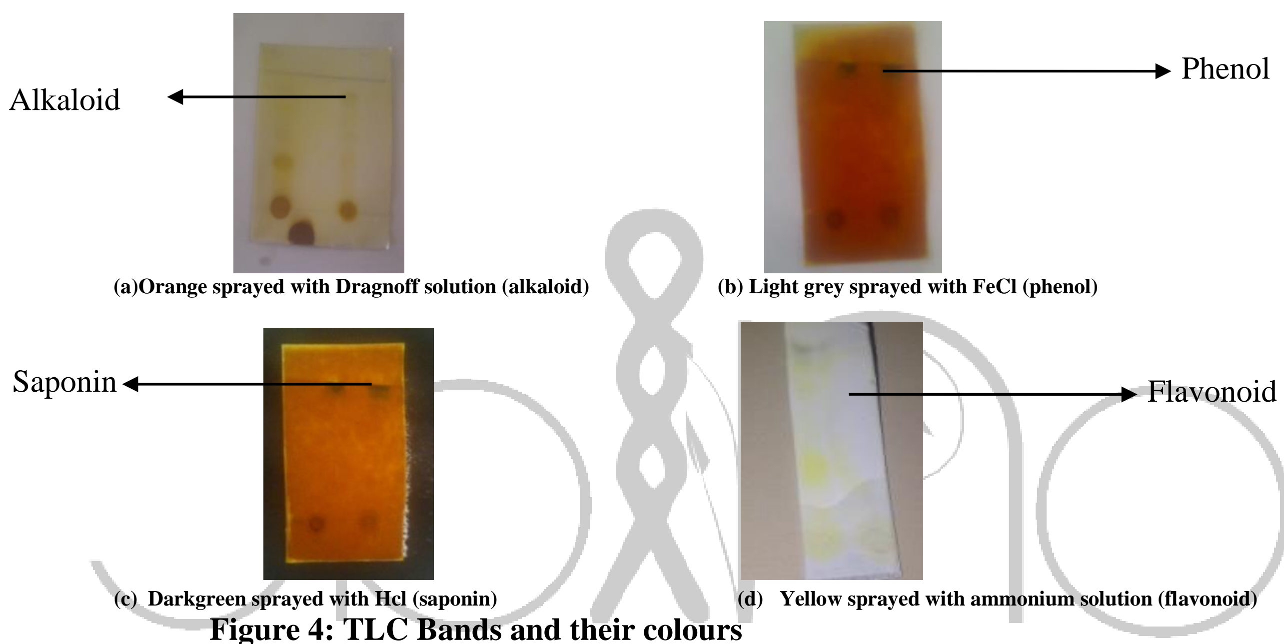
Table 2: Elution Profile of Column Chromatography of Ethanolic Leave Extract of *C. papaya* in Mobile Phase of N-Hexane: Ethyl Acetate: Water (4:2:1)

No. of Bands	Fraction	Rf values	Colour of bands	Spraying Reagent	Colour of bands appeared	Phytochemicals Detected
1	F1	0.06	Orange	Dragendorffs reagent	Orange	alkaloid
2	F2-3	0.13	Light Yellow	Ammonia solution	Yellow	Flavonoid
3	F4	0.25	Light green	Ammonia solution	Dark grey	Flavonoid
4	F5-15	0.38	Dark green	Conc. Hcl	Dark brown	Saponins
5	F16	0.44	Light grey	$FeCl_3$	Red	Phenol



(a) F1-4, (b) F5-8 (c) F9-12 (d) F13-16

Figure 3: TLC Chromatogram of *Carica papaya* fractions eluted from column chromatography



(a)Orange sprayed with Dragnoff solution (alkaloid) (b) Light grey sprayed with FeCl (phenol)

(c) Darkgreen sprayed with Hcl (saponin) (d) Yellow sprayed with ammonium solution (flavonoid)

Figure 4: TLC Bands and their colours

Suceptiblity of the Different Fractions against Test Organisms

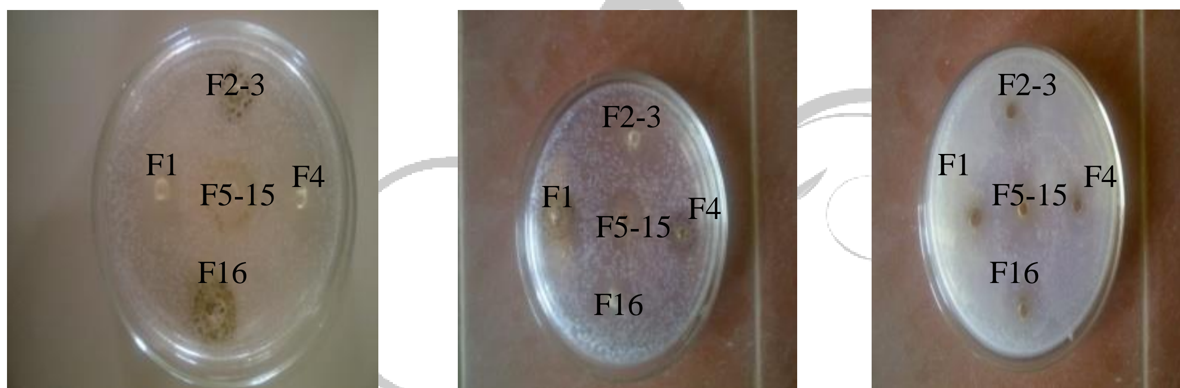
The antimicrobial activity of the extracts were located at Rf values of *Carica papaya* leaf extract is found between 0.14- 0.43. The study discovered that fractions 5-15 which was mainly saponins and phenol for *Carica papaya* leaf extract has the highest antimicrobial activity against the 3 uropathogens. The highest inhibition zones of the two plants were 20.00mm.

Carica papaya, F1 did not have activity against *E.coli*, *S.aureus* and *C.albicans*, F 2-3 also did not have activity against *E.coli*, *S.aureus* and *C.albicans*, F4 showed activity against *E.coli* with zone of inhibition of 6.12mm, 5.00mm against *S.aureus* and 2.03mm against *C.albicans*, F 5-15 had activity against *E.coli* with zone of inhibition of 6.12mm, 5.0mm against *S.aureus* and 2.03mm against *C.albicans*, F16 shows no activity against *E.coli*, *S.aureus* and *C.albicans.* All the data are presented in Table 3. Figure 5 shows the inhibition zones of the different fractions from column chromatography against the uropathogens.

Table 3: Antimicrobial Activity of the Different Fractions of *Carica papaya* Leave Extract against the three Uropathogens

Fraction	*E. coli*	*Staph aureus*	*Candida albicans*
F 1	-	-	-
F2-3	-	-	-
F4	6.12	5.00	2.03
F 5-15	20.01	18.00	8.02
F16	-	-	-

Key: - = No inhibition, F = Fraction



(a) Fractions *of C.papaya E.coli* (b) Fractions of *C.papaya S.aureus* (c) Fractions *of C.papaya C. albicans*

Figure 5: Inhibition zones of the fractions from column chromatography against the uropathogens
Key: F = Fraction

Identification of Test Organisms

Each organism was confirmed by their specific biochemical tests. Confirmatroy tests carried out to identify the isolates were Gram staining reaction, indole test, catalase production, coagulase test and germ tube test. It was observed that *E. coli* was negative for Gram staining reaction, positive for indole test, negative for catalase, coagulase tests, and germ tube test. *Staphylococcus aureus was* catalase positive, positive for gram staining reaction, negative forindole test, and coagulase positive and germ tube test negative. *C. albicans* was only positive for germ tube test negative for Gram staining reaction, indole test, catalase production and coagulase test as shown in table 4.

Table 4: Identification of Urinary Tract Infection Isolates

Gs	In	Cat	Cao	Gtt	Identity of isolates
-	+	-	-	-	*E. coli*
+	-	+	+	-	*Staph aureus*
+	-	-	-	+	*C.albicans*

Key: **Gs**-Grams staining Reaction, **In**-indole; **Cat**- catalase; **Coa**-coagulase,
Gtt-germ tube test, +**:** Positive, **-:** Negative.

Antimicrbial Activity of Aqueous Extract of *Carica Papaya* Leaf on the Uropathogens

C.papaya has 5.1±0.5mm as the highest zone of inhibition at a concentration of 500mg/ml and 0.5±0.5mm as the lowest zone of inhibition at a concentration of 31.25mg/ml.

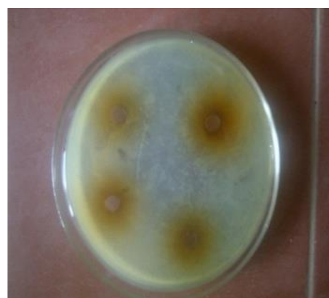
Table 5 and figure 6 shows the suceptibility of aqueous extracts of *Carica papaya* leaves on the three UTI isolates and standard strains. It was equally observed that all the three isolates were inhibited at concentration of 500mg/ml with varying zones of inhibitions. Also at 250mg/ml and 125mg/ml all the three isolates were inhibited. The LSD was calculated against each organism with *E.coli* having the highest LSD of 1.21 and *S.aureus* ATCC 6538 having the least LSD of 1.01.

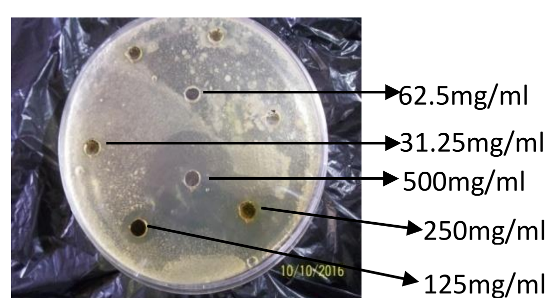
Table 5: Suceptibility of Aqueous Extract of *Carica papaya* Leaf on the three Urinary Tract Infection Isolates and Standard Strains

Conc(mg/ml)	*E.coli* (mm)	*S.aureus* (mm)	*C.albicans* (mm)	*E.coli* ATCC11775 (mm)	*S.aureus* ATCC 6538 (mm)	*C.albicans* ATCC10231(mm)
500	5.1±0.5**a**	4.1±0.2**a**	5.1±0.3**a**	5.0±0.5**a**	4.1±0.5**a**	4.8±0.2**a**
250	3.9±0.5**ab**	3.7±0.2**ab**	4.2±0.3**ab**	4.0±0.5**ab**	3.7±0.3a**b**	4.1±0.3**a**
125	3.2±0.2**b**	2.5±0.5**b**	3.2±0.3**b**	3.1±0.2**b**	2.7±0.2**b**	3.2±0.2**b**
62.5	0.5±0.5**c**	0.5±0.5**c**	1.0±1.0**c**	1.5±0.6**c**	0.5±0.5**c**	2.3±0.4**c**
31.25	-	-	-	-	-	-
LSD=	1.36	1.22	1.80	1.40	1.01	0.81

Footnote: Means tagged with different letter alphabet are significant at P=0.05
Key: - = No inhibition

(a_1)*C.papaya* against *E. coli*


(a_2) *C.papaya* against *S.aureus*



(a_3) *C.papaya* against *C.albicans*

(b_1)*C.papaya* against against *E.coli* ATCC 11775 **(b_2) *C.papaya* against *S.aureus* ATCC 6538 (b_3) *C.papaya* against against** *C. albicans* ATCC 1023

Figure 6: Suceptibilty of aqueous extract of *C.papaya* leaves against the UTI Pathogens when compared with Standard Pathogens

Ethanolic Extracts Of *Carica papaya* Leaf

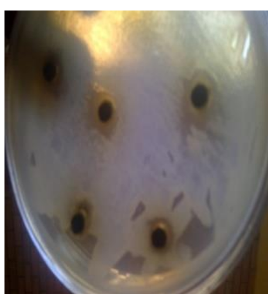
The ethanolic extract of *Carica papaya* exhibited strong activity against *E.coli, S. aureus* and *C. albicans*. Table 6 and Figure 7 shows the antimicrobial activity of ethanol extracts of *Carica papaya* leaves on the three UTI isolates and standard strains. It was equally observed that all the three isolates were inhibited at concentration of 500mg/ml with varying zones of inhibitions. The heighest zone of inhibition was seen against *E.coli* at concentration of 500mg/ml. At concentration of 250mg/ml, 125mg/m and 62.5mg/ml all the three isolates were inhibited. It was observed that at concentration of 31.25mg/ml, none of the organisms was inhibited. The LSD was also calculated against each organism.

Table 6: Antimicrobial Activity of Ethanolic Extract of *Carica Papaya* Leaf on the Three Urinary Tract Infection Isolates and Standard Strains

conc(mg/ml)	*E.coli* (mm)	*S.aureus* (mm)	*C.albicans*(mm)	*E.coli* ATCC11775 (mm)	*S.aureus* ATCC 6538 (mm)	*C.albicans* ATCC10231(mm)
500	24.7±0.5**a**	24.0±0.0**a**	21.5±0.5**a**	23.7±0.5**a**	22.5±0.5**a**	22.0±0.5**a**
250	23.3±0.1a**b**	22.7±0.5**a**	20.0±0.0 **b**	22.8±0.5**ab**	21.1±0.1**a**	19.0±0.0b
125	19.6±0.5**b**	17.5±0.5**b**	14.5±0.5**c**	19.2±0.1**b**	17.5±0.5**b**	14.5±0.6**c**
62.5	3.0±3.0**c**	5.5±0.5**c**	6.5±0.5**d**	3.5±2.5**c**	5.5±0.5**c**	6.9±0.2**d**
31.25	-	-	-	-	-	-
LSD=	5.01	1.41	1.41	4.30	2.06	0.93

Footnote: Means tagged with different letter alphabet are significant at P=0.05

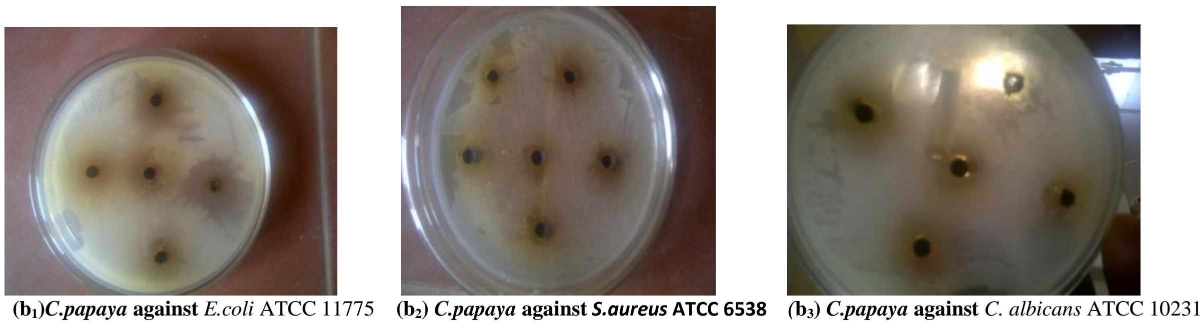
Key: - = No inhibition



(a_1) *C.papaya* against *E. coli*

(a_2) *C.papaya* against *S.aureus*

(a_3) *C.papaya* against *C.albicans*



(b1) *C.papaya* against *E.coli* ATCC 11775 (b2) *C.papaya* against *S.aureus* ATCC 6538 (b3) *C.papaya* against *C. albicans* ATCC 10231

Figure 7: Suceptibilty of ethanolic extract of *C.papaya* leaves against the UTI Pathogens

Positive and Negative Controls

Positive controls like Nitrofurantoin were used against *E coli,* Streptomycine was used against *S. aureus* and Fluconazole was used against *C.albicans*. The zones of inhibitions were 25.6±0.5, 20.0±0 and 18.0±1.0 against *E. coli*, *S.aureus*, *and C.albicans* respectively at the concentration of 500mg/ml. The zones of inhibitions for the standard strain were: - 21.1±0.1, 20.5 ±2.5 and 18.0±1.0 at the concentration of 500mg/ml for *E. coli* ATCC 11775, *S.aureus* ATCC 6538, *and C.albicans* ATCC 10231 (Table 7 and figure 8).

It was observed that all the three isolates were inhibited at concentration of 500mg/ml with varying zones of inhibitions 25.6±0.5, 20.0±0, 18.0±1.0, 21.1±0.1, 20.5±2.5 and 18.1±0.0 against *E.coli, S.aureus, C.albicans, E.coli* ATCC11775, *S.aureus* ATCC 6538 and *C.albicans* ATCC10231 respectively. Also at 250mg/ml and 125mg/ml all the three isolates were inhibited at concentration of with varying zones of inhibitions but at concentration of 62.5, only *E.coli was* inhibited with zones of inhibition of1.00mm. At 31.25 none was inhibited.

Comparing the zones of inhibition of the antibiotics to that of plant extracts, it was observed that the heighest zone of inhibition of the control was 25.6±0.5mm while the highest zone of inhibition of the Plant extracts was 24.7±0.5mm. There is statistical difference as the difference between the zones of inhibition of the plant extract to that of the antibiotics was just as little as 0.9±0.0mm. The negative control (sterile distilled water) did not show any antimicrobial activity in both the aqueous and ethanolic leave extracts as presented in plates below.

Table7: Susceptibility of Test Organisms to Positive and Negative Controls

POSITIVE CONTROLS	NITROFURANTION		STREPTOMYCIN		FLUCONAZOLE	
Conc. of positive controls	***E.coli***	***E.coli* ATCC11775**	***S.aureus***	***S.aureus* ATCC 6538**	***C.albicans***	***C.albicans* ATCC10231**
500	25.6±0.5	20.0±0.	18.0±1.0	21.1±0.1	20.5±2.5	18.1±0.0

250	18.6±1.0	16.6±1.0	10.0±1.0	10.0±1.0	12.0±1.0	11.5±0.5
125	8.5±0.5	5.5±0.5	4.3±0.5	3.5±0.5	2.2±0.0	2.0±0.0
62.5	1.0±0.0	-	-	-	-	-
31.25	-	-	-	-	-	-
Negative Control (Sterile Distilled water)	-	-	-	-	-	-

Key: = No inhibition

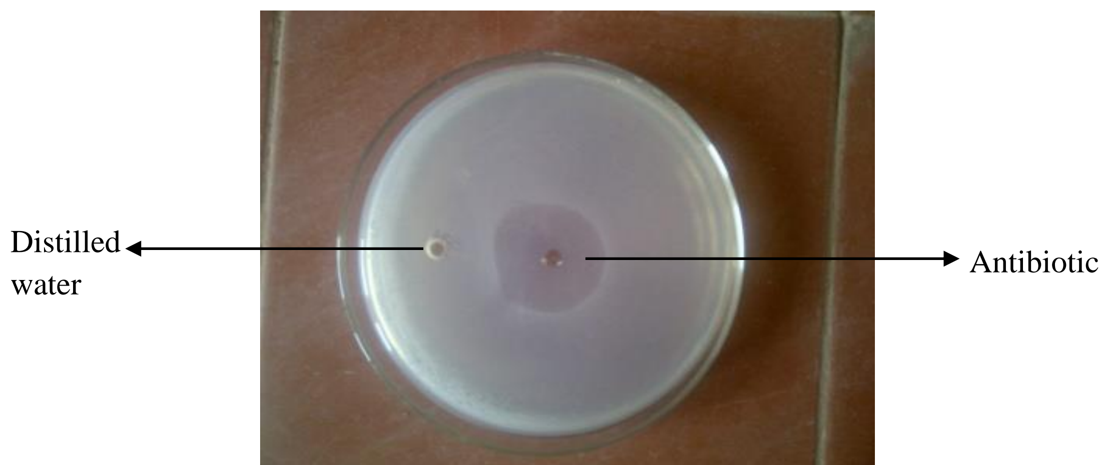


Figure 8. Positive and negative controls
NOTE: Positive control – Antibiotics; Negative control - Distiled water

Minimum Inhibitory Concentrations (MIC) of *Carica Papaya* Leaves on the Test Isolates

The Minimum Inhibitory Concentration (MIC**)** was determined using broth dilution method. In the dilution, tests microorganisms were observed for their ability to produce visible growth on a series of five broth dilutions containing dilutions of the antimicrobial agent. It was observed that each microorganism has a level of antimicrobial agent which inhibits their growth. The lowest concentration of an antimicrobial agent in (mg/ml) that, under defined *in vitro* conditions, prevents the appearance of visible growth of a microorganism within 24hours was taken as the MIC.

In this research, all the microorganisms (*E coli, S. aureus* and *C.albicans*) used in this research were inhibited at varying concentrations of between 500(mg/ml) to 31.25(mg/ml). The Minimum Inhibitory Concentrations of the ethanolic extract of *Carica papaya* leaves showed remarkable activities against the test organisms depending on the UTI.

Carica papaya has MIC 62.5mg/ml against all the test isolates except *C.albicans* which was inhibited at concentration of 125 mg/ml as shown in Table 8.

It was seen that *C.albicans* and *C. albicans* ATCC 10231 have higher MIC in both plants than the other tests organisms. The extract sterility control was carried out by culturing the extracts on sterile Mueller Hilton agar plates and incubated overnight; it was observed that the extracts were sterile as there was no growth observed.

Table 8: The MIC Results of Ethanolic Extract of *Carica papaya* Leaf on the Test Isolates

Clinical/typed strains	500(mg/ml)	250 (mg/ml)	125(mg/ml)	62.5(mg/ml)	31.25(mg/ml)	ESC	MIC
***S. aureus* ATCC 6538**	+	+	+	-	-	-	62.5
S.auerus	+	+	+	-	-	-	62.5
***E.coli* ATCC 11775**	+	+	+	-	-	-	62.5
E.coli	+	+	+	-	-	-	62.5
***C.albicans* ATCC 10231**	+	+	-	-	-	-	125
C.albicans	+	+	-	-	-	-	125

Key: + = Growth -= No growth ESC = Extract sterility control
Diameter of zone of inhibitions shown by different concentration of the extract (mm)

Minimum Bacteriostatic Concentration (MBC) and Minimum Fungistatic Concentration (MFC) of *Carica papaya*

Carica papaya has MBC of 125 mg/ml against *S.aureus* ATCC 6538, *S.aureus, E.coli* ATCC, *E.coli and* Minimum fungustatic concentration (MFC) of 125mg/ml against *C.albicans* ATCC 10231 *C.albicans.* This indicates that both extracts demonstrated bacteriocidal and fungucidal activity against the three microorganisms. The extract sterility control shows no growth after overnight incubation for 24hours (Table 9).

Table 9: The MBC and MFC Results of the Ethanolic Extract of *Carica papaya* Leaf on the Test Isolates

Clinical/typed strains	500(mg/ml)	250 (mg/ml)	125(mg/ml)	62.5(mg/ml)	31.25(mg/ml)	ESC	MIC
***S.aureus* ATCC 6538**	+	+	-	-	-	-	125

S.auerus	+	+	-	-	-	-	125
***E.coli* ATCC 11775**	+	+	-	-	-	-	125
E.coli	+	+	-	-	-	-	125
***C. albicans* ATCC 10231**	+	+	-	-	-	-	125
C.albicans	+	+	-	-	-	-	125

Key: + = Growth -= No growth ESC = Extract sterility control
Diameter of zone of inhibitions shown by different concentration of the extract (mm)

DISCUSSION

Phytochemical screening conducted on *Carica papaya* leaf extracts revealed the presence of compounds which are known to exhibit biological as well as physiological activities (Sofowora, 1993). The phytochemical tests of the aqueous and ethanolic extracts of *C.papaya* showed that they contained Tannins, Alkaloids, Flavonoids, Saponins, Glycosides, Steroids, and Phenolics. This is in affirmation to the result obtained by Cowan (1999) who reported that plant extracts possess' phytochemicals. The presence of the secondary metabolites in *C. papaya*, was an indication that these plants are of pharmacological importance, and also justified their potential use as drug in the study area. Furthermore, the plants contained phenolic compounds (polyphenols and flavonoids) which are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.,* 2007). It has some biological properties such as antiapoptosis, antiaging, anticarcinogenesis, antiinflammation, antiatherosclerosis, cardiovascular protection, improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. In this study it was observed that alkaloids, flavonoids, saponins and phenol were high in concentration. This result is also in accordance to the work of Nweze *et al.,* 2004 who observed the presence of alkaloids, flavonoids, saponins and phenol in high in concentration and the presence of these phytochemical bases in *Carica papaya* leaves were responsible for their antimicrobial activities.

However from the phytochemical analysis of the plant extracts, some of these secondary metabolites were absent (anthroquinone), some in low and moderate concentrations like Glycosides and a few in high concentrations like saponins, alkaloid, and flavonoids. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide range of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996). The presence of alkaloids and saponins

in the present work is in agreement with the opinion of Kasolo *et al.* (2010) who noted that saponins and alkaloids are two of the active constituents. For instance, alkaloids are nitrogen-containing naturally occurring compounds, commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms. Also, the presence of saponin in both plants is in agreement with previous findings of Yusha'a *et al.* (2011). The activity of plant extracts against bacteria have been studied for years, but in more intensified way during the last three decades. During this period, numerous antimicrobial screening evaluations have been published based on the traditional Chinese, African and Asian uses of plant-based drugs (Suffredim *et al.*, 2004).

The aqueous extracts of *C.papaya* showed weak antimicrobial activities. This maybe as a result of the extraction solvents used, water is more polar, so non polar compounds may not have been extracted as can be seen from the yield of the aqueous extract which was greater than the ethanolic extract, this means that a lot of bio-active compound in them may not have been extracted by water. This result is contrary to the result of Lapornik *et al.* (2005) who reported that water is a better extraction solvent as compared to ethanol. This work is in accordance with Das *et al.* (2010) who reported that water soluble flavonoids have no antimicrobial significance and water soluble phenolics only important as antioxidant compound.Though traditional healers use primarily water to extract, plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract (Lapornik *et al.* 2005). In the present study, the results of antimicrobial property of the plant extracts against tested organisms varied depending on bacteria tested and concentration. Active compound(s) may be present in insufficient quantities in the extracts to show activity with the dose levels employed (Taylor *et al.,* 2001).

The ethanolic extracts exhibited strong activity against all the UTI pathogens at varying concentrations. In general the zone of inhibition decreased with decrease in concentration of the extracts and increase with increased in concentration of the extracts. The strong activities shown by the ethanolic extracts maybe as a result of the extraction solvent (ethanol) or that the compounds in the leaves were less polar since ethanol is polar. The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. Ethanol was considered to be more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells (Cowan *et al.,* 1999). This peculiar high levels of zones of inhibition reveals the antimicrobial efficacies of these plants. This is in agreement with Adebolu and Salu (2005) who demonstrated the antimicrobial activity of some medicinal plants against bacteria. Again this work reveals that ethanol could probably be among the best solvent for the extraction of fresh leaves of *C.papaya*. This could be because ethanol diffuses easier into the medium than cold or hot water. This is in agreement with Okigbo and Mmeka

(2008) who attributed the good antimicrobial properties of ethanolic extracts against the isolates at low concentration of plant extract used. Again, it is in agreement with the findings of Obi and Onuoha (2000).who reported alcohol to be the best solvent for the extraction of most plant active principles of medical importance. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Wang *et al.,* 2010). Since nearly all of the identified active components from plants against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol extraction (Cowan *et al.,* 1999). This is similar to the findings of Obi and Onuoha (2000), who reported alcohol to be best solvent for the extraction of most plant active principles of medical importance.

The broad spectrum activities observed in ethanolic extract of *Carica papaya* leaf extracts were as a result of their activity against both Gram negative and Gram positive organisms. *Carica papaya* leaf extracts shows strong antimicrobial activities on both Gram negative and Gram positive organisms maybe as a result of their possession of alkaloids, saponin flavonoids and phenols in high concentration. The activity of ethanolic extracts against Gram-negative is considerably significant because Gram-negative bacteria tend to have higher intrinsic resistance against most antimicrobial agents (Ndukwe *et al.*, 2005). In addition, it was observed that the antimicrobial activity of the plant materials were higher against the Gram-negative organism as compared to the gram- positive. This phenomenon is contrary to that of Matasyoh (2007) and one reason for this may be the fact that Gram negative bacteria are more resistant to the action of antimicrobial compounds compared to their Gram negative counterparts as a result of the more complex cell wall of the former (Aulton, 2002). This resistance may also be due to the lipid content of the membranes of the different groups of the microorganisms and the differences in the rate of permeability of active phytochemicals of *C. papaya.* There have been a greater number of studies showing antimicrobial activity of *C. papaya* against bacteria, fungi, virus and human intestinal protozoan parasites (Bazzaz etal., 2021).

The negative controls (sterile distilled water) did not show any antimicrobial activity in both the aqueous and ethanolic leave extracts. Ethanol was not inhibiting the uropathogens rather the bioactive compounds in the plant extracted that was extracted with ethanol and the ethanol used in the extraction was evaporated via rotary evaporator. Positive controls like nitrofurantoin, streptomycine and fluconazole inhibited the uropathogens at varying concentrations as the plant extracts. It was observed that the difference between the zones of inhibition of the plant extract to that of the antibiotics was very little; this means that the extracts contain antimicrobial agents just as the antibiotics.

The Minimum Inhibitory Concentrations of the ethanolic extract of *Carica papaya* leaves showed remarkable bacteriocidal and fungucidal activities on the test organisms. The low minimum inhibitory concentrations observed in ethanolic extracts of the leaf extracts of *Carica*

papaya on *Escherichia coli, Staphylococcus aureus and C. albicans* is of great significance in the health delivery system, since it could be used as an alternative treatment to orthodox antibiotics in the treatment of diseases due to this isolates, especially as they frequently develop resistance to known antibiotics (Singleton, 1999), and will reduce the cost of obtaining health care.The lower the MIC, the higher the usefulness of the plant extract since they are normally used to evaluate the efficacy of chemotherapeutic agent under standard conditions and also support the sensitivity tests.

Thin-layer chromatography revealed that the ethanolic extracts of *Carica papaya* have some bioactive compounds in them showing some bands while aqueous extract of the two plants showed no band after many trails of different solvent system. This may be that the plants contain non polar compounds and as a result, cannot dissolve in a polar solvent like water. Column chromatography study carried out on *C.papaya* showed that the plants possess active compounds like phenols, phenolic acids, saponins and alkaloids that can be separated using hexane, ethyl acetate and water. This is in agreement with Cowan (1999) who reported that simple phenols, phenolic acids, saponins and alkaloids are the main components of plant origin that have antimicrobial activity.

CONCLUSION

The leaf extracts of *Carica papaya* leaves have exerted strong, good and promising antimicrobial activities against the Uropathogens. The broad spectrum effects on both the Gram positive and Gram negative bacteria was impressive. This present study also demonstrates that herbal medicines can also be effective as modern medicines to combat some pathogenic Microorganisms. It was equally observed that the actual bioactive phytochemicals in *Carica papaya* as chromatographically identified are majorly flavonoids, saponins and phenols. The Plants extracts may contain many phytochemicals and show antimicrobial activities but not all phytochemicals in a particular plant extract that actually contributed to the antimicrobial activity as can be seen in the case of *Carica papaya.*

The findings now serve as a steping stone in the development of new antimicrobial drugs for indigenous and foreign pharmaceutical industry.

This study therefore recommends that:

1. *Carica papaya* leaves having shown some antimicrobial activities can further be investigated for possible use in formulation of antimicrobial compounds.
2. The purified compounds *Carica papaya* should be tested on a larger group of organism *in vitro* and *in vivo* for cytotoxic effects on animals since this work was just on in-vitro activities.
3. That the use of *C. papaya* in the treatment of infections or diseases caused by; *E. coli, Staph aureus C.albicans* should be encouraged
4. Government and her agencies should sponsor more researches on plants and encourage the use so as to make them better alternatives to modern medicine.

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