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Antibacterial Activity And Sequential Extraction of Carica Papaya (PAW PAW) against Multi Drug Resistant Uropathogenic Gram Negative Bacilli

G. Lavanya[1] and M. Gopu[2]
Assistant Professor, PG and Research Department of Microbiology[1]
Assistant Professor, Department of Biotechnology[2]
Kamban College of Arts and Science for Women, Tiruvannamalai, Tamil Nadu, India
Shanmuga Industries Arts and Science College, Tiruvannamalai, Tamil Nadu, India

Abstract: *Urinary tract infection (UTI) is a general term which encompasses microbial colonization of the urine and infection of the urinary tract structures involving kidney, ureters, bladder, and urethra. Although UTIs occur in all age groups irrespective of gender, some clinical studies reveal that the overall occurrence of UTIs is more in females than in males due to anatomical structure. Some microorganisms become more virulent in high glucose concentration. Unreasonable and incorrect antibiotic prescription for UTI may induce the development of antibiotic resistant uropathogens. Many beta - lactam resistant bacteria produce beta lactamase enzyme that inactivates or degrade antibiotics and leading to extended spectrum cephalosporin and even carbapenem resistance. Carbapenemases represent the most diverse class of beta-lactamases. They are capable of efficiently hydrolyzing a wide range of beta- lactam antibiotics including penicillin, cephalosporins, monobactams and carbapenems. The production of beta-lactamases by uropathogens complicates treatment because their presence not only implies resistance to beta-lactam antibiotics but is also associated with resistance to other families of antibiotics. The emergence of multidrug resistance is a serious public health issue for the management of UTI..*

Keywords: Urinary tract infection

I. INTRODUCTION

Urinary tract infection (UTI) is a spectrum of disease caused by microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the urethral meatus. The infection bacteria invade the urethra and bladder with a compromised body defense mechanisms and decreased urine flow **(Monali*et al* .,2015).**.

Most urinary tract infection are caused by bacteria ,but some are caused by fungi in rare cases by viruses .uropathogenic have shown a slow but steady increase in resistant to several antibiotic over the last decades. overuse and use of incomplete course of antibiotic as well as empirical antimicrobial therapy has been the major contribution factor in the development of multidrug resistant bacteria (**Shresth*a et al*.,2012).**

The emergence of multidrug in Gram negative Uropathogenic is a major global concern. Gram negative bacteria especially *E. coli* , are common causes of both community –acquired and hospital acquired UTIs. These organisms can acquire genes that encodes for multiple antibiotics resistance mechanisms , including extended –spectrum-Beta lactamase (ESBLs). manily of the CTX –M family and less frequently of the SHV and OXA families , and other antimicrobial agent carbapenem enzyme, aminoglycoside, sulphanoamides, fluoroquinilones is also escalating rapidly .one to two third of *E coli* producing ESBL were is also escalating rapidly.The multidrug resistant *pseudomonas* and *klebsiella* is a noticeable problem in different parts of the world .

The present study was conducted to achieve the resistant profile among Gram negative UTIs isolates against prescribed antibiotics. further analysis was done to identify the prevelance of multidrug resistant strain and detection of ESBL positive isolates from phenotypic conformation tests.

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II. CARBAPENEMASE PRODUCING BACETRIA

Carbapenem are normally reffered to as the antibiotics of last resort and are bactericidal for both Gram positive and Gram negative organisms. they are known to be the most effective antibiotics against MDR gram negative bacilli .some kind of strains synthesis the enzyme named as Carbapenemase that initiate the activity against the MDR antibiotics (CARBAPENEM).

Because of these kind of Riddles , Now overcome the antibiotic resistance of UTI isolates by introducing the medicinally valuable Terrestrial plant against these MDR strains.

The medicinally valuable Terrestrial plants against UTI isolates are *Carica papaya, Ocimum*, *Cuscuto reflex, Datura* and *cedrusdodara*. Out of that *carica papaya* contain predominant level of nutrient and antimicrobial properties.

III. ABOUT CARICA PAPAYA

Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E. In addition to all this, it contains a digestive enzyme-papaintha effectively treats causes of trauma, allergies and sports injuries. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some form of cancer. It is reported that it helps in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is a good source of fiber.

Papaya effectively treats and improves all types of digestive and abdominal disorders. It is a medicine for dyspepsia, hyperacidity, dysentery and constipation. Papaya helps in the digestion of proteins as it is a rich source of proteolytic enzymes. Even papain-A digestive enzyme found in papaya is extracted, dried as a powder and used as an aid in digestion. Ripe fruit consumed regularly helps in habitual constipation. It is also reported that papaya prevents premature aging. The fruit is regarded as a remedy for abdominal disorders, The skin of papaya works as a best medicine for wounds. Even the pulp left after extracting the juice from papaya as poultice on the wounds.

IV. COLLECTION OF SAMPLE

For the collection of urine sample Ethical clearance is must .The sample are collected from the various hospital around Chennai. Sample are transporated safely by the means of ‘Zip-lock bag’ over the perforated sponge.

V. ISOLATION AND IDENTIFICATION OF ISOLATES

60 urine samples from UTI patients were collected from pathological laboratory. For the isolation of UTI causing strains, loop full of urine sample was streaked on the Nutrient agar and Macconkey agar plate and incubated at 37 ℃ for 24hrs. Next day individual colonies were selected and identified on the basis of morphological, cultural and biochemical characteristics.

VI. IDENTIFICATION OF BACTERIA

Gram's Staining:

- A thin smear was made from the colonies of agar plate and heat fixed.
- The smear was covered with 2-3 drops of crystal violet for one minute.
- The slide was washed with water and then covered with gram's iodine for one minute.
- Again the smear was washed to decolourise the slide gently by adding acetone/alcohol till it destains the gram's iodine.
- Then the slide was counter stained with safranin for 30 seconds.
- Once again the slide was washed with water blot dried with tissue paper and viewed under the oil immersion microscope.

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Biochemical Tests:

Indole Test:

- Inoculate the tubes of trytone broth with the test organisms and incubate at 37°c for 24 hours.
- After incubation, add 0.2ml of kovac's reagent and shake.
- Allow to stand for few minutes.
- Observe the results.

Methyl Red Test:

- Inoculate the tubes with the test organisms and incubate at 37 ºC for 24 hours.
- After incubation , add 5-6 drops of methyl red solution and shake.
- Allow to stand for few minutes.
- Observe the results.

Voges Proskaver's Test:

- Inoculate the tubes with the test organisms and incubate at 37 ºC for24 hours.
- After incubation , add 0.2ml of VP reagent A and 0.2ml of VP reagent B and shake.
- Allow to stand for few minutes.

Citrate Utilisation Test:

- Simmon citrate agar was prepared, sterilized dispensed into sterile test tubes.
- Slants were made and inoculate with the organisms
- The test tubes were incubated at 37 ºC for24 hours.
- Observe the results.

Urease Test:

- Slants were made and incubated with the test organism.
- The test tubes were incubated at 37°C for 24 hours.
- Observe the results.

Triple Sugar Agar Test:

- Slants were made and incubated with the test organism.
- The test tubes were incubated at 37°C for 24 hours.
- Observe the results

Catalase Test:

This test demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produce an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*.

Oxidase Test:

- Some bacteria posess the enzyme oxidase that forms the part of electron transport systems.
- The enzymes oxidase the reagent N-N tetra methyl paraphenylenediaminedidrochloride to a coloured product indophenols.
- When the growth of the organism was rubbed over the filter paper contaminating this reagent, purple colour developed.
- Oxidase disc was placed on the clean slide and 24 hours growth of culture was placed over the disc.

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Mannitol Motility Test:
Mannitol Motility Test Medium is designed to differentiate bacteria on the basis of their motility and ability to ferment mannitol.

Antimicrobial Susceptibility Test :
The susceptibility of isolates to antimicrobial agents was examined by an agar diffusion method using paper disks containing the different antibiotic concentration.On MHA o.5 MCfarland standardized isolates were swabbed and incubated at 37 ℃ for 24 hours .the zone of inhibition was measured and compared with CLSI guidelines .the organisms reported as either sensitive , intermediate sensitive or resistant to antimicrobial agents tested. *E.coli* ATCC 25922, *Enterococcus* ATCC 29212, *Klebsiella*ATCC 70063, *pseudomonas* ATCC 27853 used as control strains of CLSI guidelines recommendation.

Phenotypic Conformation of ESBLS :
Isolates were screened for ESBLs production by using disc diffusion of placed on the inoculated plates containing Muller- Hinton agar according to CLSI guidelines recommendation .

Confirmation Test for ESBLS :
Phenotypic confirmation test for ESBL producer were done by combination disc method .

A. Combination Disc Method
The CDT detects the production of ESBL enzymes based on the principle that they hydrolyse cephalosporin antibiotics and are inhibited by clavulanic acid .cefepime alone and in combination with clavulanic acid is preferred in isolates with inducible AMPc enzymes as the antibiotics is stable to AMPC beta-lactamase. AMPC enzymes interfere with clavulanic acid synergy and detection ESBL production in such isolates using cefataxime or ceflazidime is challenging .the test is cheap and easy to perform ,and interpretation is straightforward. the sensitivity and specificity of this test using cefotaxime and ceftazidine alone in the combination with clavulinic acid have been reported to be 96% and 100% respectively, however the sensitivity of this test can be further increased by using cefepime alone and in combination with clavulanic acid .

B. Modified Double Disc Syrnige Method
All the strains which showed a diameter of less than 27mm for cefataxime , and less than 25 mm for ceftriaxone ,were selected for checking the ESBL production . the ESBL production was determined by the modified double disc synergy test (MDDST)by using a disc of amoxicillin – clavulanate (20/10g)along with four cephalosporin .A Lawn culture of the organisms was made on a mullerhinton agar plate,a disc which contain amoxicillin-clavulanate(20/10g)was placed in the center of the plate.each disc are placed apart from 15mm to 20mm,center to center of that the amoxicillin – clavulanatedisc.any distortion or increase the zone towards the disc of amoxicillin – clavulanate was considered as positive for the ESBL production.e.coli 25922 was used as a negative control for the ESBL production.

VII. PHENOTYPIC CONFORMATION TEST FOR CARBAPENEMASE PRODUCTION

A. Modified Hodge Test
A 0.5 McFarland dilution of the Escherichia coli ATCC 25922 in 5 ml of broth or saline was prepared. A 1:10 dilution was streaked as lawn on to a Mueller Hinton agar plate. A 10 µgmeropenem or ertapenem susceptibility disk was placed in the center of the test area. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35 ± 2oC in ambient plate was incubated overnight at for 16-24 hours. Quality control of the carbapenem disks were performed according to CLSI guidelines. Quality control of the following organisms . After 24 hrs, MHT Positive test showed a clover leaf-like indentation of the *Escherichia coli* 25922 growing along the test organism growth streak within the disk diffusion zone. MHT Negative test showed no growth of the *Escherichia coli* 25922 along the test organism growth streak within the disk diffusion.

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Collection Of Plant Materials

Disease free, fresh, young, and green leaves, stem, seed and flesh were collected from the *carica papaya* plants. The leaves were washed thoroughly 3–10 times in sterile distilled water. Then, they were air-dried under shade at room temperature for 8 days and finely powdered using a blender.

Crude Extract Preparation

This is the same procedure adopted for preparing hexane and ethanol extracts. The crude extract from the leaves, stem, seed and flesh of *carica papaya* was prepared by following procedure. 100g of plant extract powder are soak in both low polarity and high polarity solvent hexane and ethanol And the maken crude are leave it for 24-48 hours of soaking .after soaking the extract were filtered by using whatsman no:1 filter paper and it undergoes for evaporation to get the final concentrated extract. Add 2 ml of DMSO to the above extract for the final stock .

Thin Layer Chromatography (TLC) :

The volume of the spots applied on the chromatographic plates was 5mL, corresponding to approximately 300mg for each dry extract. Chromatography was performed in the following solvent systems: toluene-acetone (8 :2) for hexanic and dichloromethanic extracts; toluene-chloroform-acetone (40 :25 : 35) for all extracts; *n*-butanol-acetic acid glacial-water(50 : 10 : 40), upper phase, for ethanolic and acetic acid extracts.The chromatograms were observed first without chemical treatment, under UV 254 and UV 365 nm light, and then using the spray reagents.

$$Rf = \frac{DISTANCEFROMORIGINTOANALYTEXA}{DISTANCEFROMORIGINTOSOLVENTFRONTXT}$$

Qualitative Phytochemical Screening of Plant Extract

The portion of the dry extract was subject to the Phytochemical screening using various method. Phytochemical screening was performed to test for alkaloids, saponin, tannins, falvanoids, Carbohydrate and Glycosides.

1. Test for Tannin :

To 1ml of papaya plant extract are added in tubes ,2 ml of 5 % of ferric chloride was added formation of dark blue or greenish black indicates the presence of tannins .

2. Test for Saponin :

TO 2ml of papaya plant extract are added to tubes ,2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. formation of 1cm layer of foam indicates the presence of saponins.

3. Test for Flavonoids :

To 2ml of papaya extract are added in tubes ,1ml of 2N sodium hydroxide was added. presence of yellow colour indicates the presence of flavonoids.

4. Test for Alkaloids :

TO 2ml of papaya plant extract are added in tubes ,2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagents are added.presence of green color or white precipitate indicates the presence of alkaloids.

5.Test for Quinones :

To 1ml of papaya extract are added in tubes.1ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinines.

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6. Test for Cardiac Glycoside:

TO 0.5ML of papaya extract are added to tubes,2ml of glacial acetic acid and few drop of 5%ferric chloride were added.this was under layered with 1ml of concentrated sulphuric acid .formation of brown ring at the interface indicates the presence of cardiac glycoside.

7. Test for Terpenoids :

To 0.5ml of papaya plant extract in tubes,2ml of chloroform was added and concentrated sulphuric acid was added carefully.formation of red brown colour at the interface indicates the presence of terpenoids .

8. Test for Triterpenoid :

To 1.5ml of papaya plant extract are added in tubes ,1 ml of libemann –buchard reagent (acetic anhydride +concentrated sulphuric acid) was added. Formation of bluish green color indicates the presence of triterpenoid.

9. Test for Steroids and Phytosteroids

To 1ml of papaya extract are added in tubes equal volume of chloroform is added and subjected with few drop of concentratedsulphuric acid, appearance of brown ring indicates the presence of steroids and appearance of luish brown ring indicates the presence of phytosteroids.

VIII. GAS CHROMATOGRAPHY AND MASS SPECTROPHOTOMETER

Gas chromatography plays a role in separation and introduces target substance into an ms system by directly injecting analytes into a chromatographic column after injecting and heating of plant crude extract (*carica papaya* leaves, stem, seed and flesh).

Antibacterial Activity of Carica Papaya Againt ATCC Culture

The antibacterial activity of *carica papaya* were first held on ATCC sensitive strains , such as *E.coli, pseudomonas , klebsiella , Enterobacteracea*and *staphylococcus* .And the zone of inhibition are observed after 24 hours of incubation .

Test for Antimicrobial Activity :

Agar well diffusion method was adopted to assess the antibacterial activity of *caricapapaya* leaf, stem, seed and flesh extracts against pathogens. The crude extracts were further diluted with diluent at the concentration ranging 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml. For the test, Muller-Hinton agar plates were swabbed with test organism, and the wells (8mm) were filled with different concentration of the extracts. After incubation for 24 hrs, the antibacterial efficiency of the extract were determined by measuring the zone of inhibition formed around the well.

Minimal Inhibitory Concentration :

Minimum Inhibitory Concentration is defined as the lowest concentration of an antimicrobial that will inhibited the visible growth of a microorganisms after overnight incubation and minimal bactericidal concentration as the lowest concentration of antimicrobial that will prevent the growth of an organisms after sub-culturing on antibiotic free medium. .

IX. DETERMINATION OF MINIMUM INHIBITORY CONCNETRATION (MIC)

Macrodilution Method

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate in test tubes. To 0.5 ml of varying concentrations of the extracts (5, 25, 50, 75, 100, 125, 150, 175 and 200 mg/ml) in test tubes, Nutrient broth (2 ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. A tube containing Nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37 ℃ for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity.

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Microdilution Method :

In this method 96 well microtitter plate are used .inital step to adding 100 µl of lb broth to the microtitter plate, after that adding 100 µl of seeded culture into the each well . finally adding 100 µl of plant extract it leads to serial dilution up to 10 dilution. the positive control consist it only contain plant extract and lb broth. the negative control consist it only contain seeded culture and lb broth.

Microdilution Method:

The MIC values were defined as the lowest concentration of extracts that inhibited growth. Extract MIC a value was evaluated using a microtiter dilution assay according to **Elshikh*et al.,*** with a slight modification: assays were carried out under aseptic conditions in 96 well microtiter plates **(Nunc, Roskilde, Denmark).**

- The first column of each microtiter plate was filled with100 µL of test materials (from 1 mg/mL extract stock solution), and the 2nd to 10th wells were filled with 50 µL of MHB. A two-fold serial dilution (throughout 2nd to 10th wells) was achieved by transferring 50 µL of test material wells in the first column to subsequent wells of each row, so that each well had 50 µL of test material in serially descending concentrations.
- From wells in the 10th columns, 50 µL solutions were removed. The working solution of extracts was diluted across the 96-wells using a two-fold serial dilution to give final testing concentrations of 1,000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.9, and 1.95 µg/mL.
- Each microtiter plate had a set of 2 controls: (a) test organisms without test extract as a (positive control; 11th wells), and (b) test extract without test organisms (12th wells), as a control for contamination during plate preparation. Aliquots (20 µL) of bacterial, yeast or fungal suspensions (test organisms) were added to each well.
- The plates were incubated in a temperature-controlled incubator at 37°C for 24 h for bacterial and at 48 hrs for yeast or fungi. After the incubation period, were observe the results.

X. DETERMINATION OF MINIMAL BACTERICIDAL CONCENTRATION

The bactericidal activities of the extracts obtained from *carica papaya* (leaves , stem ,seed and flesh extracts) were tested . the number of the bacteria in the initial microorganism suspension was counted by the surface plate method. After ascertaining the MIC, the number of bacteria was counted in each of the tubes of broth that showed no visible turbidity after overnight incubation, and was compared with the number of bacteria in the initial microorganism suspension. According to NCCLS (1997), the lowest concentration of the extract solution that allowed less than 0.1% of the original inoculum to survive was taken to be the minimum bactericidal concentration.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar by streaking. Nutrient agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37℃ for 24 h. After incubation the concentration at which subjected to no visible growth was seen was noted as the Minimum Bactericidal concentration (MBC).

XI. RESULT

In the present study *carica papaya* aqueous extract are used to activate against the Multidrug resistant UTI strains. The various extract of *caricapapaya* act against various pathogenic organisms . The total number of UTI isolates were fifty five .

Morphology characteristic feature of UTI isolates are performing by gram staining. Biochemical test such as indole production test, citriate utilization test , urease production test , oxidase test and Triple sugar ion for H^2S production. The biochemical test were carried whether it is positive or negative to conform the given sample. The reagents are used to observe the colour changes during reaction.

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Antimicrobial Susceptibility Test Pattern

The AST are held to determine the pathogenic isolates susceptible to given antibiotics. Among the total number of isolates, highest resistant was seen for cefataxime/clavulomic acid, cefoxitin&meropenem. All isolates showed 100% sensitive to fosfomycin antibiotics , And some isolates shows multidrug resistant (ESBLs and carbapenem resistant). Out of 55 isolates which were showing intermidate (or) susceptible zone .i.e 16mm -21mm for meropenem (6.9%)were positive for carbapenemase production by MHT .

Carbapenemase production organisms were *e.coli* 2.4%,followed by *pseudomonas* 1.3%, *klebsiella* 1.7%, *acinetobacter* 1.2% and *enterobacteracea* were 1.4%. ESBLs analysis are carried out by double diffusion syrengy method .

Selection of Plant:

In this present study, we selected the plant named as *carica papaya* (leaves,stem,seed and flesh).The above whole plant are extracted by using the solvent such as hexane and ethanol (low polarity and high polarity solvent).

Sequential Extraction of Plant :

The leaves, stem, seed and flesh of *carica papaya* were prepared by dissolving 100g of plant powder in 300 ml of respective solvents. Such as hexane and ethanol .It can be filtered by whatmann no.1 filter paper and are kept at room temperature for evaporation or artificial evaporator are used for evaporation process.The amount of yield percentage were obtained from this plant extract are :

The dry weight of *carica papaya* leaves hexane extract was = 0.645 g.
The dry weight of *carica papaya* leaves ethanol extract was = 2.186 g.
The dry weight of *carica papaya* stem hexane extract was = 0.701 g.
The dry weight of *caricapapaya* stem ethanol extract was = 3.921g.
The dry weight of *carica papaya* seed hexane extract was = 0.765 g.
The dry weight of *carica papaya* seed ethanol extract was = 4.097g.
The dry weight of *carica papaya* flesh hexane extract was = 0.767g.
The dry weight of *carica papaya* flesh ethanol extract was = 3.565g.

In this study phytochemical compound such as flavonoid , steroid and saponin absent in *carica papaya* stem and leaves. Further the extraction of *carica papaya* are proceed to GC-MS for both molecular structure and bioactive compound identification.

Carica papaya antibacterial activity were analysed against *e.coli*followed by *pseudomonas ,klebsiella , acinetobacter, proteus , citrobacter and enterobacteracea* from UTI infection.Among the bacterial organisms maximum growth suppression were in *e.coli , klebsiella* and *pseudomonas* ,when compared with other organisms . The maximum antibacterial activity are shown in hexane extract compared than other aqueous extract,.

Antibacterial Activity of *Carica Papaya* :

Antibacterial activity of *carica papaya* were analysed against ATCC sensitivity strains,the maximum zone size are observed in hexane extract when compared to ethanol. the zone of inhibition for ATCC strains were between 12- 22 mm in diameter.

Among the bacterial organisms maximum growth suppression were in *klebsiell*a (19mm-22mm) , *e.coli*(15mm-20mm) and *pseudomonas* (18mm-23mm).when compared with other isolates. The extract are diluted and minimal inhibitory concentration was determined .the MIC of *carica papaya*leaf , stem , seed and flesh was found to be 1µg/1ml of UTI isolates. the well diffusion assay shows that the leaf, stem, seed and flesh extract of *carica papaya* (200µg/ml)were more active against isolates showing 15mm-20mm inhibition zone , respectively.

The minimal inhibitory concentration (MIC) of the extract was found to be very low. the MIC of 250µg/ml proves that the extract were effective and the MIC value is an indicator of the antimicrobial activity of extract .

The minimum inhibitory concentration of macrodilution was found to below. The MIC of 200 µg/ml proves that extract were effective against pathogenic strains. Minimum bactericidal effects were exhibited with various degrees in all the hexane and ethanol extracts showing the susceptibility of UTIs against extract of *caricapapaya* . It seems

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possible that the sample tested was possessed the antimicrobial activity. The lowest MBC (200 µg/ml) was obtained in this study.

XII. DISCUSSION

One reason for choosing terrestrial plant is that they are readily available **(samie, obi ,bessong&namirata, 2005).** Of the entire world flora , 250,000 species have been identified and used for curative purposes **(patwardhan , warude , pushpanagadan&bhatt , 2005** Bioactivity of plant extract significantly varied based on the solvents used for extraction , and depends on geographical source **(cervenka*et al.*,2009),** harvest time **(MCGimpsey , *et al.*, 1994)** , storage condition , soil condition , drying method and route of extract **(Bernard *etal*.,2014).**The benefit of plant properties against microorganisms can only be achieved by using a specific solvent and solvent concentration in extraction of the plant materials. In this present study two solvent are used high- polarity solvent and low- polarity solvent (hexane and ethanol).

The extensive use of antimicrobial agent over the last 50 years has led to the emergence of bacterial resistant and to the dissemination of resistance genes among pathogenic microorganisms **(chambers, 2001**) The antibacterial activity of extract *carica papaya* has been shown in some study .but antibacterial activity of *carica papaya* against UTI infection has not been studied **.(shapna*et al*.,2010** ; **meghashri*et al*.,2009**).The present study support the view that leave, stem, seed and flesh of *carica papaya* extract might be useful against UTI pathogen. The findings of this study propose that *carica papaya* can inhibit the growth of UTI resistant isolates.

The hexane extract of *carica papaya* had promising MIC value against UTI bacteria especially *e.coli , pseudomonas* and*klebsiella* .Although in some studies , it has been reported that *carica papaya* extract has antibacterial activity against several bacteria **(nirmala et al,.2011**).In this report , antibacterial activity of this plant extract against UTIs are evaluated.Theprevelance of UTI , considered as the major problem because of developing resistant strains. when compared to synthetic drug herbal drug have fewer side effects and are more economically. This in – vitro study suggests *carica papaya* as a candidate which can help us to control the risk of UTI infection. The effect of this drug are more beneficial if it incorporated in the form of antibiotics (Oral route)

XIII. CONCLUSION

The extract of *carica papaya*(leaves , stem , seed and flesh) is an source for the treatment of UTI infection , while it has been widely observed and accepeted that the medicinal values of plants lies in the bioactive phytochemicals are present in the *carica papaya*. The antibacterial activity of extract *carica papaya* showed the antibacterial spectrum toward the uropathogens , And shows high zone of inhibition . This study has brought to knowledge about the benefictsof sequential extraction of *carica papaya* for its antibacterial potential and medicinal values against the uropathogenic gram negative isolates.

BIBLIOGRAPHY

[1]. Aravind.G ,DebjitBhowmik , Duraivel. S , Harish. G .traditional and medicinal uses of caricapapaya .(Journal of Medicinal Plants Studies .Volume :1,2018.

[2]. Anibijuwon and A.O. Udeze.Antimicrobial Activity of Carica Papaya (Pawpaw Leaf) on Some Pathogenic Organisms of Clinical Origin from South-Western Nigeria .journal of medicinal and pharmacology science vol- 13: 850-64, 2009 .

[3]. AshaRoshan, Navneet Kumar Verma, Anubha Gupta .A Brief Study on Carica Papaya- A Review.Journal of current trend in pharmaceutical research. Volume 2(4): 541-550 2014.

[4]. Anitha B, Raghu N, Gopenath TS, Karthikeyan M, Gnanasekaran A, Chandrashekrappa GK and Basalingappa.Medicinal Uses of Carica Papaya.journal of medical and ayurvedic research volume: 4 2018.

[5]. Amjad , Mirza IA, Abbasi SA, Farwa U, Malik N, Zia F.Modified Hodge test: A simple and effective test for detection of carbapenemaseproduction.Iranian Journal of Microbiology · December 2011.

[6]. AnkitNariya and DevendrasinhJhala .PHARMACOGNOSTIC STUDY OF CARICA PAPAYALEAF EXTRACT AS INHIBITORS OF REACTIVE OXYGENSPECIES. International research journal of pharmacy 2017 .

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
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Impact Factor: 7.301

[7]. Dai Co Viet, Hanoi, Vietnam.reasearch on biological activity of caricapapya leaves. (AJChE 2012, Vol. 12,2012) .

[8]. DamirBeatovic, DijanaKrstic-Miloševic*, SnežanaTrifunovic, JovanaŠiljegovic, JasminaGlamoclija, MihailoRistic and SlavicaJelacic. Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oils of Twelve caricapapyaL.Cultivars (2015)pp(62-75).

[9]. Dr. NageshMalik, Samreen Ahmed. Antimicrobial Activity of Carica papaya, Piper nigrum and Daturastramonium Plants on Drug Resistant Pathogens Isolated from ClinicalSpecimens.Journal of Biotechnology and Biochemistry (IOSR-JBB) 2016.

[10]. Do ThiHoaVien, Tran Van Loc. extractionand Quantification of Carpaine fromCarica papaya Leaves of Vietnam.International Journal of Environment, Agriculture and Biotechnology (IJEAB) Vol-2, Issue-5, Sep-Oct- 2017.

[11]. JyotsnaKiran Peter, Yashab Kumar, PriyankaPandey and HarisonMasih. Antibacterial Activity of Seed and Leaf Extract of Carica Papaya Pusa dwarf Linn. Volume 9, (Mar-Apr. 2014), PP 29-37 .

[12]. Kelechi Mary UKAEGBU-OBI, ChisomPrisca ANYAEGBUNAM, Emmanuel ENYA. ANTIBACTERIAL ACTIVITY OF CARICA PAPAYASEEDS ON SOME HUMAN PATHOGENS.journel of medicinal science and studies 2018.

[13]. M.Priyadharsini, SakshiBhardwaj and E.Sheeba. Isolation, identification of microbial isolates from urinary tract infection patients and evaluation of antimicrobial activity using plant extracts. International journal of current microbiology and applied science Volume 3 Number 4 (2014) pp. 153-160 .

[14]. M.E. Pascual, M.E. Carretero, K.V. Slowing & A. Villar. Simplified Screening by TLC of Plant Drugs. journal of Pharmaceutical Biology, 139-143 (2018).

[15]. Mahendra C. Gunde , Nikhil D. Amnerkar. Nutritional, medicinal an pharmacological properties of papaya (Carica papaya linn.): A review. Journal of Innovations in Pharmaceuticalsand Biological Sciences 2017.

[16]. `N. Nirosha and R. Mangalanayaki.Antibacterial Activity of Leaves and Stem Extract of Carica papaya L. international journal of advance medicine,pharmacology,biology and chemistry.Vol. 2(3), 2013).

[17]. Orhue P.O and MomohA.R.Antibacterial activities of different solvent extract of caricapapya fruit on gram positive and gram negative organisms.International Journal of Herbs and Pharmacological Research IJHPR, 2013,(pg no :234-236).

[18]. Owolabi, A.O., A bah, K. A. and Oranusi, S. Invitro antimicrobial antoxidant activity of carica papaya leaf and stem bark extract on selected clinical isolates .(volume 4 and year 2017).

[19]. PurbowatiningrumRiaSarjono. Antioxidant and antibacterial activities of secondary metabolite endophytic bacteria from papaya leaf (Carica papaya L.)Journal of Biotechnology and Biochemistry 2019.

[20]. Ramapparagavendra And gurumurthy , D. Mahadevan. In vitro antimicrobial activity of various plant latex against resistant human pathogen.International Journal of Pharmacy and Pharmaceutical Sciences Vol 3, 2011.

[21]. Shanjida Islam Tumpa, Md. IqbalHossain, TasneemaIshika. Antimicrobial activities of Psidiumguajava, Carica papaya and Mangiferaindicaagainst some grampositive and gramnegative bacteria .Journal of Pharmacognosy and Phytochemistry 2015.

[22]. S. Akhila and N. G. Vijayalakshmi. PHYTOCHEMICAL STUDIES ONCARICA PAPAYALEAF JUICE.International Journal of Pharmaceutical Sciences and Research · February 201

[23]. Subandi and Anis Nurowidah.The Potency of Carica papaya L. Seeds Powder as Anti- Obesity 'Coffee' Drinks .Journal of current trend in pharmaceutical research 2019.

[24]. TatyasahebPatil, SnehalPatil, AnupritaPatil, ShreedeviPatil. Carica Papaya Leaf Extracts – An Ethnomedicinal Boon. International Journal of Pharmacognosy and Phytochemical Research 2014;pp ; 260-267.

[25]. VimalKishor Singh, IshitaGoyal, AbhishekSaini, Ramesh Chandra. Studying the Effect of Carica papaya Leaf Extract on the Shelf Life of Platelets.International Journal of Science and Research (IJSR) 2015.

[26]. Wemambu II, Ajose DJ and Eni CC.Antibacterial Effect of Carica papaya Root Extract on Some Selected Pathogens from Clinical Isolates.journel of medicinal science and studiesVolume 1 July 2018 .

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[27]. YidnekachewWelde and AbebeWorku . Identification and extraction of papainenzyme from papaya leaf in adigrattowen, northern Ethiopia.. Journal of Medicinal Plants Studies 2018; pg no: 127-130.