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MULTIFUNCTIONAL BIOACTIVITY OF *Mangifera indica* SEED EXTRACT: ANTIMICROBIAL, ANTIOXIDANT, AND THERAPEUTIC POTENTIAL AGAINST UTI PATHOGENS

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Abstract

The present study investigates the antimicrobial, antifungal, anti-inflammatory, antioxidant, phytochemical, and antiproliferative properties of the ethanol extract of *Mangifera indica* (mango) seed, along with its chemical profiling using GC–MS analysis. Mango seeds were collected and processed to obtain ethanol extracts. Urine samples from patients with urinary tract infections (UTIs) were collected, and pathogenic bacteria were isolated and identified. The antibacterial activity of the extract was evaluated against these UTI pathogens. Additionally, fungal species were isolated from contaminated vegetables, and antifungal activity of the extract was assessed.
The antioxidant potential of the extract was determined through standard assays, while anti-inflammatory activity was evaluated using protein denaturation inhibition. Enzyme inhibition studies, including α-amylase activity, were conducted to assess potential antidiabetic properties. The antiproliferative activity of the extract was analyzed to evaluate its potential against abnormal cell growth. Qualitative phytochemical screening was performed to identify bioactive constituents present in the extract. Furthermore, GC–MS analysis was carried out to characterize the chemical compounds responsible for the observed biological activities. The study also explored the application of mango seed extract in water quality assessment and as a potential food additive.
The findings suggest that ethanol extract of *Mangifera indica* seed possesses significant bioactive properties, indicating its potential as a natural source for antimicrobial, therapeutic, and nutraceutical applications.

INTRODUCTION

Urinary tract infections are a serious health problem affecting millions of people each year. A urinary tract infection (UTI) that occurs when bacteria enter and multiply anywhere along the normally sterile urinary tract (Svanborg and Godaly, 1997).

90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram-positive bacteria (Lane and Takhar, 2011). Most of the urinary tract infections are caused by gram-negative bacteria like *Escherichia coli, Klebsiella sp., Proteus vulgaris, Pseudomonas aeruginosa, Acinetobacter* and *Serratia*(Woodford and George,2011). Estrogen deficiency plays an important role in the development of

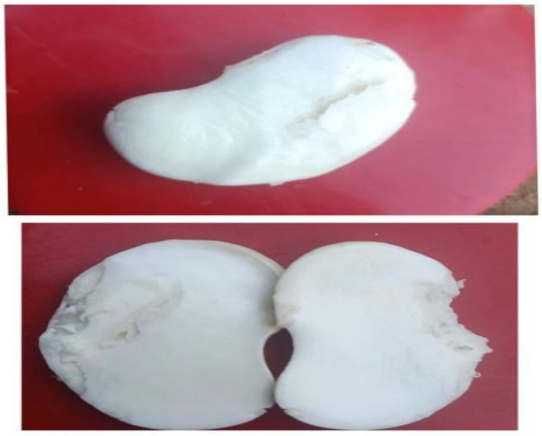
bacteriuria (Raz, 2011). Urinary tract infections (UTIs) are the most common bacterial infections in women, and their incidence rises in the postmenopausal period mainly because of lower estrogen production (Carettoet *al.,*2017).WHO stated that overuse and misuse of available antibiotics of patients getting drug resistant strains and lack of discovery of the new antimicrobials drug by the pharmaceutical industry make the crisis more sever and life threaten condition UTI infected patients(Ludden *et al.,* 2015). Multidrug-resistant bacteria are emerging and spreading rapidly, threatening our ability to treat common infectious diseases. Indiscriminate use of commercial antibiotics is one of the main reasons for drug-resistance. According to the World Health Organization (WHO), drug-resistant diseases could cause 10 million deaths each year by 2050 and damage the global economy significantly (WHO, 2020). Based on this alarming growth of uropathogens that are resistant to existing drugs and the side effects of antibiotics, new therapeutic agents that are less expensive and have fewer adverse effects need to be developed (Raz, 2011). Thus, it is very crucial to identify products with antimicrobial properties that could be utilized to develop novel and efficient antibiotics.

It is expected that by the year 2050, the value of international trade of medicinal plants and their products would reach USD 5 trillion (Zahra*et al.,* 2020).Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rates than modern medicines(Mann *et al.,* 2008). Plant"s secondary metabolites (e.g., alkaloids, phenols, terpenes, etc) are bioactive and provide defense against herbivores and microbes (Nazir *et al.,* 2020). Plants possess extensive therapeutics bioactive coupled with varied chemical structures. In this regard, medicinal plants can be used to overcome socioeconomic and health impact caused by multidrug resistant bacteria, including MRSA and multidrug resistant gram-negative bacteria such as *E. coli* and *K. pneumonia (*Gadisa*et al.,*2019),(Khameneh *etal.,*2019).

Therefore, there is a need to develop new infection fighting strategies to control(Gunda and Kommidi,2020).Different parts of plants such as the leaf, root, flower, fruit, peel, bark, seed, stem, rhizome, etc can be used for therapeutic purposes as they contain a high number of bioactive compounds with therapeutic potential (Ifesan*et al.,* 2013).The aim of this study was to evaluate antimicrobial activityof solvent extract of Mango seed againstUTI bacteria and also screeningof itsphytochemicals, antioxidant,anti-inflammatory and antiproliferative activities.

MATERIALS AND METHODS

COLLECTION OF SAMPLE



Fresh mango (*Mangifera indica L.)* were collected from Thoothukudi market,Tamilnadu,Indiain the time period of October2022 -January 2023. The samples were authentic by the professor of botany Department,KamarajCollege,Thoothukudi.

The seeds were removed from mango kernel, chopped into small pieces, shadow dry by couple of weeks and grinded to a fine powder using electronic mixerand stored in air-tight bottles at room temperature until for further use.

EXTRACTION OF MANGO SEED

10g of driedmango seed powder was soaked in 80ml of ethanoland the flask was wrapped with cotton and kept in a shaker for 3days. The supernatant was filtered using Whatman Filter paper No. 1 and the filtrate was poured into a petri plate for evaporation. Then the residue was measured and dissolved in (DMSO) dimethyl sulfoxide to reach a concentration of 1mg/ml. Then ethanol extract of *Mangifera indica* seed were stored in sterile tubes for further studies.

YIELD OF SOLVENT EXTRACT

The percentage yield of solvent extract of*Mangifera indica* seed was determined at the end of the extraction. The % yield was calculated using the equation.

$$\% \text{ yield} = \frac{\text{mass of extracted solvent liquid}}{\text{mass of } \textit{Mangifera indica} \text{ seed powder (solid)}} \times 100$$

ISOLATION OFUTI PATHOGENS

The midstream urine samples were collected from UTI infected patients from Government Hospital, Pasuvanthanai, Thoothukudi,Tamilnadu,India. The collected samples were transferred aseptically with ice bags and processed in our microbiology laboratory. Isolation of bacteria from the UTI samples were done by streak plate method.

The sample were streaked on different selective agar media such as EMB agar, Pseudomonas isolation agar, Mannitol salt agar, Urease agar, SS agar.The plates were incubated at 37°C for 24-48 hours.After incubation, isolated colonies were observed and selected for the study.

IDENTIFICATION OF MICRO ORGANISMS

All isolates were subjected to Gram's staining and Bio chemical tests according to Bergy's manual.

PREPARATION OF INOCULUM

Fresh culture of UTI pathogenic bacteria was prepared from refrigerator slant culture, a loopful colony was inoculated into Nutrient broth (10ml) and incubated for 24 h at 37^{O}C in an incubator. After 24h incubation, turbidity was adjusted to 0.5Mc Farland standard (10^8 CFU/ml) used for the study.

TO DETERMINE ANTIBIOTIC \ SUSCEPTIBILITY AGAINST UTI BACTERIA

Antibiotic susceptibility of standard commercial antibiotics against UTI bacteria was done by agar disc diffusion method.Commercial antibiotics were selected for this study was Streptomycin (10mcg/disc), Methicillin (10mcg/disc), Vancomycin (30mcg/disc), Levofloxacin (5mcg/disc), Chloramphenicol(30mcg/disc), Ampicillin(10mcg/disc), Ciprofloxacin (5mcg/disc) Tetracycline(30mcg/disc). To prepare the Muller Hinton agar plates and allowed for solidification. The organisms were seeded on the agar medium. After that, placed the standard antibiotics disc on the surface of the agar plates using sterile forceps. The plates were aerobically incubated at 37º C for 24 hours. After incubation, the susceptibility of standard commercial antibiotics against UTI bacteria were observed.

ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED AGAINST UTI BACTERIA

The antibacterial activity of ethanol extract of mango seed against UTI bacteria was evaluated using the agar well diffusion method. Muller–Hinton agar plates were prepared, and 0.1 mL of bacterial culture was spread on the surface. Wells (8 mm) were made and filled with different concentrations of extract (50, 100, 150, and 200 µL). The plates were incubated at 37°C for 24 hours, and the zone of inhibition was measured in millimeters (mm).

DETERMINATION OF MINIMUM INHIBITION CONCENTRATION (MIC)

The minimum inhibitory concentration (MIC) of ethanol extract of mango seed was determined by the broth dilution method. Different concentrations (100–500 µL/mL) were prepared in nutrient broth, and a loopful of standardized bacterial culture (10^8 cells/mL) was added. The tubes were

incubated at 37°C for 24 hours. Microbial growth was assessed by turbidity (OD value), and MIC was defined as the lowest concentration showing no visible growth.

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION

Minimum bactericidal concentrations (MBCs) of ethanol extract of mango seed were determined by according to the MIC values. 10µl of suspension from each tube in the MIC assays were spread on the nutrient agar plate. The plates were incubated aerobically at 37°C for 24hrs. MBCs were defined as the lowest concentration of the extract that produced no growth visible on the plate.

ISOLATION OF FUNGI

The spoiled vegetables were taken in a sterile container from vegetable market Thoothukudi. The collected samples were taken in sterile container, transported and processed in our microbiology lab. Fungal media Rose Bengal Chloramphenicol agar media was prepared and the samples were inoculated on the surface of sterile plates. The plates were incubated at 25^0C for 3 days. After that, fungal morphology was identified by Lactophenol cotton blue staining method.

IDENTIFICATION OF FUNGAL ISOLATES

The identification of fungi was done by studying the cultural characteristics of each isolate. Afterfive days, the fungi grew well on each plate. The fungi morphologywas observed by cello tape method of lactophenol cotton blue staining. A clear cellophane tape was taken and hold it securely with forceps and press the lower,sricky side to the surface of the fungal colony. Tape was gently pulled away from the fungal colony.By doing this aerial hyphae was ahered to the tape.Then placed it on a small frop of LPCB stain on a glass slide. Then fungal morphology was observed under the microscope.

ANTIFUNGAL ACTIVITY FOR ETHANOLEXTRACT OF MANGO SEED

The antifungal activity of ethanol extract of mango seed was evaluated using the agar well diffusion method. Rose Bengal Chloramphenicol agar was prepared, sterilized (121°C for 15 min), and poured into sterile Petri plates. Fungal conidia were swabbed onto the agar surface, and wells were made using a sterile cutter. Different concentrations of extract (50, 100, 150, and 200 µL/well) were added. Plates were incubated at 30°C for 48 hours, and antifungal activity was assessed by measuring the zone of inhibition in millimeters (mm).

DETERMINATION OF MINIMUM FUNGAL CONCENTRATION (MFC)

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration

(MFC) of ethanol extract of mango seed against isolated fungi were determined by the broth microdilution method. Different concentrations (100–500 µL/mL) were prepared in nutrient broth and incubated for 48 hours. For MFC determination, samples from MIC tubes were spread on Rose Bengal Chloramphenicol agar and incubated at 35°C for 48 hours. MIC was defined as the lowest concentration inhibiting fungal growth, while MFC was the lowest concentration showing no growth on agar.

MICROSCOPIC OBSERVATION OF FUNGICIDAL ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED

The *Rhizopus stolonifer*wasgrown on glass slide by slide culture technique. After 92 hours, fungal growth observed on the slide was taken for this study. One slide was control. Directly stain with Lactophenol cotton blue and observe its morphology under microscope. Another slide was an experimental one. The fungal growth observed slide was treated with adding 0.5ml of ethanol extract of mango seed. This slide was kept in 24hours. After that, stained with lactophenol cotton blue and observe the mycelial morphology under the microscope.

DETERMINATION OF ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED

Antioxidant activity of ethanol extract from mango seed was done by Phosphomolybdenum assay (Prieto *et al.,* 1999). An aliquot of 0.1mL of sample solution of different concentrations (1000µl/mL)treated with 1mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C in a water bath for 90 min. The samples were cooled to room temperature and their absorbance was recorded at 765 nm. Ascorbic acid was used as the positive control. Antioxidant capacity was estimated by using following equation:

Antioxidant activity % = [(Ac-As)/Ac] x100, where Ac was the absorbance of control and As was the absorbance of extract sample

PRELIMINARY ASSAY OF ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED

The α-amylase inhibition assay was performed by the 3, 5-dinitrosalicylic acid (DNSA) method. Take 6 test tube then add 1ml of starch to control and test sample tubes. Add 0.5ml of amylase enzyme to all the tubes and the tubes were incubated at 37º C for 30mins. And add 1ml of DNSA reagent to all the tubes and heat the tubes at 95ºC for 15 mins. Then read the absorbance at 510nm. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below:

The % α-amylase inhibition was plotted against the extract concentration and the IC_{50}values were obtained from the graph.

% ofInhibition = [Absorbance Control-Absorbance Test)/Absorbance control] ×100

PROTEIN DENATURATION INHIBITION ACTIVITY OF ETHANOL EXTRACT FROM MANGO SEED

The Protein Denaturation Inhibition activity of ethanol extract of *Mangifera indica* seed was performed by the reaction mixture consisted of 0.1ml of bovine serum albuminto six tubes. The test tubes 1 to 5 represent various concentration 200-1000µl/ml of sample. Standard tube contains 1000µg/ml of aspirin drug. Then allow the tubes for incubation at 40ºC for 10 minutes. After incubation, add 1 ml of reagent C (Mixed reagent-Copper sulphate-0.5ml, Sodium Potassium Tatarate-0.5ml, and Reagent A-99ml) to the tubes and add 0.3ml of Folins phenol reagent to all the tubes. Then incubate at dark for 30minutes. Finally read the absorbance was measuredspectrophotometrically at 660nm for control test 0.05ml distilled water.

The percentage inhibition of protein denaturation wascalculated by the following formula:

% Inhibition = [Absorbance

Test/Absorbance control] ×100.

ANTIBIOFILM ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED

The extracts that can prevent initial cell attachment was investigated through the biofilm inhibition assay using Sandasi *et al*. (2008). In this experiment to take five sterile test tubes, each carrying 1 ml of Nutrient broth. 0.1ml, 0.5ml and 1ml of standardised concentration of culture (1.0×10^6 CFU/mL) of *Pseudomonas aeruginosa* was added into the first three tubes. Forth test tube and fifth tube was added with 0.1ml of culture and incubated at 37 °C for 4 h without shaking. After incubation, 1ml (1mg/ml) aliquots of ethanol extract of mango seed was added in the experimental tubes (Forth test tube) and fifth tube was added with 1ml DMSO. Then all test tubes were incubated further at 37 °C for 24 h without agitation. While70% DMSO assisted as controls. The biomass was quantified using the modified crystal violet staining method (Djordjevic *et al*., 2002).

Crystal violet staining assay

The biofilm assay was performed with slight modifications of Sandasi et al. (2008). Test tubes were washed, dried, and stained with 1 mL of 1% crystal violet for 15 minutes. Excess stain was removed by washing, and biofilm formation was observed as a purple ring on the tube walls.For semi-quantitative analysis, 1 mL of ethanol was added to

destain the biofilm. The destaining solution was transferred to a fresh tube, and absorbance was measured at 450 nm using a colorimeter. The mean absorbance was calculated, and percentage inhibition of biofilm was determined.

Percentage of inhibition = OD of control-OD of Experimental / OD of control X 100

ANTIPROLIFERATIVE ACTIVITY OF ETHANOL EXTRACT FROM MANGO SEED AGANIST BREAST CANCER

Cell culture maintenance: Breast Cancer (MDA MB 231) cell lines were procured from the cell repository of National Centre for Cell Sciences (NCCS), Pune, India. Dulbecco`s Modified Eagle Media (DMEM) was used for maintaining the cell line, which was supplemented with 10% Fetal Bovine Serum (FBS). Penicillin (100 U/ml), and streptomycin (100 µg/ml) were added to the medium to prevent bacterial contamination. The medium with cell lines was maintained in a humidified environment with 5% CO2 at 37°C.

MTT assay: The cytotoxicity of the sample on Hela cells was determined by the method of Mosmann, (1983).Cell viability assay, MDA MB 231 viable cells were harvested and hemocytometer diluted in DMEM medium to a density of 1× 104 cells/ml was seeded in 96 well plates for each well and incubated for 24 h to allow attachment. Then the cells were treated with the sample of different concentration (100 to 800µg/ml) were applied to each well. All the treated cells were incubated at 37º C in a humidified 95% air and 5% CO2 incubator for 24 h. After incubation, the drug containing cells were washed with fresh culture medium and the MTT (5mg/ml in PBS) dye was added to each well, followed by incubated for another 4 h at 37ºC. The purple precipitated formazan formed was dissolved in 100 µl of concentration DMSO and the cell viability was absorbance and measured 540nm using a multi well plate reader. The results were expressed at the percentage of stable cells with respect to the control. The half maximal inhibitory concentration (IC_{50}) values were calculated.

Inhibitory of cell proliferation (%) = $\frac{\text{Meanabsorbenceofthecontrol} - \text{Meanabsorbenceofthesample}}{\text{Meanabsorbanceofthecontrol}}$ X **100**

The IC_{50} values were determine from the sample does responsive curve where inhibition of 50% cytotoxicity compared to vehicle control cells. All experiments were performed in triplicate.

PHYTOCHEMICAL ANALYSISOF ETHANOL EXTRACT OF MANGO SEED BY QUALITATIVELY

Test for Tannins, Alkaloids, Saponins, Flavonoids, Giygosides were performed. Ferric chloride test confirmed tannins by a

blue-black color, while Marqu's reagent indicated alkaloids through turbidity formation. These tests help identify important medicinal constituents present in the sample.

GC-MS ANALYSIS OFETHANOL EXTRACT OF MANGO SEED

Gas chromatography – mass spectrometry was used for identification of the mango seed extract by using apparatus type Shimadzu GCMS-QP2010Ultra. The 2 micro litres of ethanol extract from *Mangifera indica* seed extract (ethanol extract) was injected into the GC-MS system on a 30-m glass capillary column with a film thickness of 0.25 mm (30 m 60.2 mm i.d. coated with UCON HB 2000) using the following temperature program: initial oven temperature of 40°C for 4 min, increased to 25°C at 158°C/ min, and then held at 25°C for 10 min. GC-MS was run under computer control at 70 eV. Chemical ionization was performed using ammonia as the reagent gas at 95 eV. The solvent (dichloromethane) peak was seen at 2.4 min during the GC- MS analysis followed by compound peaks. Identification of unknown compounds was made by probability-based matching using the system such as, WILEY Registry™95, NIST05 and NIST05s. MS data library and comparing the spectrum obtained through GC-MS compounds present in the *Mangifera indica* seed sample were identified.

APPLICATION OF ETHANOL EXTRACT OF MANGO SEED

1.Application of Ethanolic Extracts in Homemade Tomato Paste

Tomato was purchased from a local market Thoothukudi, washed, cut to small pieces and mixed with water (1:2 w/v); then, the salt was added (2%), and the mixture was crushed in blender and then overheated at 80–100 ^{0}C_ with continuous steering until the desired texture was reached. The obtained paste was divided into sterile screw-capped glass bottles. One tube was added with(1%) extract and mixed with the tomato paste. Control (without add extract) was maintained. The treated samples and the control were stored at room temperatures, and examined every four days for the appearance of bacteria or fungi (Downes and Ito,2001).

2.Application of Ethanolic Extracts in municipal water- for water quality

Two sterile screw-capped tubeswere taken for the experiment. Both tubes taken with 5 ml of municipal water. One tube was added with(1%) extract and mixed with it. Control (without add extract) was maintained. The treated water and the control were stored at room temperatures, and examined every four days for the appearance of bacteria (Abdalla *et al.,* 2007)

2.1Total Viable Count

Total viable counts of untreated water and treated water samples (with ethanolicextracts

of mango seed) water quality were determined in room temperature after 24h. Nutrient agar medium was used (Manual,1990), and the plates were incubated for 48 h at 37 ºC. Total viable count and Coliform bacteria (EMB agar) was calculated directly in colony forming units (CFU/ ml).

RESULT

Based on the staining and Bergey's manual of biochemical characteristics, isolatedUTIpathogens were identified as *Staphylococcus aureus,E.coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi and Shigellaflexneri.* The biochemical characteristic of UTI pathogenic bacteria was shown in (Table 1). (Plate 1) shows morphologyof UTI bacteriaon selective media.

BIOCHEMICAL CHARACTERISTIC OF UTI PATHOGENIC BACTERIA

S.NO	PATHOGENS	Indole	MR Red	VP	Citrate	Oxidase	Catalase
1	*Escherichia coli*	+	+	-	-	-	+
2	*Staphylococcus aureus*	-	+	+	+	-	+
3	*Proteus mirabilis*	-	+	-	+	-	+
4	*Pseudomonas aeruginosa*	-	-	-	+	+	+
5	*Salmonella typhi*	-	+	-	+	+	+
6	*Shigella flexneri*	Variable	+	-	-	-	+

The % of yield of solvent extraction of ethanol was 35,(Table 2).

PERCENTAGE OF YIELD OF SOLVENT EXTRACTION

S. No	SOLVENT FOR EXTRACTION	% OF YIELD
1	Ethanol	35

The antibacterial activity of ethanol extract of mango seed was evaluatedagainst the UTIpathogenic bacteria showed excellent activities observed at the concentration of 200µl/well. The maximum antibacterial activity of ethanol extract of mango seed showed against *Escherichia coli*and *Proteus mirabilis*was 11mmrespectively and followed by *staphylococcus aureus*was 10mm,*Pseudomonas aeruginosa* and *Salmonella typhi*was 9mm and *Shigellaflexneri* was 8mm. The antibacterial activity ofethanol extract of mango seed against UTI bacteria was showed in Table 3, (Plate 2).

ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED AGAINST UTI BACTERIA

UTI PATHOGENS	CONCENTRATION OFETHANOL EXTRACT OF MANGO SEED(µl/well)			
	50	100	150	200
	ZONE OF INHIBITION (mm)			
Staphylococcus aureus	5	8	9	10
Escherichia coli	6	7	9	11
Pseudomonas aeruginosa	5	7	8	9
Proteus mirabilis	6	8	9	11
Salmonella typhi	5	6	7	9
Shigella flexneri	3	5	6	8

The Minimum inhibitory concentrationof ethanol extract of mango seed (MIC) was showed in Table 4, (Plate 3). The Minimum inhibitory concentration of ethanol extract of Mango seed against all UTI bacteria was 500µl/ml. The Minimum bactericidal concentration of ethanol extract of Mango seed against all UTI bacteria was shown in Table 5, (Plate4).

MIC VALUE OF ETHANOL EXTRACT OF MANGOSEED

S.NO	UTI PATHOGENS	ETHANOL EXTRACT OF MANGOSEED CONCENTRATION (µl/ml)	OD VALUE (620nm)
1	*Staphylococcus aureus*	100	0.52
		200	0.5
		300	0.48
		400	0.45
		500	0.41
2	*Escherichia coli*	100	0.54
		200	0.52
		300	0.49
		400	0.43
		500	0.4
3	*Pseudomonas aeruginosa*	100	0.54
		200	0.5
		300	0.48
		400	0.44
		500	0.42
4	*Proteus mirabilis*	100	0.53
		200	0.48
		300	0.45
		400	0.43
		500	0.41
5	*Salmonella typhi*	100	0.56
		200	0.54
		300	0.45
		400	0.41

6	*Shigellaflexneri*	100	0.5
		200	0.49
		300	0.45
		400	0.44
		500	0.39

MINIMUM INHIBITORY AND MINIMUM BACTERICIDAL VALUE OF ETHANOL EXTRACT FROM MANGO SEED

S.NO	UTIPATHOGENIC BACTERIA	MIC VALUE(µl/ml)	MBC VALUE(µl/ml)
1	*Staphylococcus Aureus*	500	500
2	*E. coli*	500	500
3	*Pseudomonas Aeruginosa*	500	500
4	*Proteus mirabilis*	500	500
5	*Salmonella typhi*	400	400
6	*Shigellaflexneri*	500	500

The result of susceptibility of commercial antibiotics against UTI bacteria was shown in Table 6, (Plate5). All the isolated UTI bacteria were highly resistant against selected commercial antibiotics.

TO DETERMINE SUSCEPTIBILITY OF COMMERCIAL ANTIBIOTICS AGAINST UTI BACTERIA AND DIARRHEAL BACTERIA

TEST ORGANISMS	COMMERCIAL ANTIBIOTICS				
	Streptomycin (10mcg)	Methicillin (10mcg)	Ampicillin (10mcg)	Tetracycline(30mcg)	Chloramphenicol(30mcg)
Escherichia coli	Resistance	Resistance	Resistance	Resistance	Resistance
Staphylococcus aureus	Resistance	Resistance	Resistance	Resistance	Resistance
Pseudomonas aeruginosa	Resistance	Resistance	Resistance	Resistance	Resistance
Proteus mirabilis	Resistance	Resistance	Resistance	Resistance	Resistance

Salmonella typhi	Resistance	Resistance	Resistance	Resistance	Resistance
Shigellaflexneri	Resistance	Resistance	Resistance	Resistance	Resistance

TEST ORGANISMS	Levofloxacin(5mcg)	Vancomycin(30mcg)	Ciprofloxacin(5mcg)
Escherichia coli	Resistance	Resistance	Resistance
Staphylococcus aureus	Resistance	Resistance	Resistance
Pseudomonas aeruginosa	Resistance	Resistance	Resistance
Proteus mirabilis	Resistance	Resistance	Resistance
Salmonella typhi	Resistance	Resistance	Resistance
Shigellaflexneri	Resistance	Resistance	Resistance

Fungi isolated from spoiled vegetables were identified as *Aspergillus flavus, Rhizopus stolonifer* and *Aspergillusglaucus* based on the conidial arrangements by Lactophenolcotton blue staining and fungal morphology on Rose Bengal agar Plate.

Rhizopus stolonifer showed broad hypha and scarcely septate,rhizoids and stolons are presented, Sporangio spores appeared and white in colour naturally.*Aspergillus flavus* showed green powdery colonies on rose Bengal agar plates. In microscopic observation smooth conidoispores and conidia observed. *Aspergillus glaucus* colonies on rose Bengal agar at 25ºC showed grayish-turquoise to deep yellow central.Reverse is pale yellow or pale brown and septate hyphae,conidial heads are observed in microscopic.(Plate 6).

The ethanol extract of mango seed showed highest inhibitory effects against all the three isolatedfungal strains.The highest zone of inhibition was observed in (9mm) diameter against *Rhizopus stolonifer* at the concentration of 200µl /well. The zone of inhibition of 8mmobserved against *Aspergillus flavus.* The minimum zone of inhibitionobserved in 6mm against *Aspergillus glaucus*.The antifungal activities of ethanol extract ofmango seed against fungi isolated from spoiled vegetables and bread showed inTable 7, (Plate7).

ANTIFUNGAL ACTIVITY TABLE OF ETHANOL EXTRACT OF MANGO SEED

FUNGI ISOLATES	CONCENTRATION OFETHANOL EXTRACT OF MANGO SEED(µl/well)

	50	100	150	200
	ZONE OF INHIBITION (mm)			
Rhizopus stolonifer	6	7	8	9
Aspergillus glaucus	Nil	1	3	6
Aspergillus flavus	Nil	Nil	3	8

The value of Minimum inhibitory concentration of ethanol extract of mango seed was shown in Table 8, (Plate8). The value of Minimum fungicidalconcentration of ethanol extract of mango seed was shown in Table 8, (Plate 9).

MINIMUM INHIBITORY AND MINIMUM FUNGICIDAL CONCENTRATION VALUE OF ETHANOL EXTRACT OF MANGO SEED

FUNGI	MIC(µl/well)	MFC(µl/well)
Rhizopus stolonifer	400	400
Aspergillus flavus	400	400
Aspergillus glaucus	400	400

MICROSCOPIC OBSERVATION OF FUNGICIDAL ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED

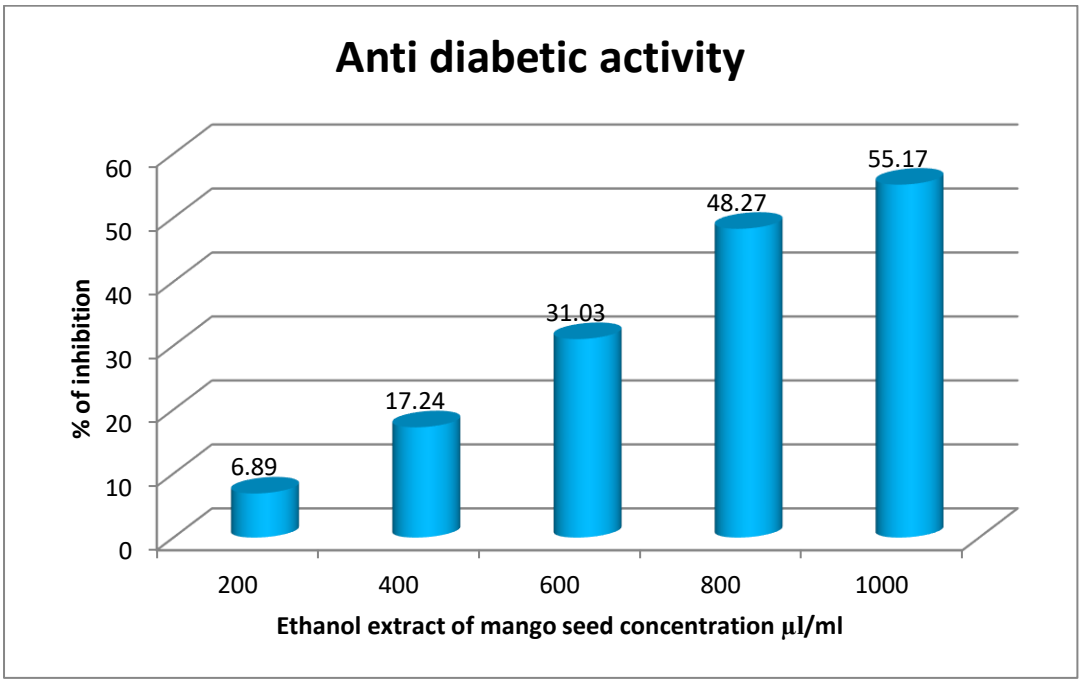
Microscopic observation of fungicidal activity of ethanol extract of mango seedshowed conidial detachment from conidiophores. The microscopic observation exhibited excellent fungistatic activity of ethanol extract of mango seed (Plate 10).

The α amylase inhibitory activity of ethanol extract of Mango seed was expressed as percent inhibition was 55.17%. Alpha amylase inhibition assay ethanol extract of mango seed used to treat to diabetes. 1000µl/ml ofethanol extract of mango seed showed that 50% α-amylase inhibitory activities.% of IC_{50} value was 856.290µl/ml.This results exhibited considerable αamylase inhibitory activities. Further, this study may be supports its usage in ethnomedicines for management of diabetes.The results indicated as the presence of antidiabetic activity of ethanol extract of mango seed showed in Table 9. (Plate 11).

ALPHA AMYLASE INHIBITION ASSAY OF ETHANOL EXTRACT OF MANGO SEED

SAMPLE CONCENTRATION µl/ml	ABSORBANCE@ 510 nm	% INHIBITION	IC_{50}VALUE

Control	0.29		
200	0.27	6.89	856.290µl/ml
400	0.24	17.24	
600	0.20	31.03	
800	0.15	48.27	
1000	0.11	55.17	

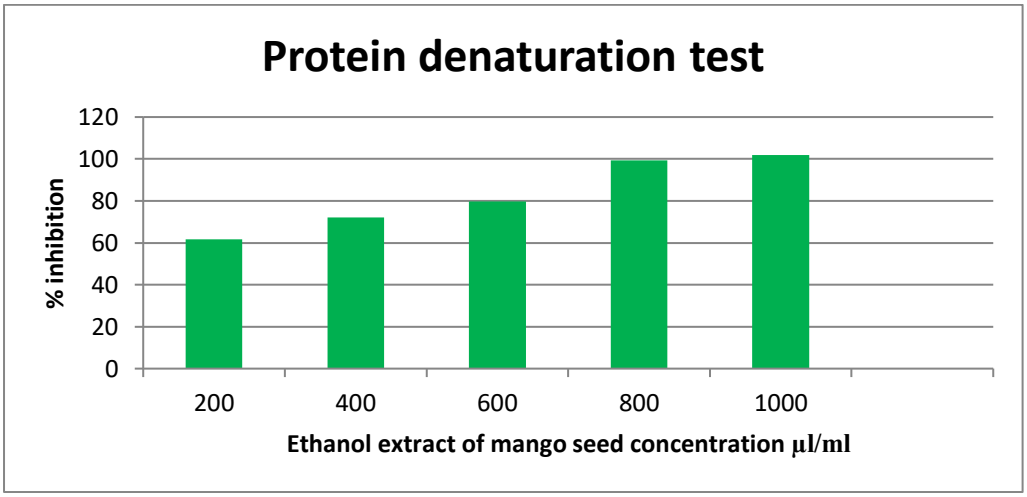


The protein denaturation activity of ethanol extract of mango seed was expressed as percent inhibition was 101.85%. IC_{50} value ofproteindenaturation was 440.92µl/ml.This result showed that the extract had excellent anti-inflammatory activity. The percentage inhibition of protein denaturation of ethanol extract of mango seedshowed in Table 10, (Plate 12).

PROTEIN DENATURATION INHIBITION ACTIVITY FOR MANGO SEED

SAMPLE µl/ml	ABSORBANCE @520nm	% INHIBITION	IC_{50}VALUE
Control	1.620		
200	1.000	61.72	
400	1.170	72.22	
600	1.290	79.62	

800	1.610	99.38	440.92µl/ml
1000	1.650	101.85	



The antioxidant activity of ethanol extract of mango seed was 92% .Theantioxidantactivity of ethanol extract of mango seed was showed in Table11, (plate 13)

ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF MANGO SEED

SAMPLE	CONCENTRATIONµl/ml	OD @680 nm	% ANTIOXIDANT ACTIVITY
Ascorbic acid	1000	1.700	99.9
Ethanol extract of mango seed	1000	0.820	92

ANTIBIOFILM SCREENING INHIBITION OF BIOFILM FORMATION

The effect of antibiofilm activity of ethanol extract of mango seed was conducted against *P. aeruginosa.* The results showed that the ability of these extracts to prevent theattachment of biofilm on the wall of glass tube, But the control tube showed that the appearance of thin adherence of bacterial film on the wall of glass tube(Plate14), So the result exhibited the formation of biofilm on the wall of glass tube by *P. aeruginosa.*

The percentage inhibition of antibiofilm activity of ethanol extract of mango seed (1ml) was 43. This result showed that the extract have excellent anti-biofilm activity.

Table12 showed thatthe percentage inhibition of Antibiofilm activity of ethanol extract of mango seed.

Table Percentage inhibition of antibiofilm activity of ethanol extract of mango seed.

S.NO	TEST CULTURE Inoculum (ml)	SAMPLE CONCENTRATION (mg/ml)	OD VALUE (450nm)	% OF BIOFLIM ACTIVITY
1	0.1	-	1.30	43
2	0.5	-	1.35	
3	1	-	1.09	
4	0.1	Add 1ml of ethanol extract of mango seed	0.74	
5	0.1	1(DMSO) CONTROL	1.32	

The results of antiproliferative activity of ethanol extract of mango seed against breast cancer cells showed that(Plate15).Photomicrograph (20X) represents morphological changes in Breast cancer cells such as shrinkage, detachment, membrane blebbing, and distorted shape induced by aquas extract of mango seed treatment (600 µg and 800 µg/ml for 24 h) as compared with control. Control showed normal intact cell morphology and their images were capture by Fluorescent Microscope. This results were indicated that ethanol extract of mango seed had better Antiproliferative activity against breast cancer cells showed Table13.IC_{50} value ofantiproliferative activity of ethanol extract of mango seed against breast cancer cells was 478µg/ml.

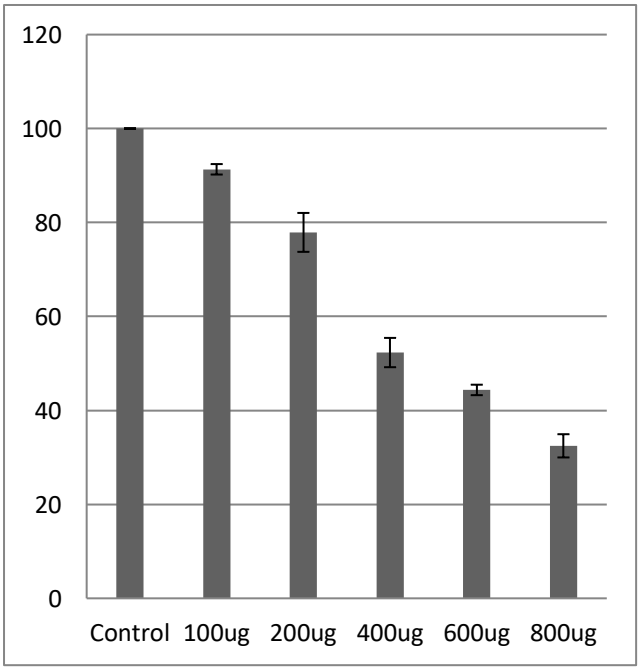
ANTIPROLIFERATIVE ACTIVITY OF ETHANOL EXTRACT FROM MANGO SEED AGANIST BREAST CANCER Cell viability

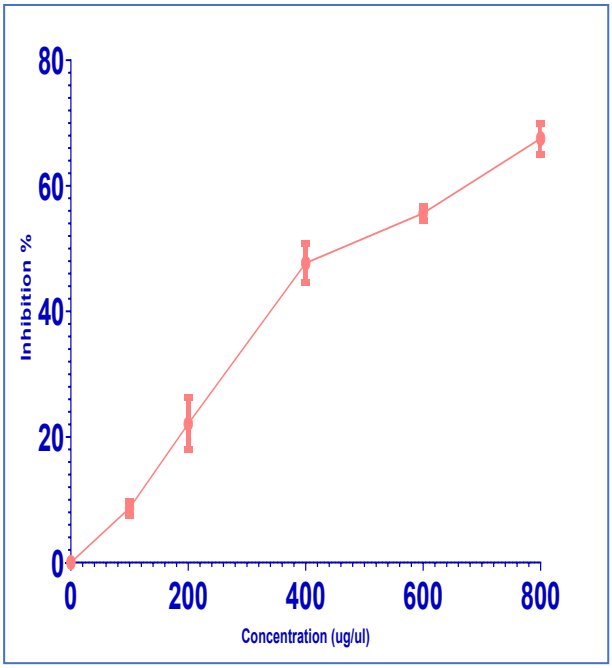
Control	100ug	200ug	400ug	600ug	800ug
100	90.64	74.39	55.60	43.68	30.24
100	92.61	82.47	51.94	45.65	35.13

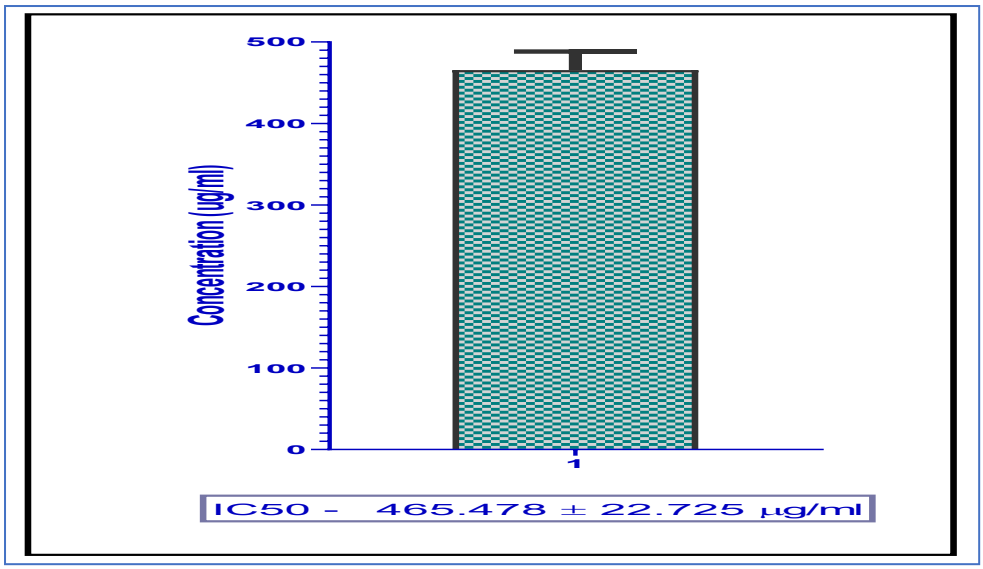
	100	90.73	76.83	49.41	43.77	32.03
Average	100	91.33	77.90	52.32	44.36	32.47
SD	-	1.11	4.14	3.12	1.11	2.47

Inhibition %

Control	100	200	400	600	800
0	9.36	25.61	44.40	56.32	69.76
0	7.39	17.53	48.06	54.35	64.87
0	9.27	23.17	50.59	56.23	67.97







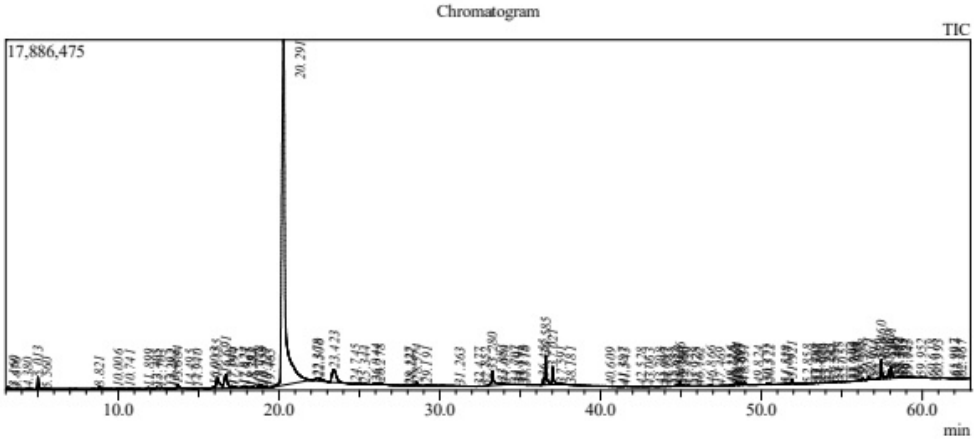
The results of phytochemical analysis of ethanol extract of mango seedextract showed the presence of Alkaloids, Terpenoids, Flavonoids, Saponins, Glycosidesand Tannins (Table 14),(Plate 16).

PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF MANGO SEED

TEST SAMPLES	PRESENCE OR ABSENCE OF SAMPLES
Saponins	Presence
Tannins	Presence
Flavonoids	Presence
Alkaloids	Presence
Glycosides	Presence

GC MS analysis of ethanol extract of mango seed showed the presence of numerous bioactive compounds such as 1,2,3-Benzenetriol, beta-D-Glucopyranose,1,6-anhydro, Oleic Acid, 5-Hydroxymethylfurfural, n-Hexadecanoic acid etc(Figure 1).

Figure 1 **GC-MS ANALYSIS OF ETHANOL EXTRACT OF MANGO SEED**



Peak Report

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	Name
1	20.291	19.530	21.947	270383975	69.61	17615208	56.45	1,2,3-Benzenetriol
2	23.423	23.160	24.060	12393071	3.19	653917	2.10	beta-D-Glucopyranose,1,6-anhydro-
3	36.585	36.500	36.910	10655050	2.74	1490278	4.78	Oleic Acid
4	16.691	16.493	16.963	9209753	2.37	691852	2.22	5-Hydroxymethylfurfural
5	33.280	32.900	33.800	8304068	2.14	760359	2.44	n-Hexadecanoic acid
6	16.155	16.040	16.493	7790697	2.01	559048	1.79	5-Hydroxymethylfurfural
7	37.021	36.910	37.740	7461133	1.92	905267	2.90	Octadecanoic acid
8	57.460	57.357	57.587	5264121	1.36	974986	3.12	beta-Sitosterol
9	58.061	57.990	58.290	4713242	1.21	592880	1.90	Bromoacetic acid, 4-pentadeey
10	5.013	4.900	5.450	3674746	0.95	616639	1.98	Furfural

IUPAC NAME	MOLECULAR FORMULA /PubChem CID	MOLECULAR WEIGT	RT	AREA	AREA%	ACTIVITY	REFERENCE
1,2,3-Benzenetriol	C6H6O3/ 10787	126.11	20.291	27038395	69.61	Antimicrobial activity and Antioxidant activity	G. GnanaPriyanka Beulah *et al,* 2019.
beta-D-Glucopyranose, 1.6-anhydro-	C6H10O5/11947765	162.14	23.423	12393071	3.19	Production of Antibiotics and Bioethanol production and Antioxidant and Anti-inflammatory	Kehinde OluwakemiFagbemi*et al,* 2022.
Oleic Acid	C21H42O2Si/	354	36.585	10655050	2.74	Antioxidant,Antiinflammatory and	Ö. Özşen Batur *etal,*2019.

	5366433					Antidiabetic activity.	
5-Hydroxymethyl furfural	C6H6O3/237332	126	16.691	9209753	2.37	Food additives,scavenging activity, anticancer activity and antiproliferative activity.	Ling Zhao*et al,* 2013.
n-Hexadecanoic acid	C16H32O2/985	256	33.280	8304068	2.14	Antioxidants, hypocholesterolemic, nematicide, and pesticide andAnti inflammatory activity.	Vasudevan Aparna *et al,* 2012.
5-Hydroxymethyl Furfural	C6H6O3/237332	126	16.155	7790697	2.01	Food additives,scavenging activity, anticancer activity, antiproliferative	Ling Zhao*et al,* 2013.
Octadecanoic acid	C18H36O2/5281	284	37.021	7461133	1.92	antimicrobial activity, acaricidal activity, Cytotoxic and antitumor activity, antiproliferative and antioxidants activity	Nalinee Kumari *et al,*2020.
Beta-Sitosterol	C29H50O/6432744	414	57.460	5264121	1.36	anxiolytic &sedative effects, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, anticancer, ant	Shyamaladevi Babu, Selvaraj Jayaraman,2020.

						ioxidant, cardioprotective and antidiabetic	
Bromoacetic acid,4-pentadecyl ester	C17H33BrO2/536338	348	58.061	4713242	1.21	antibacterial activity , Antioxidant activity	Aastha Bhardwaj *et al,* 2014.
Furfural	$C_5H_4O_2$/7362	96.08	5.013	3674746	0.95	antimicrobial activities andantityrosinase activity.	MejdiSnoussi*et al,* 2022.

Total Viable Count of Ethanol Extract of mango seed treated municipal water showed excellent reduction of bacterial count compared to control after 5 days. (Table15). *E.coli*count was greatly reduced in the ethanol extract of mango seed treated municipal water after 5 days. *E.coli* is an indicator organism of water pollution. (Plate17).

TOTAL VIABLE COUNT OF ETHANOLIC EXTRACTS ofmango seed**TREATED MUNICIPAL WATER**

S.NO	DURATION	TOTAL VIABLE COUNT		EMB /*E. coli* COUNT	
		CONTROL	SAMPLE	CONTROL	SAMPLE
1	24 hours	TNTC	63	TNTC	30
2	Day 3	TNTC	20	TNTC	5
3	Day 5	TNTC	Nil	TNTC	Nil

Ethanolic Extract of mango seedin food preservation of Homemade Tomato Paste showed excellent reduction of total viable COUNT and no fungal growth after two weeks. The results of ethanolic extract of mango seed treated Homemade Tomato Paste revealed that excellent food preservation in long periods compared to control (Table 16), (Plate18).

APPLICATION OF ETHANOLIC EXTRACTS of mango seed **IN FOOD PRESERVATION-HOMEMADE TOMATO PASTE**

S.NO	DURATION	TOTAL VIABLE COUNT		FUNGAL PLATE COUNT	
		CONTROL	SAMPLE	CONTROL	SAMPLE
1	48 hours	TNTC	19	Nil	Nil
2	Day 7	20	4	TNTC	Nil
3	Day 14	TNTC	Nil	20	Nil

DISCUSSION

Urinary tract infection is one of the most common infections over the past several decades (Fair and Tor,2014). These infections are a serious public health problem because they are recurrent in many patients and can lead to severe sequelae, such as sepsis, pyelonephritis, kidney damage, and premature delivery, and multi-resistant strains (Flores-Mireles *et al.,*2015). So, in this work concentrated the isolation of pathogenic bacteria from urine of Urinary tract infected patients.

Al-Jiffri*et al.* (2011) stated that the most common etiological agent of uncomplicated UTI is *E. coli*, which is present in about 80%-90% of cases. In the present study, isolated uropathogenic bacteria were identified as*, E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis,Salmonellatyphi and Shigellaflexneri.*Similarly,Blake.(2006) stated that the major causative microorganisms of rUTIs are members of the Enterobacteriaceae family, aerobic Gram-negative bacteria that are present in the intestinal microbiota, such as the genera *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*, *Salmonella*, and *Shigella*. 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram positive bacteria (Bhat*et al.,* 2011). Similarly, most of the isolated bacteria were gram-negative except *Staphylococcus aureus.* Same result was supported by (Lane and Takhar,2011).

Due to their indiscriminate use, antibiotic therapy has been globally jeopardised by the marked increase in antibacterial resistance among common bacterial pathogens (Alam*et al.,* 2020).Kostakioti*et al.* (2012) specified thatchronic recurrence of urinary tract infections, resulting in the frequent use of antibiotics or long-term antimicrobial prophylaxis that exposes patients to the consequences of chronic use of these drugs and long-lasting changes in normal microbiota of the vagina and gastrointestinal tract and also got resistant strains. In the present work,all the isolated UTI bacteria were highly resistant against selected commercial antibiotics.Also,WHO report showed that overuse and misuse of available antibiotics and lack of discovery of the new antimicrobials drug by the pharmaceutical industry make the crisis more sever and life threaten condition UTI infected patients(Ludden *et al.,* 2015).

Cho and Chung. (2017) reported that urinary tract infected *Escherichia coli* and other types of bacteria can develop resistance, which allows them to release an

enzyme called extended spectrum -lactamase (ESBL), which breaks down many types of antibiotics used to treat a UTI and renders them inactive.Emerging and re-emerging of MRSA, extended-spectrum β-lactamases producing organisms (ESBL), is a global public health challenge in worldwide. Therefore, there is a need to promote the development of alternative antimicrobial agents considering the current emergency of new strains, ineffectiveness of the existing therapies, ineffective awareness and prevention measures (Frieri*et al.,*2017). So, searching for innovative antibiotics from natural products should be ultimately an important segment of modern medicine to overcome the various health impacts caused by multidrug resistant microbes (Dadgostar,2019).

Tayel*et al.* (2019) reported that several aqueous and hydro-alcoholic extracts obtained from different plants were screened for their antibacterial effects against human pathogenic bacteria and demonstrated remarkable antibacterial effects on multidrug resistant bacteria including MRSA and ESBL producing bacteria. In this view, the present work concentrated to extract bioactive compounds from waste material of mango seed.Mango (*Mangifera indica* L.) is a member of the Anacardiaceae family and is cultivatedin more than 90 countries in tropical and subtropical regions, with hundreds of cultivars (Asif et al., 2016). According to the mango variety and its weight, 33–85% constitutes flesh, 7–24% peel and 9–40% seed (w/w). With respect to mango juice, the market of fruit juices, mainly those madefrom orange, apple, mango, mixed fruits, and others, is expected to grow at 2.1% from 2021-2026 (EMR,2020).Therefore, during mango processing, 35–60% (w/w) of agro-industrial waste is generated every yearWall-Medrano *et al,* (2020).

Kumar *et al.* (2021) stated that Plant-based medicines are drawing significant interest due to their lower toxicity and minimal side effects. In the present work, we selected mango seeds for solvent extraction and aim for to screen its antimicrobial activities against UTI pathogens.The antibacterial activity of ethanol extract of mango seed was evaluated against the UTI pathogens showed excellent activities observed at the concentration of 200µl/well. The maximum antibacterial activity of ethanol extract of mango seed showed againstand *Pseudomonas aeruginosa* was9mmrespectively and followed by *E. coli* and *Proteus mirabilis* was

11mm,was10mm, *Staphylococcus aureus* was 8mm.

Stoilova*et al.* (2005) reported that mango leaf extract have the ability to kill the fungi such as *A.flavus*, and *A.fumigatus*.The ethanol extract of mango seed showed highest inhibitory effects against all the three isolatedfungal strains.The highest zone of inhibition was observed in (9mm) diameter against *Rhizopus stolonifer*at the concentration of 200µl /well. The zone of inhibition of 8mmobserved against*Aspergillus flavus.*Theminimum zone of inhibitionobserved in 6mm against *Aspergillus glaucus*.

Microbial biofilms are communities of bacteria, embedded in a self-producing matrix, forming on living and non-living solid surfaces. Parsek and Singh. (2014) reported that biofilm-associated cells have the ability to adhere irreversibly on a wide variety of surfaces, including living tissues and indwelling medical devices as catheters, valves, prosthesis, and so forth. And also, Musk *et al.* (2005) estimated 75% of bacterial infections involve biofilms that are protected by an extracellular matrix. The present workthe percentage inhibition of antibiofilm activity of ethanol extract of mango seed (1ml) was 43. This result showed that the extract have excellent anti-biofilm activity.

Inhibition of biofilm formation can be explained by the presence of flavonoids, previously reported such as quercetin, kaempferol, naringenin, and apigenin, which are capable of reduce biofilm synthesisbecause they can suppress the activity of the autoinducer-2 responsible for cell-to-cell communication (Vikram *et al.,*2010).

Many plants can successfully combat infections because of synergistic effects of their different pharmacologically active compounds (Hemaiswarya*et al.,*2008). In the present study, such synergistic antimicrobial effects of ethanol extract of mango seed were observed against antibiotic resistant UTI pathogenic bacteria.

Ethanol extract of Mango seed show a beneficial effect on health due to the presence of flavonoids, which have health-related properties, which are based in their antioxidant activity(Ginestra*et al.,* 2009). The high content of polyphenolic compounds in plant extracts is often correlated with their significant antioxidant activity (Gaweł-B eben*et al.,*2020).

Phytochemical screening results of ethanol extract of mango seed showed in the presence of different functional groups likeAlkaloids, Terpenoids, Flavonoids, Saponins, Glycosides and Tannins. Aqeel *et al.* (1989) stated that alkaloids that intercalate into DNA and its presence could explain the antimicrobial activity of the extract. Akiyama *et al.* (2002) reported that flavonoids cause bacterial death by inhibiting DNA or RNA synthesis and tannins including possible inhibition of extracellular microbial enzymes.

Stefanachi*et al.* (2018) stated that with thepresence of phenolic structure (benzopyran-2-one, or chromen-2-one derivatives) are coumarins, likewise flavonoids, good drug adepts thanks to a variety of pharmacological activities, including antimicrobial, antioxidant, and anti-inflammatory effects.

In the present study GCMS analysis exhibited that the presence of number of bioactive compounds likeβ-sitosterol ,1,2,3-Benzenetriol, beta-D Giucopyranose,1,6-anhydro, Oleic Acid, 5-Hydroxymethylfurfural, n-Hexadecanoic acid etc.in theethanol extract of mango seed.

Kumari *et al.* (2020) reported that Hexadecanoic acid and Octadecanoic acid have antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory activity. Octadecanoic acid, 2,3-dihydroxypropyl ester have strong anti-microbial and anti-cancerous compound isolated from *Cenchrus biflorus* plant. Same compound present in ourethanol extract of mango seed. Similar result supported byAparna*et al.* (2012) stated that n-Hexadecanoic acid to have antioxidant, anti-inflammatory, hypocholesterolemic and cancer prevention activities

One of the important compounds of ethanol extract of mango seed is β-sitosterol.Babu *et al.* (2020) reported that β-sitosterol (SIT) is the major compound, found plentiful in plants. It has been evidenced in many *in-vitro* and *in-vivo* studies that SIT possesses various biological actions such as anxiolytic & sedative effects, analgesic, immunomodulatory, antimicrobial, anticancer, anti – inflammatory, lipid lowering effect, hepatoprotective, protective effect against NAFLD and respiratory diseases, wound healing effect, antioxidant and anti-diabetic activities.

Additionally, the presence of Hydroxymethylfurfural, 1,2,3-Benzenetriol,and oleic acid, methyl ester

observed in the *ethanol* extract of mango seed.1,6-Anhydro-beta-D-glucopyranose have been reported with different activities such as antibacterial, antifungal, antitubercular, anticancer, antioxidant and other prophylactic activities (Fagbemi*et al.,* 2022). According to Zhao *et al.* (2013) the results showed thatHydroxymethylfurfural(5-HMF) showed higher antiproliferative activity on human melanoma A375 cells.

1,2,3-Benzenetriol (Pyrogallol) the compounds found in the study are reported to possess antioxidants, anti-inflammatory, anticancer, cytotic, diuretic and antimicrobial activities (GnanaPriyanka Beulah *et al.,* 2019).

The presence of these bioactive compounds can show bactericidal or bacteriostatic effects on multidrug resistance pathogen bacteria and also as precursor for develop antibiotics for treating infectious agents, mainly from urinary tract infections causing bacteria (Rossiter*et al.,* 2017).

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