



THERAPEUTIC POTENTIAL OF AROMATIC PLANT LEAF (MENTHA PIPERITA L.) AND FRUIT EXTRACT (FLACOURTIA JANGOMAS LOUR) AGAINST THE UTI BACTERIAL PATHOGENS

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ABSTRACT

Medicinal plants are considered colossal producers of bioactive therapeutics agents. Fruit is an essential part of the human diet and is of great interest because of its richness in phytochemicals. The present study was chosen the objectives are assessment the antibacterial activity against the dominantly affected seven Urinary Tract Infected (UTI) pathogenic bacterial species plant leaf extracts and fruits such as *Mentha piperita* (L.), *Flacourtia jangomas* (Lour) Raeusch. Experimental UTI pathogenic organisms named as *Alcaligenes* sp., *Kliebsiella* sp., *E. coli*, *Proteus* sp., *Staphylococcus* sp., *Pseudomonas* sp., Hence the current result has been clearly depicted that the methanol extract of *Mentha piperita* leaf expressed the potential significant antibacterial activity against the five UTI pathogenic organisms such as *Alcaligenes* sp., and *Klebsiella* sp. *Proteus* species and *Pseudomonas* sp., than other two experimental extracts of benzene and ethanol. From the present result clearly noticed three elucidated compounds are act as an antibacterial bio-compounds such Octadecanoic acid methyl ester, 1, 2, 4, 5-tetramethyl Piperone and α -Propyl tetradecanol present in methanol leaf extract of *M. piperita* (L.) plant. Similarly *F. jangomas* (Lour.) fruit extract (methanol), possessed the antibacterial property biologically effective compounds named as *Lavandulol*, α -*Humulene* and *Terpineol*. Therefore, both experimental plant leaf and fruit extract possessed significant antibacterial activity against the tested seven UTI clinical pathogenic organisms Furthermore nanoparticle study also denoted both experimental methanol extracts of *M. piperita* (L.), (leaf) *F. jangomas* (Lour) expressed the significant nanoparticle production. It was noticed the range between 21.50 to 41.03nm and 60.52 to 19.22nm. for *M. piperita* (L.) *F. jangomas* fruit juice extract respectively. From the current research clearly showed that methanol extract of both experimental samples could be act as a potential traditional plant based medicines for urinary tract infected diseases.

KEYWORDS : *Mentha piperita* (L.), *Flacourtia jangomas*, Urinary Tract Infection., GCMS, SEM-XRD

INTRODUCTION

The urinary system, also known as the renal system, it is a system that maintains the volume and composition of body fluids within normal limits. It consists of the kidneys; each one consists of millions of functional units called nephrons (Abrink *et al.*, 2004). The purpose of the renal system is to eliminate wastes from the body, regulate blood volume and blood pressure, control level of electrolytes and metabolites, and regulate blood pH. Following filtration of blood and further processing, wastes (in the form of urine) exist the kidney via the ureters, tube made of smooth muscle fibers that propel urine towards the urinary bladder, where it is stored and subsequently expelled from the body by urination (voiding). The female and male urinary systems are very similar differing only in the length of the urethra (Dugdale and David, 2011). Previously Tanagho and Mcaninch., (2004) published the urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging from asymptomatic presence of bacteria or fungi in the urine to severe infection of the organs of the system with resultant species Urinary tract infection is defined also as the growth of a known bacterial pathogen more than 10000 cfu /ml tested with a positive dipstick or urinalysis (Zorc and Levine, 2005). *Mentha piperita* leaf Extract caused by consumption of contaminated foods with pathogenic bacteria and/or their toxins has been of great concern to public health. Controlling pathogenic microorganisms would reduce food-borne outbreaks and assure consumers a continuing safe. (Karanika, 2001; Seema and Isac Sobana Raj, 2023).

The exploration of naturally occurring *M. piperita* plant leaf extract act as antimicrobials for urinary infected pathogens hence this experimental plant extract increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives (Tassou *et al.*, 2000). *Flacourtia jangomas* contains bioactive component counting tannins, carbohydrates, fats, minerals, ascorbic acid, tartaric acids, proteins amino acids and phenolic compounds (Ghani, 2003).

Due to the evolution of multidrug-resistant pathogens, the analysis for the novel cure all substitutes had gained acceptance of the capacity of medicinal plant extracts for curing infections (Mishra and Padhy., 2013; and Marasini *et al.*, 2015). In the present study have investigated the antibacterial potential against UTI pathogenic bacterial organisms and determine the production of nanoparticles of methanol extract from both experimental samples of *M. piperita* (L.) (leaf) *F. jangomas* (fruit)

MATERIALS AND METHODS

Sample collection

The experimental cultures UTI bacterial pathogenic organisms were collected from the urinary tract infected patients from the government hospital Neyyattinkara, Thiruvananthapuram district in stored a clean centrifuge tube and serially diluted the microbes were stored nutrient broth for further studies unknown dominant colony also been collected for further identification of the particular dominant UTI pathogenic organism from affected patients.. Then the experimental plant *M. piperita* leaves were collected from the vegetable market in Parassalai.

Assessment of minimum inhibitory concentration (MIC)

A pure culture of a specified microorganism grown over night, then diluted in growth supporting broth to the concentration. A stock dilution of the antimicrobial test substance is made at approximately 100 times the expected MBC /MIC. Further 1:1 dilutions are made in test tubes or 96 well microtiter plates. A modification of the dilution method for the determination of MIC and MBC was used. All dilutions of the test products are inoculated with equal volumes of the specified microorganism. The 1 ml of tested organism are serially diluted in 9 ml of sterile distilled water and it make up with 10 ml. The dilution for this sample is 10^{-2} . The diluted sample into take various concentrations, 10^{-3} and 10^{-4} was prepared. The above 1 ml of sample was mixed with 1 ml of experimental extracts (*Pepper mint*, *Pandanus amaryllifolius*, *Piper betle*, *Citrus limon*, *Citrus sinensis*, *Flacourtia jangomas*) and add 3ml of distilled water to make up with 5 ml. And the test

products are carefully to take the optical density at 520nm. The above treated all samples were examined for the optical density at 520 nm.

GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

The phytochemical investigation of methanolic extract of *Mentha* leaf was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra-ver.: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z for GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. Relative quantity of the chemical compounds present in each of the extracts of *P. mint* was expressed as percentage based on peak area produced in the chromatogram. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2. and the results were compared by using Wiley.

The PCR primers which target the 16s rRNA sequence analysis

Polymerase chain reaction (PCR) amplification of 16S rDNA gene. The method of Bulut *et al.* (2005) was used. Amplification of 18S rDNA gene - ITS region, was performed by using the following primer pairs. Forward (18S ITS For), 5'-AGAGTTTGATCCTGGCCTCAG-3 and reverse (18S - ITS Rev), 5'-CAAGGCATCCACCGT-3/, 18S rDNA V3, forward 5'-CCTAGGGGAGGCAGCAG-3 and 16S rDNA V3, reverse, 5'-ARRACCGCGCTGCTGC-3/'. The forward 5'-CCTACGGGAGGCAGCAG-3' and reverse, 5'-ATTACCGCGCTGCTGG-3', primers used occupied positions 341-358 and 518-534, respectively of the V3 region in the 16S ribosomal DNA of *Staphylococcus aureus* CMRSA-1. The primers specify about 200 bp of the PCR products (as could be seen on the gel after electrophoresis). The V3 primer pair was used for ease of sequencing of the gene, using the variable region 3 (V3), for the genetic identification of the isolates. Each of the polymerase chain reactions (PCR) was performed in a 50 µl reaction volume containing 50 µg genomic DNA as the template, 10 µl of 0.2 mM deoxy nucleoside triphosphates, dNTPs, 10 µl of 2.5 mM MgCl₂, 10 pmol each (0.1 µl volume) of the DNA primer in PCR buffer (Promega, UK), and 10 µl of 1.25 units Taq DNA polymerase (Promega, UK) and 18.9 µl distilled water. Amplification conditions were as follows: an initial denaturation step of 5 min at 94°C, 40 amplification cycles, each consisting of 1 min denaturation at 94°C, 1 min annealing at 42°C, and 1 min elongation at 72°C. Reactions were terminated with a final extension step for 10 min at 72°C. PCR amplification was performed in a Thermocycler (K). Gel electrophoresis of 16S rDNA PCR Products Electrophoresis of the amplified 18s rDNA PCR products were performed on the Bio-Rad contour - clamped homogenous electric field (CHEF) DRII

electrophoresis cell. This was done through 1.5% (w/v) agarose gel (Biogene, Germany) in 0.5 X TAE buffer at 84 V for 1.5-2 h. This was prepared by boiling 1.5 g of agarose powder in 100 ml of 0.5X TAE buffer. A 100 bp ladder (Promega, U.K) and 1 Kb DNA ladder (Promega, U.K) were used as molecular size markers sequencing and analysis of 18S rDNA gene Purification of PCR 16S rDNA gene 75 µl of the PCR 18S rDNA amplified products (obtained above) were resolved in 1% agarose gels with the conditions earlier described. PCR products were resolved by gel electrophoresis, using an agarose gel (1.5%; Biogene) that was stained with of 0.5 µg/ml ethidium bromide, in 1xTAE buffer at 84 V for 1.5 - 2 h. The DNA bands were then visualized using a UV transilluminator (Amersham Pharmacia Biotech, UK) with 313 nm emission and pictures were taken using Fuji Film Imaging system FT1-500 (Amersham Pharmacia Biotech, UK). The resulting bands in agarose gel were carefully excised with sterile scalpels and then purified the Wizard PCR preps DNA purification kit (Promega, USA). The purified DNA was kept at 4°C until used. Drying of the purified 18S rDNA genes to a 50 µl of the purified DNA, 0.1 µl of sodium acetate buffer (3M, pH 5.0) and 2.0 µl of 100% ethanol were added. This was then incubated at -20°C for 1 h. It was brought out and left to stand at room temperature for 5 min, and then centrifuged at 13,000 g at 4°C for 45 min. The liquid was removed, leaving only the DNA in the Eppendorf tubes. The DNA was dried in an incubator at 37°C for 30 min. Sequencing of 18S rDNA gene The dry DNA samples (obtained using V3 primers) were sequenced using a computer analytical sequencer (MGW - Biotech, Germany) with the V3 and V5 primer Rev, acting as the basis according to manufacturer's instructions. The generated nucleotide sequences were subjected to analysis. Sequencing of the purified 18S rDNA DNA products was performed using the sequencing unit of the University of Nottingham; a 373 DNA sequence (Perkin-Elmer Applied Biosystems) was used with the Taq Dye Deoxy terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems).

Statistical analysis

Bacterial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented. The data collected was analyzed. The results of this experiment are presented as Mean+ SD triplicate experiments analyzed by using SPSS. Differences between mean is evaluated by one-way ANOVA at p<0.05.

RESULT

Antibacterial activity of the *Mentha piperita* plant leaf extracts result has been represented on table-1. From the present result expressed the maximum zone of inhibition was noticed against *Proteus* species (10.05±1.31cm) in the methanol extract. However, more or less similar antibacterial activity 7.56 ±2.64, 7.28 ±0.97cm also ben observed on *Proteus* species, *Staphylococcus* sp., followed by 7.0±2.13cm of similar antibacterial activity also detected against *Alcaligenes* sp., and *Klebsirilla* sp expressed in benzene, ethanol extracts respectively. Though, minimum range of antibacterial activity noticed in ethanol and aqueous extract on *Pseudomonas* sp. and *Alcaligenes* sp., of UTI organisms. Eventhough, least amount of zone of inhibition response expressed in ethanol extract against *E. coli*. Hence the current result has been clearly depicted that the methanol extract of *P. mentha* leaf expressed the potential significant antibacterial activity against the five UTI pathogenic organisms such as *Alcaligenes* sp., and *Klebsirilla* sp. *Proteus* species and *Pseudomonas* sp., than other experimental extracts.

In addition, the present result was clearly showed that maximum response of antibacterial activity in benzene extracts on *Pseudomonas* sp., (5.74±1.33) subsequently *Staphylococcus* sp., expressed the increased range of zone of inhibition (5.68±0.68, 5.21±0.11) noticed in both ethanol and methanol extracts. However, methanol extracts possessed the

remarkable antibacterial effect on six tested UTI pathogenic organisms except *Serratia* sp., alone. Moreover, another extract of benzene also exhibited second more better antibacterial effect against five treated UTI pathogenic organisms except *Alcaligenes* sp., and *E.coli* sp., (Table-2).

SEM analysis result indicated that specifically when methanol extract of *M. piperita* (L.) plant leaf expressed the nanoparticle range between 21.50 to 41.03nm Fig-2a and 2b. Similarly, second experiment sample of *F. jangomas* fruit juice extract showed nanoparticle range between 60.52 to 19.22nm. Moreover XRD pattern revealed distinct peaks at 2θ values, which can be attributed to 21, 23, 31 and 35 crystalline planes of Ag NPs. Similarly, *F. jangomas* fruit juice extract showed XRD pattern revealed distinct peaks at 2θ values, which can be attributed to 35, 38, 43, 65 and 75 (Fig-3a and 3b).

These peaks are associated with the face-centered cubic lattice. Other peaks at 2θ values in Ag NPs pattern can be ascribed to the residues of the organic content of the plant extract. These peaks reveal the crystallization of some plant metabolite moieties on the surface of the AgNPs. This is acceptable evidence to confirm the involvement of the plant extract compositions in the Ag NP formation.

The fig-4 shows the GCMS chromatogram it was clearly depicted totally ten different bioactive compounds, the major peak compound named as 1, 2, 4, 5-tetramethyl Piperone, it has denoted abundance was 320.57 represented the table: -3.

The duren have the least peak the compound for 72.58 for the *M. piperita* plant leaf methanol extracts (Fig-5. In addition, *F. jangomas* the fruit methanol extract has been depicted peak compound named as Heptadecane along with the abundance has been observed 110.64 followed by other two second most peak compounds also been noticed more or less similar named as Pinocarvone and α-Humulene both represented percentage of abundance 82.53 & 82.58 respectively.

Furthermore the least peak biocompound was observed such as Trans-nerolidol and its abundance displayed as the 30.52.

From the present result clearly noticed three elucidated compounds are act as an antibacterial bio-compounds such Octadecanoic acid methyl ester, 1, 2, 4, 5-tetramethyl Piperone and α-Propyl tetradecanol present in methanol leaf.extract of *Mentha piperita* (L.) plant. Similarly *F. jangomas* (Lou.) fruit extract (methanol).possessed the antibacterial property biologically effective compounds named as Lavandulol, α-Humulene and Terpeneol. Hence, both experimental plant leaf and fruit extract possessed significant antibacterial activity against the tested seven UTI clinical pathogenic organisms.

The antimicrobial activity the aromatic plant extract and the fruit extract are act through the bacteria specialized by the mint and *F. jangomas* it shows the better nanoparticle production than other experimental extracts.

In the GCMS analysis are also done by to check out the maximum plant compounds through our experimental extracts (Fig-5 and table-4). Apart from the current result has been identified the dominant unknown UTI experimental pathogen by sequence analysis, Such a typical UTI organism named as *Staphylococcus aureus* strain CMRSA-1”.

This organism also been conformed through like 16srRNA along with specific DNA 455bp from PCR amplification product by ethidium bromide method and the other techniques gel viewer, electropherogram too denotes total nucleotide base pairs of this sequence UTI pathogenic organism represented in Fig: - 6a, b, and c.

Table 1: - Antibacterial effect of leaf extracts from the *M. piperita* L plant against the UTI Pathogen

UTI CAUSATIVE ORGANISMS	Water (Control)	ETHANOL (mm)	BENZENE (mm)	METHANOL
<i>Alcaligenes</i> sp.,	-	6.32±0.23	7±1.03	3.51±1.03
<i>Kliebsiella</i> sp.,	3.4±0.53	2.3±0.64	7±2.31	
<i>E.coli</i>	-	-	-	2.8±0.97
<i>Serratia</i> sp.,	4.86±1.05	-	-	-
<i>Proteus</i> sp.,		3.05±0.21	7.56±2.64	10.05±1.6
<i>Staphylococcus</i> sp.,	7.28±0.97		2.45±3.19	5.68±0.68
<i>Pseudomonas</i> sp.,	6.37±1.03	4.31±0.22	2.33±1.73	5.6±0.35

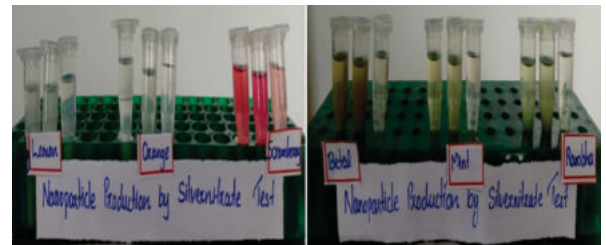


Fig 1: -Green synthesis of nanoparticle production from experimental samples in leaf and fruit extract with .1N AgNO₃

Table 2: - Antibacterial effect of *F. jangomas* fruit extract against the UTI Pathogen

UTI PATHO. ORGANISMS	Water (Control)	ETHANOL (mm)	BENZENE (mm)	METHANOL
<i>Alcaligenes</i> sp.,	2.5±0.22	3.7±0.41	-	4.11±1.05
<i>Kliebsiella</i> sp.,	1.2±0.53		1.7±2.31	1.9±0.27
<i>E. coli</i>	2.8±0.13	-	-	2.0±1.43
<i>Serratia</i> sp.,	-	-	3.12±0.11	-
<i>Proteus</i> sp.,	-	3.65±0.21	4.±0.22	3.05±0.22
<i>Staphylococcus</i> sp.,	-	5.68±0.68	1.31±0.10	5.21±0.11
<i>Pseudomonas</i> sp.,	3.16±0.08	-	5.74±1.33	4.22±1.41

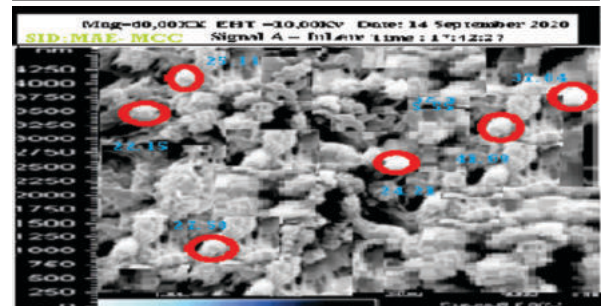


Fig 2a: - Sem view for *M. piperita* L. leaf Methaol extract with .1N AgNO₃ silver nano study

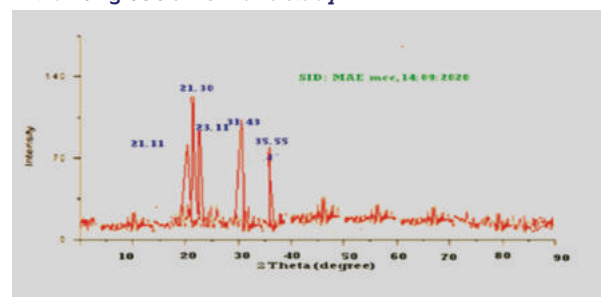


Fig 2b: - XRD pattern of *M. piperita* L. leaf Methaol extract with .1N AgNO₃

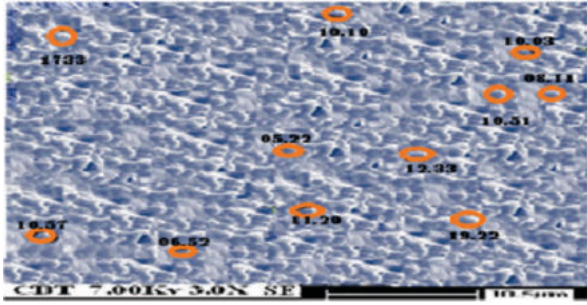


Fig 3a:- Sem view for *F. jangomas* L. fruit Methanol extract with .1N AgNO₃ silver nano study

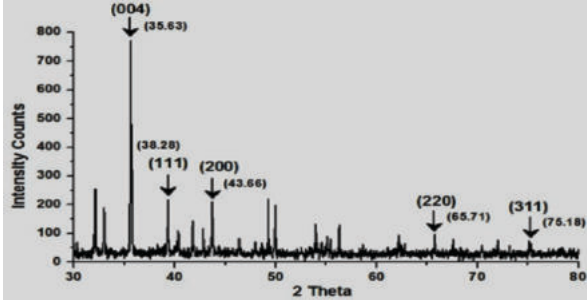


Fig 3b-- XRD pattern of *F. jangomas* fruit Methanol extract with .1N AgNO₃

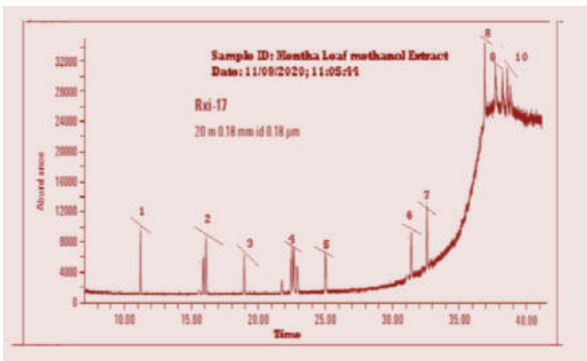


Fig 4:- GCMS Chromatogram view for the *M. piperita* L. leaf Methanol extract sample

Table 3:- Elucidated bio-compounds from *M. piperita* L. leaf Methanol extract by GCMS Analysis

S.No.	Compound(s) separated	Molecular formula	Retention time (mts)	Abundance (%)
1	Octadecanoic acid	C ₁₉ H ₃₄ O ₂	11.21	98.84
2.	2,6,10,14-tetramethylhexadecane	C ₂₀ H ₄₂	16.78	95.41
3.	Durene	C ₁₀ H ₁₄	19.32	72.58
4.	Catechintetramethyl ether	C ₁₉ H ₂₂ O ₆	22.98	93.55
5.	Trace	-	25.04	71.05
6.	Octadecanoic acid methyl ester	C ₁₉ H ₃₄ O ₂	31.82	97.38
7.	1-decanol 2, 2-dimethyl	C ₁₂ H ₂₆ O	33.51	102.43
8.	1, 2, 4, 5-tetramethyl Piperone	C ₁₀ H ₁₄	36.05	120.57
9.	Unknown	-	38.41	280.53
10.	α -Propyl tetradecanol	C ₁₄ H ₃₀ O	39.27	255.09

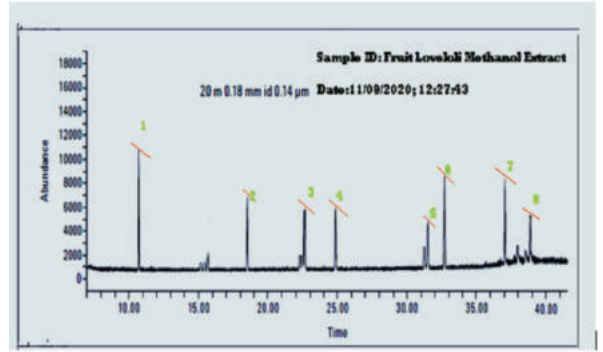


Fig 5:- Chromatogram view of sample from Fruit of *F. jangomas* by GCMS Analysis

Table 4:- Bio-Compounds elucidation of methanol extract from *F. jangomas* fruit sample by GCMS Analysis

S.No.	Compound(s) separated	Molecular formula	Retention time (mts)	Abundance (%)
1	Heptadecane	C ₁₇ H ₃₆	10.98	110.64
2.	L-Linalool	C ₁₀ H ₁₆ O	18.50	73.32
3.	Lavandulol	C ₁₀ H ₁₆ O	22.83	64.73
4.	2,6-Dimethyl-1-5,7-Octatriene-3-ol	C ₁₀ H ₁₆ O	25.10	67.41
5.	Terpineol	C ₁₀ H ₁₆ O	31.57	46.63
6.	Pinocarvone	C ₁₀ H ₁₆ O	33.51	82.53
7.	α -Humulene	C ₁₅ H ₂₄	37.83	82.58
8.	Trans-nerolidol	C ₁₅ H ₂₆ O	39.71	30.52

Name of the Identified Organism from the Urinary Tract Infected Bacterial Organisms (UTIBO): "*Staphylococcus aureus* Strain CMRSA-1

Type: species-specific signature sequences

The PCR primers which target the 16S rRNA Sequence Analysis

FB1/rULWSI - X68376- 5' GACCCTTCAAAGGTCTTAG-3'

And

P1/WXint - U54992- 5'-GACAGTGTGCTTATAACTTTTA-3'

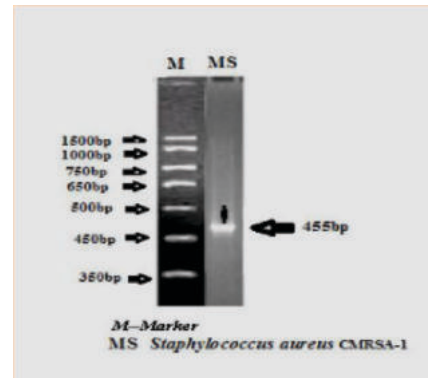


Fig 6a:- Ethidium Bromide-Stained Gel of PCR Amplification Products of *Staphylococcus aureus* Strain CMRSA-1

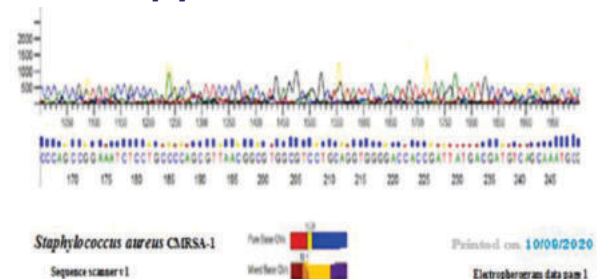


Fig 6b:- Electropherogram for the Sample Organism of *Staphylococcus aureus* Strain CMRSA-1

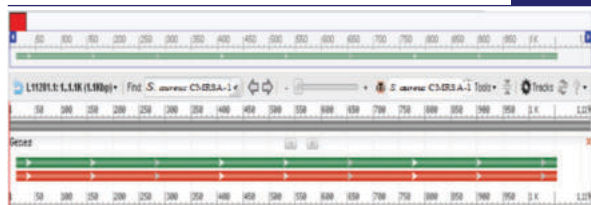


Fig 6c: - Graphic View of the Sample Organisms' Staphylococcus aureus Strain CMRSA-1

DISCUSSION

For prevention and long-term treatment of UTI, plant-based alternatives to antibiotics are appealing choices because they are cost-effective, readily available, safe, with fewer side effects, reduce antimicrobial resistance hazards, and help decrease adverse antibiotic effects and different symptoms). According to the National Institute for Health and Clinical Excellence (NIHCE) guidelines, urinary tract infection is defined by a combination of clinical features and the presence of bacteria and or fungi in urine. About 150 million people developed a urinary tract infection each year (Flores *et al.*, 2015). They are more common in women than men (Salvatore *et al.*, 2011). In women, they are the most common form of bacterial infection (Colgan and Williams, 2011). The main causative pathogens involved in recurrent urinary tract infection in women is *Escherichia coli*, which accounts for about 80% of all episodes, other significant pathogens include *Staphylococcus saprophyticus*, *Klebsiella pneumoniae* and *Proteus mirabilis* each cause about 4% of all of acute cystitis. *Citrobacter* and *Enterococci* are less likely causes of urinary tract infection in women (Echols *et al.*, 1999).

The antimicrobial resistance problem that occurs due to conventional antibiotic treatments has not been reported yet for natural plant remedies (Silverman *et al.*, 2013), that contain a wide range of active phytochemical biomolecules responsible for their beneficial effects. Plant polyphenols, such as anthocyanidins and proanthocyanidins, flavonoids, ellagitannins, and monoterpenoids, such as iridoids, are the main phytoconstituents accountable for the treatment of UTI. (Sihra *et al.*, 2018; Murray *et al.*, 2021) A notable proportion of patients who develop UTI have no discernible causes. However, UTI is mainly associated with patients with detected urinary tract abnormalities, suppressed immune systems, long-term catheter use, and recent urinary procedures (Lewis *et al.*, 2016). A lot of recent studies suggest a genetic predisposition to the recurrent symptomatic UTI. Numerous genes seem to contribute and have been strongly associated with UTI-prone patients (Bazzaz *et al.*, 2021; Murray *et al.*, 2021). The innovation in prophylactic plant-based treatment strategies should target the bottleneck points from UTI pathogenesis and search for molecules that reduce rUTI symptoms without antibiotics and those that increase the host's immune response.

Further progress in developing plant-based products to cure UTI will be supported by the advances in UTI pathogenesis. Furthermore fruit also act highest medicinal pathogenicity against the UTI pathogens, because which has more acidic to compared by the other two fruit experimental fruits. However, green synthesised nano particle production study also showed that, the *F. jangomas* fruit juice extracts possessed extremely efficient nano particles measurement size such as SEM view of the current research sample of *M. piperita* leaf extract-based nanoparticle view was clearly expressed the range between 21.99 to 31.44nm range of the nanoparticles. Similar result also been noticed from the leaf extract of *M. piperita* plant (El-Badry *et al.*, 2010). Generally, the *M. piperita* and the *Flacourtia jangomas* are highly activated as the UTI. In this context, interest in plant phytochemicals as alternative treatment strategies in bacterial infections has increased.

Fruit extracts consist of a combination of functional components, including bioactive antibacterial compounds in a urinary system (Issa *et al.*, 2011).

Flacourtia jangomas belongs to family *Flacourtiaceae*. The plant has been used traditionally for the treatment of different diseases in India (Ahmad *et al.*, 1984). bacteria were sensitive to the stem extracts with highest zones of inhibition for *Pseudomonas aeruginosa*, *Vibrio cholera* and *Salmonella typhi* and moderate zones of inhibition for *Bacillus megaterium*, *Bacillus polymyxa*, *Staphylococcus aureus*, *E. coli* and *Bacillus pumilis*, It was observed from *Flacourtia jangomas* was investigated for antibacterial activity against both gram-positive and gram-negative bacteria by Pravin *et al.* (2011.; Varaldo., 2002).

Various chemical constituents such as glycosides, flavonoids, phenolics and steroids were found. Phenolics, steroids and flavonoids were present in all the extracts of fruit from *F. jangomas* previously described the same result by Parvin, *et al.*, (2011). Antibacterial property of the *F. jangomas* fruit extract mainly due to their ability to donate electron resulting in the conversion of highly reactive free radicals to nonreactive stable molecules. Phenolic compounds may be of three types- non-flavonoids like hydroxybenzoic acid, flavonoids like flavones, flavonols, flavanones etc and type three is tannins. The n-butanol extract of *F. jangomas* showed maximum amount of antioxidant activity in reducing power assay and thiobarbituric assay which is because of the presence of flavonoids.

Medicinal plants are one of the natural resources that can be explored by humans. Various sources of secondary metabolites from plants can be used as medicines, agrochemicals, flavours, fragrances, dyes, biopesticides and food additives. Citrus fruits contain nutrients and phytochemicals that are beneficial to health. Citrus juice contains various substances including carbohydrates, fiber, vitamin C, potassium, folate, calcium, thiamine, niacin, vitamin B6, vitamin A, phosphorus, magnesium, copper, riboflavin, pantothenic acid and various phytochemicals. These substances are needed for the body. Some compounds in citrus fruit can provide additional protection for the body against chronic disease and basic nutrition. Citrus fruits also contain lots of phytochemicals, including essential oils, alkaloids, flavonoids, coumarin, psoralens, and carotenoids. The essential oil of the plant showed fungistatic and fungicidal activity that was significantly higher than that of the costlier fungicide bifonazole (Mimica-Dukic *et al.*, 2009). Menthol has been shown to be an antimicrobial and antifungal agent against ringworm and other fungal infestations of different kinds (Karaman *et al.*, 2003; and Sahin *et al.*, 2003). Menthol is also effective against dental bacteria (Al-Bayati., 2009) It has commonly been reported that Gram-positive bacteria are more vulnerable to essential oils of the plant than Gram-negative bacteria (araman *et al.*, 2003). (Al-Younis, and Argushy, 2009). One study on five flavonoids separated from *M. longifolia* extract showed that the quercetin-3-O-glucoside had the maximum antibacterial activity among the flavonoids tested (Alkroum *et al.*, 2009) Apigenin is a common dietary flavonoid that is found in *Mentha spp.* and has many biological effects including antimicrobial activity (Matsuda *et al.*, 2003; and Xiao *et al.*, 2011).

There is very scanty information available on antimicrobial characteristics of the *M. piperita* leaf extracts of this plant. Some researchers conducted experiments and found that the leaf extract (especially the methanol extract) shows no inhibitory effect as seen by zone of inhibition by agar well method (Dumaol *et al.*, 2010). Previously, several researchers agreed the present research work Aqueous, Ethanolic and methanolic extracts of *M. piperita* leaf extracts were

antibacterial agent (Parillon, and Edward., 2006; and Datta *et al.*, 2011). Agar well diffusion method was used here in order to determine the antimicrobial properties of the plant extracts against the pathogens performed earlier by (Khan, and Kumar., 2011). The Ethanolic, methanolic, ethyl acetate and Aqueous extracts of *M. piperita* possess good antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli* it can be agreed with the current research published by El-Badry *et al.*, 2010. The ethanolic extract turned out to be most effective for its antibacterial activity against all eleven selected bacterial strains. Amongst the Gram-negative bacteria, the extract showed higher activity against *Pseudomonas putida*, *Escherichia coli* and *Salmonella*. From this investigation, it was observed that *Piper betel* leaf extracts successfully inhibited growth of both groups of UTI pathogenic bacterial strains.

CONCLUSION

In conclusion, of the present research work has initially estimate the therapeutic potential of three various aromatic plant leaf and fruit extracts against the UTI pathogens. Urinary tract infection among the most common diseases in developing, as well as in the developed, world with a higher incidence rate in females. A variety of antibiotics are being employed to treat UTI according to the severity of the disease. But antibiotic drug resistance has become an emerging challenge for clinicians to overcome the UTI associated uro pathogens. From this investigation, it was observed that methanol extract of *M. piperita* was more effective against the five UTI pathogenic organisms such as *Alcaligenes sp.*, and *Klebsiella sp.* *Proteus* species and *Pseudomonas sp.*, *E.coli* than other experimental two extracts of ethanpl and benzene. Similarly the same methanol extract of *F. jangomas* also showed the better zone of inhibition against four UTI pathogenic organisms. From, the GCMS result also been clearly revealed that methanol extract of two experimental samples are contains the antibacterial effective compounds, hence it was act as potential antibiotic agents against the UTI pathogenic organisms.

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