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Antibacterial Activity and Phytochemical Screening of Mentha arvensis Linn. against Proteus mirabilis from Urinary Tract Infected Patients

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Abstract: In the present study 63 urine samples were collected from Urinary tract infected patients from various hospitals in Salem District, Tamilnadu, India. The collected samples were subjected to microscopic observation and biochemical characterization to identify the presence of bacteria The *Proteus mirabilis* were isolated on specific medium using XLD (xylose lysine deoxycholates agar deficient) , Macconkey agar , Mullen hinton agar, CLED and UTI agar. The purified *Proteus mirabilis* were performed in biochemical tests such as IMVIC tests etc. The positive isolate was used. Leaves of *Mentha arvensis Linn.* were extracted by using acetone, isopropyl alcohol and petroleum ether, the compound was purified and preliminary phytocemical studies was done. A comparative study on the total antibiotic activity of plant extract were found to be effective against the tested isolated organism *Proteus mirabilis* and MTCC 442 strain. MIC and MBC was performed by agar dilution method and the range was found to be 0.97mg/ml to 250 mg/ml. from the result that particular plant extract (*Mentha arvensis Linn.*) showed high antibacterial activity against tested organism.
Key words : UTI infected urine samples, *Proteus mirabilis,* Antibacterial activity, MIC and MBC, *Mentha arvensis Linn,* Phytochemical screening.

Introduction and Experimental

Urinary Tract Infections are the serious health problem affecting millions of people every year. These are very common infection that occurs when bacteria enter into urinary bladder and multiply anywhere along the normally sterile urinary tract. Most of the infections are caused by bacteria normally present on the skin or in the intestinal tract that invade the urinary tract. UTI'S are more common in persons aged 20-50 years. Approximately 95% of infections occur when bacteria ascend through the urethra and the bladder [1,2]. Urinary Tract Infection is remaining a major clinical problem over 50 years after introduction of antimicrobial chemotherapy. Urinary infection is defined as bacteriuria, i.e the multiplication of bacteria in urine within the renal tract .A concentration of greater then10^5 organisms/ml is regarded as significant bacteriuria. Pyuria is the presence of W.B.C (polymorphus) in the urine. And Hematuria is the presence of R.B.C in urine [3,4].The result of congenital abnormalities seen more often in males. After age of 50 years, the ratio between men and women begins to decline because of the increasing incidence of prostate disease. UTI'S in men younger than 50 years are usually caused by urologic abnormality [5].

The common uropathogens identified patients with UTI include 90% of infection organisms are enteric gram negative bacteria with *E.coli* being the most common followed by the *Proteus mirabilis, Klebsiella sps* and *Enterococcus*

in complicated UTI's, in addition to *E.coli*, there is a higher prevalence of *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Enterococci* and other aerobic gram negative bacteria of the *Enterobacteriaceae* family include *Citrobacter* and *Salmonella*. The Halmark of a UTI has been the presence of a single microorganism's of $>10^5$ colony forming units (efus) per ml in a clean catch or midstream urine specimen. There are an estimated 150 million urinary tract infections per annum worldwide. Warren *et al* reported that in the united states UTI's result in approximately 8 million physician visit per year [6].The organisms attacking any portion of the urinary system cause UTI, the kidney (Pyelonephritis) bladder (cystitis), urethra (urethritis) or urine (bacteriuria) once bacteria infect any site, all other areas are at risk. The diagnosis of lower UTI's (cystitis and urethritis). In upper UTI's (Pyelonephritis), flank pain, fever, blood in the urine (hematuria) and other physical findings may be present and then confirmed by culture [7] .

Leading etiological agents of UTI's include *E.coli* (60-70%), *Enterococcus feaecalis*, *Pseudomonas aeroginosa* and *Proteus mirabilis*. Especially *Proteus mirabilis* causes 90% of *Proteus* infection.The term *Proteus* signinifies changeability of form as personified in the homeric poems in *Proteus*, the old man of the sea who tends the sea flocks of Poseidon and has the gift of endless transformation. The first use of the term *Proteus* in bacteriological nomenclature was made by Houser 1885 who described under this term three types of organisms which he isolated from the putrefied meat [8]. *Proteus mirabilis* is small gram negative bacillus and a facultative anaerobe. It can be found in water, soil and our intestinal tract. It is a part of the normal flora of the human gastro intestinal tract. However, when this organism enters in to the urinary tract, it can cause infection or the lungs it can become pathogenic. *Proteus mirabilis* is characterized by its swarming motility, it is ability to ferment maltose, and it's an ability to ferment lactose. *Proteus mirabilis* has the ability to elongate itself and secrete a polysaccharide when it contact with solid surfaces, although it is seen as a furtherance of infection, not all patients have the symptoms associated with urethritis and cystitis and vomiting. *Proteus mirabilis* can enters the blood stream through wounds the bacteria induce inflammation response that can cause sepsis and systemic inflammatory response syndrome (SIRS). *Proteus mirabilis* infection causing fever, chills and tender prostate chills, chest pain, rales, and cough in men. symptoms for urethritis are mild including frequency of urination and pyuria symptoms are easier to distinguish and include back pain, concentration appearance urgency, hematuria as well as included frequency of urination and pyuria [9].The ability of *Proteus* organism to produce urease and to alkalinize the urine by hydrolyzing urea to ammonia makes it effective in producing on environment in which it can survive. This leads to precipitation of organic and inorganic compounds which leads to struvite stones formation. Struvite stones are composed of a combination of magnesium ammonium phosphate and calcium carbonate apatite. It can increased and the ph of the urine is elevated to decrease the solubility of phosphate ureases metabolized urea into ammonia and corbondioxide [10]. Commonly used antibiotics for Amoxacillin, Ampicillin, Ciprofloxacin and ofloxacin.

The traditional medical methods, especially the use of medicinal plants still play a major role in the developing countries. The history of the use of herbal medicine may be as old as the history of mankind. However 80% of the world's population use plants their primary source of medication. Here Plant source is used as a *Mentha arvensis Linn.* is an herbaceous perennial herb commonly referred as pudina. These leaves have high medicinal value. The aromatic leaves are used widely for flavouring foods and beverages. It is an erect aromatic herb the stem is cylindrical and the leaves are siple and opposing type. It is used as a contraceptive, carminative, antispasmodic, anti peptic ulcer agent, and has been given to treat indigestion, skin diseases, cough and colds in folk medicine [11] .

Materials and Methods

Bacterial Strains

The pathogenic *Proteus mirabilis* MTCC 442 strain was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India**.** A total of 63 clinical samples were collected from Government Hospitals in a round Salem District, Tamil Nadu, and India. For the identification of *Proteus mirabilis*, the collected samples were inoculated on Nutrient agar medium, MacConkey agar medium, *Hi*Chrome Urinary Tract Infection (UTI) agar medium, Cystine Lactose Electrolyte Deficient (CLED) agar medium, Xylose Lysine Deoxy Cholate (XLD) agar medium plates and incubated at 37ºC for 18 - 24 hours. After the incubation, the agar medium plates were subjected to morphological and biochemical characterization, i.e. Gram staining, Carbohydrate fermentation test, Indole test, Methyl red test, Voges Proskauer test, Citrate Utilization test and

Triple sugar iron agar test. Then the confirmed cells of *Proteus mirabilis* were preserved in nutrient broth containing 4% glycerol and kept in freezer at -4^0C until use.

Plant Materials

Fresh plants *Mentha arvensis Linn*. of were collected from the surroundings of Namakkal District, Tamil Nadu, India. The collected plant species were identified and confirmed by Dr. R. Selvaraj, Professor, Department of Botany, selvarajphd@yahoo.co.in, Annamalai University, Annamalai Nagar, Tamil Nadu and India.

Preparation of Crude Extracts

Fresh leaves in bulk were locally obtained from *Mentha arvensis Linn*. plant. The leaves were cleaned and shade-dried at room temperature. The dried leaves were ground into a fine powder with the help of an electrical grinder. After, 50 gm of the powder was taken in soxhlet apparatus and 200 ml of organic solvents viz Acetone, Isopropyl alcohol and Petroleum ether, were added separately to run each for 24 hours. The filtrates extractions were taken in previously weighed evaporating Petri- dishes and used rotary vacuum evaporator to remove the excess solvents. After the complete evaporation, the weight of the extracts was recorded and then labeled. The extractions stored separately at 4^0C in amber colored airtight bottles.

Phytochemical Analysis

The freshly prepared extracts were subjected to standard preliminary phytochemical analysis for the presence of Alkaloids, Flavonoids, Carbohydrates, Tannin and Phenolic Compounds [12,13]. And the results were recorded.

Preparation of Discs for Antibacterial Activities

The Observing capacity of 5mm sterile discs (*HIMEDIA*) was selected to hold 10µl to 50µl. Hence the preparation of stock solution, 10mg of each crude extract was dissolved in 1ml of DMSO. From these stock, 10µl, 20µl, 30µl, 40µl and 50µl was added on the sterile discs to get the concentration of 100µg, 200µg, 300µg, 400µg, and 500µg respectively of plant extracts. Then the prepared discs were dried in controlled temperature to remove excess of moisture and used for antibacterial activity.

Antibacterial Activity of Plant Extracts and Antibiotics

The disc diffusion method was employed to determine the antibacterial activity of the Acetone, Isopropyl alcohol and Petroleum ether, extracts of leaves of *Mentha arvensis Linn*. and different antibiotics were used. The cells of *Proteus mirabilis* (Isolated strain and MTCC 442 Strain) were spread over the Muller-Hinton agar medium using sterile cotton swab horizontally and vertically in order to get a uniform microbial growth. Then the prepared discs with compounds were placed on upper layer of the inoculated plates using sterile forceps. Then the plates were incubated for 18-24 hours at 37°C. After the incubation, the diameter of the zone of inhibition could be measured and the values were recorded [14,15].

Minimal Inhibitory Concentration (MIC)

The broth dilution technique was used where the plant extract was prepared to the highest concentration of 500mg/ml (stock concentration) in DMSO and serially diluted (two-fold) to a working concentration ranging from 0.97 mg/ml to 250mg/ml using peptone broth. And the tubes were inoculated with 0.1ml suspension of the test organisms. Control was used with peptone broth, plant extract and without test organism. After 24hours of incubation at 37^0C, the tubes were observed for turbidity. The least concentration where no turbidity was observed and it was determined as MIC value [16].

Minimal Bactericidal Concentration (MBC)

To determine the MBC, from each set of test tubes in the MIC reports, a loopful of inoculum from each tube was transferred into nutrient agar medium plates. The inoculated plates were incubated at 37^0C for 24 hours. The lowest concentration of the plant extract has shown no bacterial growth. Then the results were recorded as the MBC Value [17].

Results

In this study the number of 63 urine samples were collected from infected persons from various Govt.hospitals in Salem District, Tamil Nadu and India. All the collected samples were cultured and identified for the presence of *Proteus mirabilis*. Among these 63 clinical samples, 47 as positive and 16 as negative. From the 47 positive samples, 27 as samples infected with *Proteus mirabilis* and 20 samples infected with other UTI pathogens. From the 27 clinical samples 11 samples infected with Males and 16 samples infected with Females.The identification of *Proteus mirabilis* on the basis of Colony morphology with different media included as Nutrient agar medium, MacConkey agar medium, UTI agar media, XLD agar, CLED agar, gram staining reaction and motility test by hanging drop

method the results of biochemical analysis also recorded. The antibiotic sensitivity pattern was determined by the Disc Diffusion technique using s standard antibiotic discs against *Proteus mirabilis* MTCC –442 and clinical isolates of *Proteus mirabilis*. Ciprofloxacin and ofloxacin showed the higher zone formation in *Proteus mirabilis* MTCC-442 and clinical isolates of *Proteus mirabilis*. The antibiotic Sensitivity pattern of the *Proteus mirabilis* MTCC-442 and clinical isolates of *Proteus mirabilis* were tabulated (Fig-3).

The Plant extracts were appeared as semisolid and liquid form with viscosity. Color, amount and time taken for extracting full extract was differ among plant extracts and the three type of solvents used and tabulated (Table-2). Time taken for extracting acetone extract of *Mentha arvensi Linn.* was high and Isopropyl alcohol extract of *Mentha arvensi Linn* was low. Appearance and time taken for extracting full extracts in *Mentha arvensi Linn.* were noted and Microbial growth was absent in all the plant extracts. DMSO and empty sterile disc did not show any inhibitory effect. So inhibitory effect of the solvents was negligible.

The minimum concentration for the zone formation was in 100µg in all the plant extracts and maximum with 500µg. The highest inhibitory activity was in acetone extract and lowest inhibitory effect was in Petroleum ether of *Mentha arvensi Linn.* (Table-4). MIC and MBC values of *Mentha arvensis Linn.* from various extracts includes, Acetone extract (62.5mg/ml) and Isopropyl alcohol (31.25mg/ml) extracts of *Mentha arvensis Linn*. had high MIC and MBC value. Petroleum ether extract of (15mg/ml), had low MIC and MBC value (Table-5&6).

The Preliminary Phytochemical screening of *Mentha arvensis Linn.* on different extracts have shown the following results. Acetone extract showed the being there of alkaloids, Tannins and phenolic compounds and carbohydrates, but the absence of proteins and amino acids, flavonoids, and Phenolic compounds and Phytosterol. Isopropyl alcohol extract showed the presence of alkaloids, flavonoids, tannin and Phenolic compounds, and carbohydrates, but the absence of, proteins and amino acids, Anthraquinone glycosides, saponins and Phytosterol. Petroleum ether extract showed the presence of alkaloids and flavonoids, but the absence of proteins and amino acids, Anthraquinone glycosides, tannin and Phenolic compounds and carbohydrates. These results were shown in Table-3.

Table 1: Age group distribution in Male and Females with Urinary tract infection

Age groups (in years)	Males	Percentage	Females	Percentage
20-30	10	38.46	15	44.11
30-40	4	15.38	13	29.41
40-50	8	30.76	8	23.52
50-60	4	15.38	1	2.94
Total	26	100	37	100

Table 2: The yield of the various extracts of *Mentha arvensis Linn.*

S.No	Name of the solvents	Colour	Form	Extract weight (in grams)
1	Acetone	Brown	Jelly syrup	4g
2	Isopropyl alcohol	Green	Jelly syrup	3g
3	Petroleum ether	Light green	Oily paste	3g

Table 3: Preliminary Phytochemical Screening of Extracts of *Mentha arvensis* Linn.

S/N	Constituents / Tests	Acetone	Isopropyl alcohol	petroleum ether
1	Alkaloids			
	Mayer's test	—	—	+
	Dragendorff's test	—	—	—
	Hangers test	—	—	—
	Wagers test	—	—	—
2	Proteins & Amino acids			
	Millon's test	—	—	—
	Ninhydrin test	—	—	—
	Biuret test	—	—	—
3	Anthraquinone glycosides			
	Borntragers test	—	—	—
4	Flavonoids			
	Shimoda's test	+	—	—
	Ferric chloride test	—	—	—
5	Tannins & Phenolic compounds			
	Ferric chloride test	++	—	—
	Lead acetate test	+	—	—
	Gelatin contains NaCl test	++	—	—
6	Carbohydrates			
	Molisch's test	++	++	—
	Barfoed's test	+	—	—
	Fehling test	++	+	—
7	Saponins			
	Frothing test	—	—	—
8	Phytosterol			
	Liebermann-Burchard's test	—	—	—

Note: — = Negative (absent), + = positive (slightly present), ++ = positive (mode rately present)

Table 4: Antibacterial activity of various solvent extract of *Mentha arvensis Linn.* against *Proteus mirabilis* MTCC-442 & Isolated strain.

S. N.	Name of the solvents	*Proteus mirabilis*									
		MTCC-442					Isolated strain				
		Zone of inhibition (in mm)									
		(µg/disc)									
		100	200	300	400	500	100	200	300	400	500
1	Acetone	14	17	19	20	21	19	23	24	25	26
2	Isopropyl alcohol	15	17	19	20	21	17	20	21	23	24
3	Petroleum ether	12	15	16	18	19	15	17	18	21	22

Fig-1

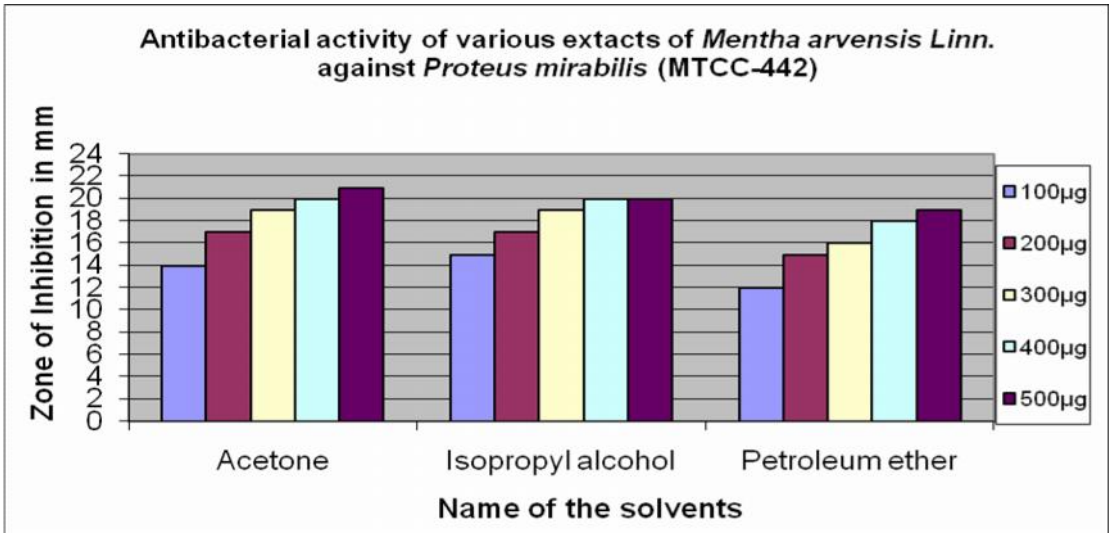


Fig-2

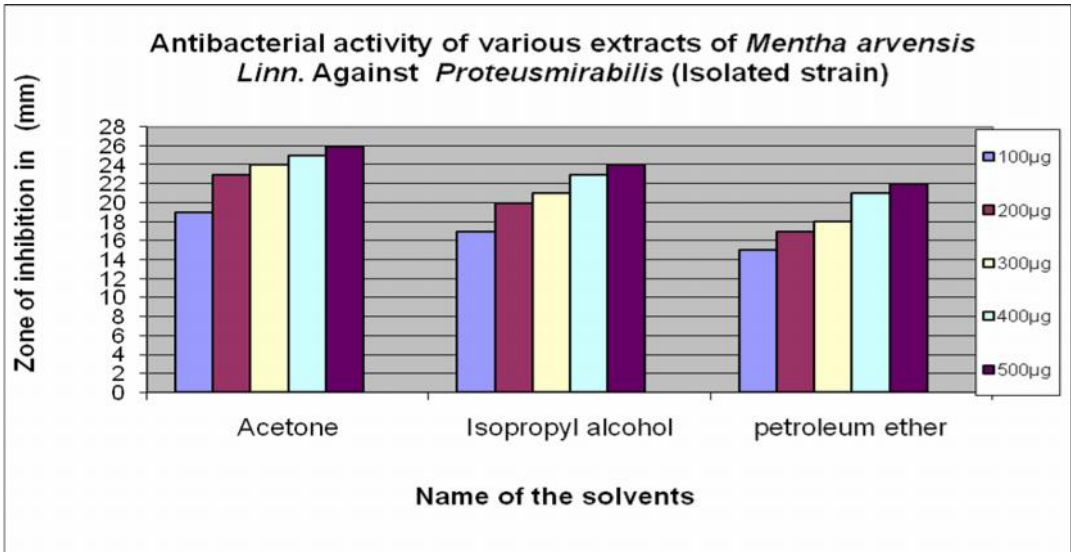


Fig-3

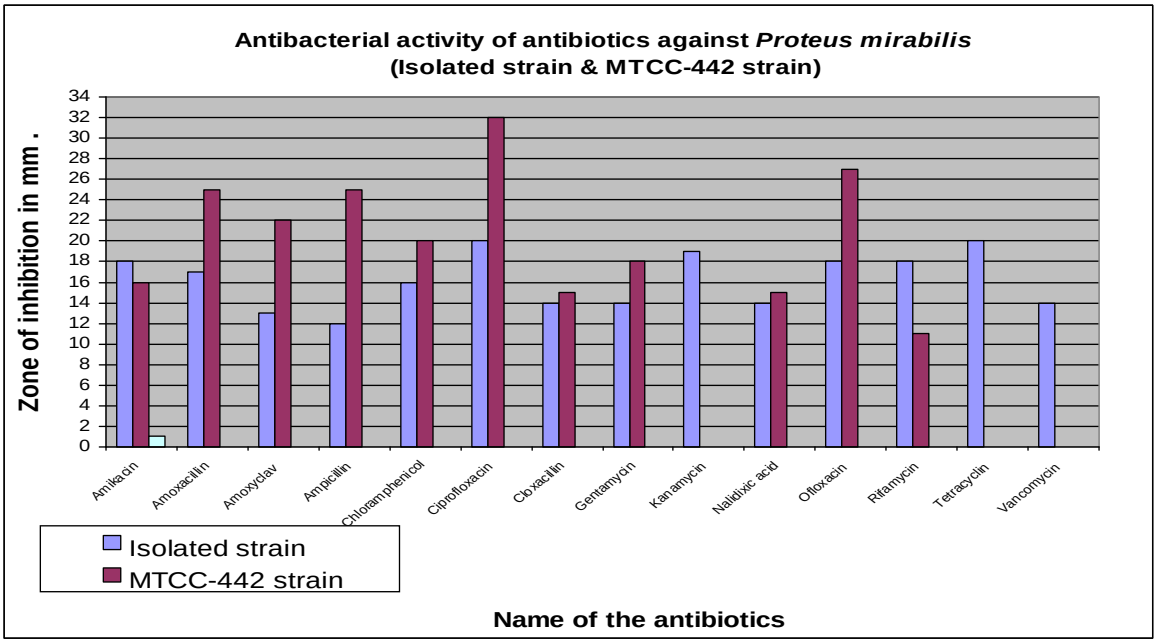


Table 5: Minimal Inhibitory Concentration (MIC) of Various Solvents Extract of *Mentha arvensis Linn.* against *Proteus mirabilis* MTCC-442 & Isolated Strain.

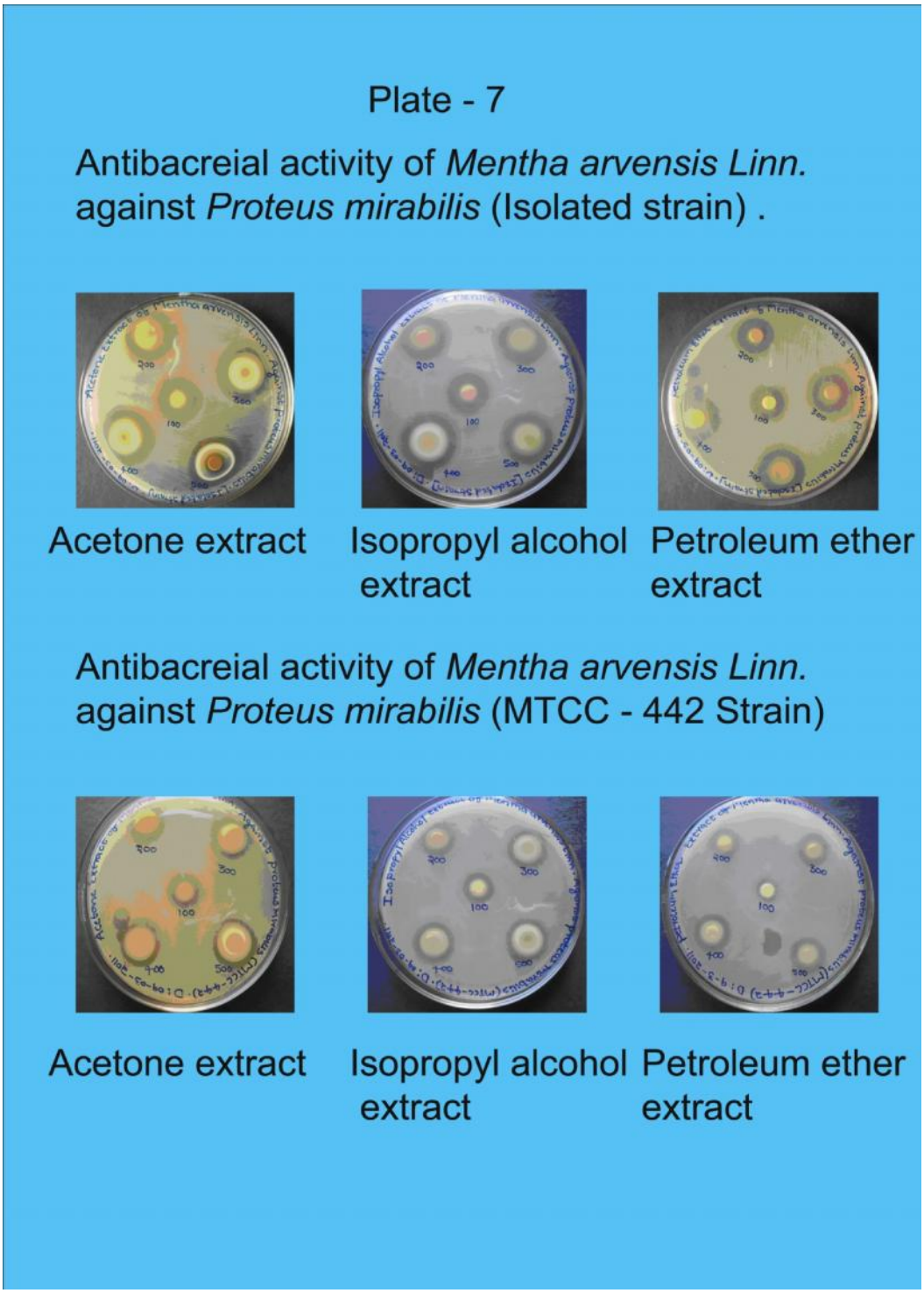
Name of organism	Name of solvents	Extract concentration (mg/ml)								
		Proteus mirabilis MTCC- 442								
		0.97	1.95	3.90	7.81	15.6	31.2	62.5	125	250
Proteus mirabilis	Acetone	+	+	+	+	+	β	-	-	-
	Isopropyl alcohol	+	+	+	+	+	+	+	β	-
	Petroleum ether	+	+	+	+	+	+	β	-	-
	Proteus mirabilis Isolated strain									
	Acetone	+	+	+	+	+	β	-	-	-
	Isopropyl alcohol	+	+	+	+	+	+	β	-	-
	Petroleum ether	+	+	+	+	+	+	β	-	-

Note: + = Turbidity observed. - = No turbidity observed. β =MIC value.

Table 6: Minimal Bactericidal Concentration (MBC) of Various Solvents Extract of *Mentha arvensis Linn.* against *Proteus mirabilis* MTCC-442 & Isolated Strain.

Name of organism	Name of solvents	Extract concentration (mg/ml)								
		Proteus mirabilis MTCC- 442								
		0.97	1.95	3.90	7.81	15.6	31.2	62.5	125	250
Proteus mirabilis	Acetone	+	+	+	+	+	β	-	-	-
	Isopropyl alcohol	+	+	+	+	+	+	+	β	-
	Petroleum ether	+	+	+	+	+	+	β	-	-
	Proteus mirabilis Isolated strain									
	Acetone	+	+	+	+	+	β	-	-	-
	Isopropyl alcohol	+	+	+	+	+	-	β	-	-
	Petroleum ether	+	+	+	+	+	β	-	-	-

Note: + = Turbidity observed. - = No turbidity observed. β =MIC value



Discussion

Urinary tract infection occurs more frequently in women than men. Incidence of UTI occurs in men younger than 50 years are usually caused by urologic abnormality. From this present study, a total of 63 urine samples were collected among them 40 samples showed positive. And remaining 20 samples were showed negative. Similar work carried out by Khan Azizm. *et al*., 2006. From their report, they collected 100 UTI samples from those 48 samples from male and 52 samples from females with UTI. In their report they found UTI caused by gram negative bacteria (*E. coli, Proteus.*sps).

Identification and screening was carried out to detect the presence of *Proteus mirabilis* using the biochemical characterization, Motility determination and colony formation on differential media like UTI agar, CLED agar, XLD agar, Nutrient agar, Macconkey agar. Likewise H.U khan, *et al*., 2006, have been identified gram

negative bacteria like *Pseudomonas* and *Proteus mirabilis* using the biochemical characterization and growth on CLED agar medium. In the present study Ciprofloxacin and Ofloxacin showed high inhibitory action against *Proteus mirabilis*. Similar work was done [22] . In another similar work was carried out and reported that Ciprofloxacin and Gentamycin should considerd for treatment for UTI.

The extraction of crude extracts from *Mentha arvensis Linn.* using three different solvents was included as acetone, isopropyl alcohol and petroleum ether. And similar work carried out by Gupta Sandeep, *et al*., 2010, and they used the solvents as chloroform, ethyl alcohol and petroleum ether for crude extraction to detect the presence of secondary metabolites. In the present study preliminary phytochemical analysis result showed the presence of alkaloids, tannins, phenolic compounds and carbohydrates but the absence of proteins, amino acids, flavanoids and phyto sterol.

Antibacterial activity of various extracts of *Mentha arvensis Linn.* against *Proteus mirabilis* (Isolated and MTCC-442) was done by using disc diffusion method. From that study acetone and isopropyl alcohol extracts showed better result than petroleum ether. Similar work was carried out [20,21]. From their study extracts of *Mentha arvensis Linn.* exhibit exceptionally good antimicrobial effects against gram negative pathogenic bacteria. Their study the leaves of *Mentha arvensis Linn* exhibited highest selectivity (17.24mm) and least antibacterial activity (15.82mm) against gram negative bacteria and similar work was carried out by Alper sener and basaran Dulger in 2009 from their report the ethanolic extract of leaves of *Verbascum sinuatum L* showed good antibacterial activity against UTI pathogens by using the same disc diffusion method and another similar work was carried out by Mohammad rahbar and Kambiz Diba, 2010 from their study Cranberry extract was showed good antibacterial activity especially on UTI pathogen in UTI patients.

From the present study MIC and MBC was observed that the broadest activity of extracts against *Proteus mirabilis* was 62.5µg/ml as MIC while the MBC of 31.2µg/ml was observed. Similar work carried out by Usman, *et al.,* 2007. They observed MIC and MBC of the extract against most gram negative organisms was 6.250mg/ml as MIC while the MBC of 1.563mg/ml was observed from their study. The extracts also showed some level activity against *E.coli* which is the common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infection in young women [18,24] .

Conclusion

In conclusion the extract of *Mentha arvensis Linn*. has showed better antibacterial activity against isolated *Proteus mirabilis* from Urinary Tract Infected patients and MTCC 442 strain. Therefore the potential of *Mentha arvensis Linn*. Product to act as a non antibiotic alternative for preventing UTI. There by reducing the total amount of antibiotics prescribed for treatment of UTI and preventing drug resistance. This study demonstrated that the extracts from the leaf of *Mentha arvensis Linn*. as effective as modern medicine to combat *Proteus mirabilis*, biological and pharmacological screening of this medicinal plant using the modern tool may leads to some new interesting drug.

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