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# Phytochemical Screening and Antibacterial Activity of *Moringa oleifera Lam.* against *Proteus mirabilis* from Urinary Tract Infected Patients

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**Abstract:** A total of 63 Urine samples were collected from infected persons from various hospitals in Salem District, Tamil Nadu, India. The collected samples were subjected to microscopic observation and biochemical characterization to identify the presence of *Proteus mirabilis.* Leaves of *Moringa oleifera* were extracted by using petroleum ether, acetone and isopropyl alcohol. Preliminary Phytochemical studies revealed that the presence of Alkaloids, Flavonoids, Carbohydrates, Tannin and Phenolic Compounds. Antibacterial activity of *Moringa oleifera* extracts were studied by disc diffusion method, which shows better activity against the *Proteus mirabilis* clinical isolate and MTCC 442 strain. MIC and MBC was performed by agar dilution method and the range was found to be 0.78mg/ml to 400mg/ml.

**Key words:** Phytochemical Screening, Antibacterial activity, *Moringa oleifera, Proteus mirabilis.*

## Introduction

Urinary tract infections are the second most common type of infection in the world. It is mainly a bacterial infection that affecting peoples throughout their lifespan [1]. These are more common in women than men, leading to approximately 8.3 million doctor visits per year. *Proteus mirabilis* commonly causes of urinary tract infections and formation of stones. This is a part of Enterobacteriaceae family. It is a small gram-negative bacillus, facultative anaerobe and posses swarming motility [2], ability to ferment maltose and inability to ferment lactose. *It* is a one type of bacteria that can be also found in water, soil and gastrointestinal tract. Commonly used antibiotics for the treatment of urinary tract infections are Clindamycin, Vancomycin, Bacitracin, Ampicillin, Chloramphenicol and Erythromycin. *Moringa oleifera* commonly referred as Moringa. It is an exceptionally nutritious vegetable tree with a variety of potential uses. These leaves have high medicinal value [3]. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti tumor [4], antipyretic, antiepileptic, anti inflammatory, antiulcer, [5] antispasmodic, diuretic [6,7], antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepato protective, antibacterial and antifungal activities[8],and are being employed for the treatment of different ailments in the indigenous system of medicine [9].

## 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, 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$^{o}$C until use.

**Plant Materials**

Fresh plants of *Moringa oleifera* LAM were collected from the surroundings of Namakkal District, Tamil Nadu, India. The collected plant species were identified and confirmed (Herbarium Voucher Number Acc. No. M/AU/113) by Dr. R. Selvaraj, Professor, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

**Preparation of Crude Extracts**

Fresh leaves in bulk were locally obtained from *Moringa oleifera* 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 Petroleum ether, Acetone, Isopropyl alcohol 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$^{0}$C 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 [10, 11]. 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**

The disc diffusion method was employed to determine the antibacterial activity of the Petroleum ether, Acetone and Isopropyl alcohol extracts of leaves of *Moringa oleifera.* 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 [12].

**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 0.78mg/ml to 400mg/ml using peptone broth. And the tubes were inoculated with 0.1ml suspension of the test organisms. Control were used with peptone broth, plant extract and with out test organism. After 24hours of incubation at 37$^{0}$C, the tubes were observed for turbidity. The least concentration where no turbidity was observed and it was determined as MIC value [13].

**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$^{0}$C for 24 hours. The lowest concentration of the plant extract has shown no bacterial growth. Then the results were recorded as the MBC Value [14].

**Table. 1 Preliminary Phytochemical Studies on Various Solvent extracts of *Moringa oleifera Lam.***

| S.No | Constituents | Name of the Tests/Reagents | Petroleum Ether | Acetone | Isopropyl Alcohol |
|---|---|---|---|---|---|
| 1 | Alkaloids | Mayer's reagent | + | + | + |
| | | Dragondroff's Reagent | - | - | - |
| 2 | Protein and Amino acids | Million's reagent | - | - | - |
| | | Ninhydrin reagent | - | - | - |
| | | Biuret test | - | - | - |
| 3 | Anthraquinone Glycosides | Borntrager's test | - | - | - |
| 4 | Flavonoids | Shimoda's test | + | - | + |
| 5 | Tannin and Phenolic Compounds | $Fecl_3$ | - | - | - |
| | | Gelatin & Nacl | + | + | + |
| | | Lead Acetate | + | + | + |
| 6 | Carbohydrates | Molisch' test | + | + | - |
| | | Fehling's test | - | - | - |
| 7 | Saponins | - | - | - | - |
| 8 | Phytosterol | Liebermann Burchared test | - | - | - |

+ - Positive, – - Negative

**Table. 2 Antibacterial activity of Various Solvent Extract of *Moringa oleifera* Lam. against *Proteus mirabilis (*MTCC-442 Strain and Isolated Strain)**

| S. No | Name of the Solvents | *Proteus mirabilis* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MTCC-442 Strain | | | | | Isolated Strain | | | | |
| | | Zone of Inhibition (in mm) | | | | | | | | | |
| | | 100 | 200 | 300 | 400 | 500 | 100 | 200 | 300 | 400 | 500 |
| | | (µg/disc) | | | | | | | | | |
| 1 | Petroleum Ether | 11 | 13 | 14 | 16 | 18 | 14 | 18 | 22 | 25 | 28 |
| 2 | Acetone | 10 | 12 | 15 | 15 | 16 | 16 | 17 | 20 | 23 | 22 |
| 3 | Isopropyl Alcohol | - | 10 | 13 | 13 | 15 | 13 | 17 | 17 | 19 | 15 |

**Table. 3 Minimal Inhibitory Concentration (MIC) of Various Solvent Extract of *Moringa oleifera* Lam. against *Proteus mirabilis* MTCC 442 & Isolated Strain**

| Name of the Organism | Name of the Solvents | Extract concentration (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Proteus mirabilis* MTCC 442 | | | | | | | | | |
| | | 0.78 | 1.56 | 3.12 | 6.25 | 12.5 | 25 | 50 | 100 | 200 | 400 |
| *Proteus mirabilis* | Petroleum Ether | - | - | - | β | + | + | + | + | + | + |
| | Acetone | - | - | - | - | β | + | + | + | + | + |
| | Isopropyl Alcohol | - | - | - | - | β | + | + | + | + | + |
| | | *Proteus mirabilis* Isolated strain | | | | | | | | | |
| | Petroleum Ether | - | - | - | - | β | + | + | + | + | + |
| | Acetone | - | - | - | - | - | β | + | + | + | + |
| | Isopropyl Alcohol | - | - | - | - | - | β | + | + | + | + |

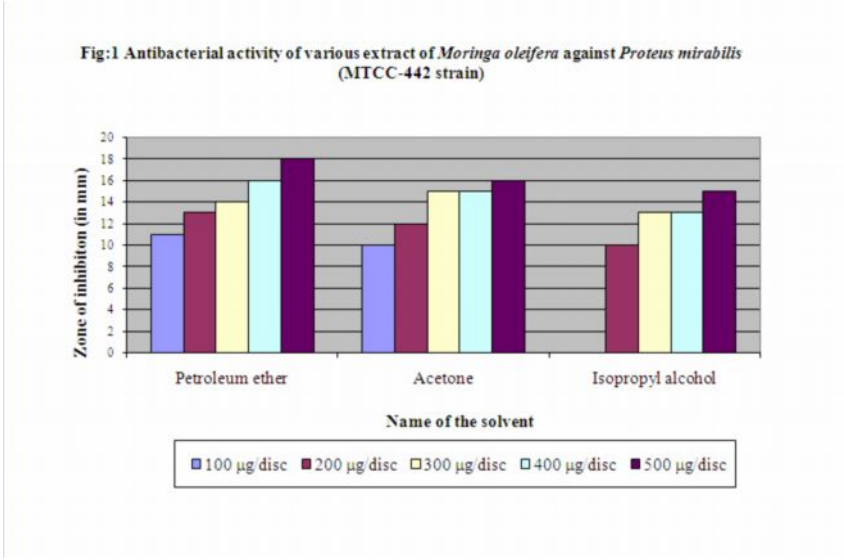
– Resistance (growth of bacteria or turbidity)

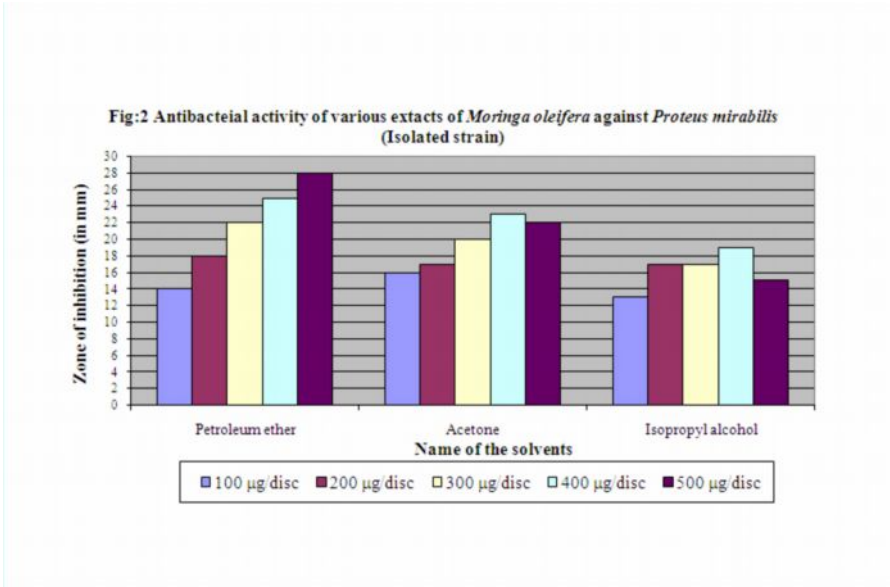
+ = Concentration showing no turbidity, β = MIC Value

**Table. 4 Minimal Bactericidal Concentration (MBC) of Various Solvent Extract of *Moringa oleifera* Lam. against *Proteus mirabilis* MTCC 442 & Isolated Strain**

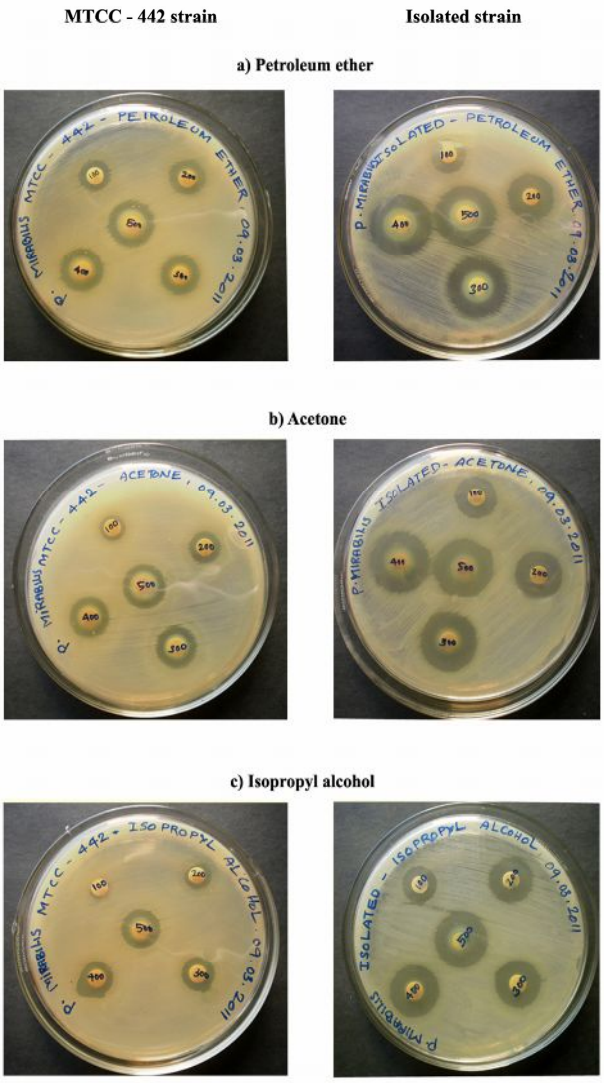
| Name of the Organism | Name of the Solvents | Extract concentration (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ***Proteus mirabilis* MTCC 442** | | | | | | | | | |
| | | **0.78** | **1.56** | **3.12** | **6.25** | **12.5** | **25** | **50** | **100** | **200** | **400** |
| *Proteus mirabilis* | Petroleum Ether | - | - | - | β | + | + | + | + | + | + |
| | Acetone | - | - | - | - | β | + | + | + | + | + |
| | Isopropyl Alcohol | - | - | - | - | β | + | + | + | + | + |
| | ***Proteus mirabilis* Isolated strain** | | | | | | | | | | |
| | Petroleum Ether | - | - | - | - | β | + | + | + | + | + |
| | Acetone | - | - | - | - | - | β | + | + | + | + |
| | Isopropyl Alcohol | - | - | - | - | - | β | + | + | + | + |

– = Resistance (growth of bacteria or turbidity),
+ = Concentration showing no turbidity, β = MIC Value



**Antibacterial activity of various extract of *Moringa oleifera* Lam. against *Proteus mirabilis***




## Results and Discussion

In our present study, 63 urine samples were collected and possessed 47 as positive and 16 as negative. From the 47 positive samples, 27 samples (10 male and 17 as female) infected with *Proteus mirabilis* and 20 samples infected with other urinary tract pathogens. Similar work was carried out by Sowmiya, *et al*., (2009) and reported as from the collection of 25 UTI samples, 68% of samples were as female and 32% as male. The isolated *Proteus mirabilis* strain could be conformed by morphological characterization and biochemical analysis were recorded. Preliminary phytochemical analysis was performed for the presence of Alkaloids, Flavonoids, carbohydrates, Tannin and phenolic compounds (Table.1). The similar work have been carried out by Gayatri Dewangan, *et al*., (2010) [15] and used the acetone, chloroform and methanol extraction to detect the secondary metabolites.

According to antibacterial activity with *Moringa oleifera* leaves, the highest inhibitory activity in petroleum ether and the lowest inhibitory activity in isopropyl alcohol extract against *Proteus mirabilis* were recorded (Table.2). Ankinpelu and Onakoya (2006) [16] have been reported the MIC of

*Psidium guajava* extract against the test organisms varied between 0.313mg/ml and 0.625mg/ml while that *Mangifera indica* ranged between 1.25mg/ml and 10.0mg/ml. In our present study, MIC and MBC values of *Moringa oleifera* from various extracts includes, Petroleum ether (6.25mg/ml), Acetone (12.5mg/ml) and Isopropyl alcohol (12.5mg/ml) against *Proteus mirabilis* (Both Isolated strain and MTCC-442 strain) were also recorded (Table. 3 & 4).

The result of the antibacterial assay shows the antibacterial activity of leaves of *Moringa oleifera* Lam. From the above evidence, plant extracts have high antibacterial compounds against *Proteus mirabilis* and that they can be used in the treatment of urinary tract infection. This study reported that the precious therapeutic value of Drumstrick tree or *Moringa oleifera*. Thus, the activity guided phytochemical studies may lead to development of novel agents for various disorders. In future, *Moringa oleifera* may help to discover new chemical classes of antibiotic substance that could serve as selective agents against infectious diseases.

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