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ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* STEM BARK AGAINST URINARY TRACT INFECTIONS PATHOGENIC BACTERIA

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ABSTRACT

Objective: To find out the effect of *Moringa oleifera* stem bark on various human pathogens causing urinary tract infections.

Materials and Methods: 30 samples of urine were collected at Sir Sundar Lal Hospital, Banaras Hindu University, from July 2010 to July 2011. Test samples were cultured immediately after collection and cultured bacterial stain was identifies by comparison of certain characteristics with standard. Sensitivity of extracts of *M. oleifera* [ethanolic extract (EtMO) and hydroalcoholic extract (HyMO)] and ciprofloxacin were done by disc diffusion method.

Results: In our study, a high incidence of *E. coli* (66.67%), *K. pneumonia* (16.67%) and *P. mirabilis* (10.00%) were found. 80% of the cultured samples were responded to EtMO while 83.33% samples to the HyMO. Moreover, 85% *E. coli*, 50% *P. aeruginosa*, 33.33% *P. mirabilis* and 20% *K. pneumonia* were found to be resistant to ciprofloxacin.

Conclusion: Thus, the study establishes the importance of *M. oleifera* stem bark used in Ayurveda for the management of UTI.

Keywords: *M. oleifera*, extract, Urinary Tract Infections, resistance.

INTRODUCTION

Urinary tract infections (UTI) is a global health condition affected about 8.3 million of people each year.[1] Primarily the UTI was caused by gram–negative bacteria and involvement of gram–positive bacteria was very less. *Escherichia coli* (80%), *Proteus mirabilis*, *Klebsiella pneumonia*, and *Enterobacter aerogenes* were common gram–negative bacteria while *Staphylococcus saprophyticus* (10–15%), *Enterococci*, and *Staphylococcus aureus* were gram–positive bacteria.[2] Uncontrolled use of antibiotics resulted in bacterial resistant, which converted into a threatening condition to the present world.[3] *Staphylococcus, Pseudomonas* and *Escherichia* were most susceptible bacteria for development of multi drug resistance (MDR). In recent years, the problem of the drug resistance to human pathogenic is growing day by day and the outlook for the use of drugs in future aspect is uncertain.[4] Therefore, the world looks at some alternative and effective medicine particularly of natural origin with ultimate goal to provide efficient drugs to the patient. Numerous efforts have been done worldwide to

find out novel antimicrobial agents from plants[5-8] which may act through different mechanisms other than those currently used antimicrobials and might be noteworthy in clinical practice for the management of MDR microbial strains.[9] These practices are based on traditional[10] and cultural uses.[11] Therefore, there is a vital need for thoroughly investigating such plants not only on laboratory bacterial strain but also clinically isolated microbe to develop them into magic bullet to treat various disease conditions.

Horse radish tree *(Moringa oleifera Lam.* Fam. Moringaceae) is indigenous to sub-Himalayan regions of northwest India, Southeast Asia, Africa, the Pacific and Caribbean Islands. Fresh stem bark is used for the treatment of fractured bones and Cattle dysentery.[12] In Indian ethnotherapeutic system of medicine, the plant is used for *mutra rogas* (urinary disorder), *jvara* (fever), *vidradhi* (abscess), *shotha* (edema), *shula* (pain), *krimi* (helminthes), *abhishyanda* (conjunctivitis) and *vrana* (wound) where micro-organism may involve in pathogenesis.[13, 14] The stem bark used as antibacterial[15-19] and antifungal[19] agent against varieties of both gram positive and gram negative bacteria. It also has emmenagogue, abortifacient and antifertility effect.[20] Previously we reported that the drug was found effective in the management of *mutrakrichha* (UTI). In the present study we reports its effect on clinically isolated bacteria from the patients of UTI.[21]

MATERIALS AND METHODS

Preparation of plant extract

The drug was collected Varanasi and identification of drug was carried out in Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical sciences, Banaras Hindu University, Varanasi. Coarse powder of plant material was prepared with the help of mechanical grinder and 20# sieve. Powdered material (250g) was extracted by soxhlet extraction using ethanol (2L) for 7 day. Another extract was prepared by cold maceration process using hydoalcoholic solvent (30:70) for 24 h (shaking frequently for 6 h and the allowed to stand for 18 h). Both The extracts were filtered through Whatmann No. 1 filter paper separately and concentrated using rotary evaporator (Perfit India, Pvt. Ltd.) below 60°C to generate the crude extracts of *M. oleifera* bark and were finally stored in dessicator for further studies.

Collection of urine and isolation of bacterial isolates

Mid stream urine samples were collected in a sterile wide mouthed container from clinically diagnosed cases of UTI from the Dravyaguna Out Patient Department, Ayurveda wing, Sur Sunder Lal Hospital, Institute of Medical sciences, Banaras Hindu University, Varanasi **(Ref: Dean/2010–11/92).** Loops full of urine specimen were speckled in to the nutrient agar and Mac Conkey agar incubated at (37±2°C) for 24 h. Next day individual colonies were selected and the isolated bacterial strains were examined according to Bergey's Manual of Determinative Bacteriology.[22] Various morphological, physiological and biochemical tests were performed to identify correct bacteria.

In vitro Antimicrobial assay

Solutions of different concentration (50 mg/mL to 400mg/mL) of the test samples were made by dissolving calculated quantity of the samples in calculated volume of Dimethyl sulfoxide (DMSO). Several dried and sterilized filter paper discs [prepared from Whatman No. 1 filter paper (6 mm di-

ameter)] were placed on nutrient agar medium uniformly swabbed with the pathogens separately. Standard antibiotic Ciprofloxacin (10.0 µg/mL) and only solvents were used as a positive and negative control respectively. 10 µL of test, standard drug and solvent were placed on the disks separately using micropipette. After that the plates were kept at low temperature (4°) for 24 h to before incubation at 37°C for 24 h for maximum diffusion. Distinct zone of inhibition of surrounded media were measured.[23,24]

RESULTS AND DISCUSSION

Multi drug resistant (MDR) bacteria become a challenge before the health practitioners and microbiologist.[25] MDR and numerous side effects of available antibiotics are key factors which increases interest of scientist to find new antimicrobial agent from natural source.[26] The aim of present study was evaluate *in vitro* antimicrobial efficacy of the *M. oleifera* stem bark against some human clinical bacterial isolates (*Klebsiella spp. Pseudomonas spp. Proteus spp*. and *E. coli*). 4 bacterial strains were isolate from these patients viz. *E. coli, K. pheumoniae, P. aeruginosa, P. mirabilis* and named as E1-E20, K1- K5, Ps1- Ps3 and P1- P2 respectively. Subsequent to the isolation procedures bacteria of different types were identified. There has been a high incidence of *E. coli* (66.67%), *K. pneumonia* (16.67%), *P. mirabilis* (10.00%). The results of antibiotic susceptibility test of the bacterial isolates indicated that 85% *E. coli*, 50% *P. aeruginosa*, 33.33% *P. mirabilis* and 20% *K. pneumonia* were resistant to ciprofloxacin.

The antibacterial efficacy of various plant extracts showed varied level of inhibition against the human pathogenic bacteria. In the present study two different extracts of moringa stem bark, EtMO and HyMO were evaluated against clinically isolated bacteria. In case of *E. coli* and *K. pneumonia* isolates, the highest zone of inhibition was 20.33±0.88 mm and 15.00±0.58 mm which were exhibited by EthMO. HyMO exhibited highest activity against *P. mirabilis* (17.67±0.67 mm). HyMO also exhibited significant inhibitory effect against *E. coli* and *P. aeruginosa* (15.00±0.58 mm) isolates. Thus, the study ascertains the value of *M. oleifera* stem bark used in Ayurveda for the management of UTI, different kind of fever, wound etc.

CONCLUSION

The present work shows that most of the studied plants are potentially a good source of antimicrobial agents and demonstrates the importance of this plant in UTI medicine and alternative healthcare. It validates their traditional uses from a scientific point of view. It is important to find new plant and to obtain documentation on traditional remedies before they disappear. Further studies should to bring to bear the active agents in the therapeutic process.

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Table 1. Antimicrobial activities against *E. coli* isolates.

Isolated bacteria	EtMO	HyMO
E1	10.67±0.33	13.33±0.33
E2	12.33±0.88	13.00±1.00
E3	18.00±0.58	15.33±0.88
E4	18.33±0.33	16.00±0.58
E5	-	-
E6	20.33±0.88	12.00±1.00
E7	10.33±0.88	12.67±0.67
E8	10.00±0.58	14.00±0.58
E9	12.67±0.67	12.67±0.33
E10	14.00±0.58	10.33±0.88
E11	11.00±0.58	10.00±0.58
E12	10.33±0.88	12.33±0.88
E13	11.33±0.33	15.00±0.58
E14	-	-
E15	11.67±0.67	11.33±0.33
E16	-	10.00±1.00
E17	11.33±0.33	-
E18	14.67±0.33	13.00±1.00
E19	11.67±0.88	12.33±0.33
E20	11.00±1.53	15.67±0.88

Table 2. Antimicrobial activities against *K. pneumonia* isolates.

Isolated bacteria	EtMO	HyMO
K1	11.33±0.88	13.67±0.67
K2	15.00±0.58	15.00±0.58

K3	11.67±0.88	12.67±0.67
K4	-	-
K5	12.00±0.58	11.67±0.67

Table 3. Antimicrobial activities against *P. aeruginosa* isolates.

Isolated bacteria	EtMO	HyMO
P1	14.00±1.15	14.67±0.67
P2	-	11.00±0.88

Table 4. Antimicrobial activities against *P. mirabilis* isolates.

Isolated bacteria	EtMO	HyMO
Ps1	12.67±0.67	17.00±0.11
Ps2	-	-
Ps3	16.33±0.33	17.67±0.67

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