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Antibacterial Potential of *Emblica officinalis*, *Terminalia bellerica*, *Terminalia chebula* and Triphala against UTI Pathogens

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ABSTRACT

The aqueous and ethanol extracts of three medicinal plants and their combined formulation (triphala) were investigated to evaluate their antibacterial activity against 100 multidrug resistant pathogens. Urinary tract infection (UTI) isolates belonging to different bacterial genera (*E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus)* by agar well diffusion method.. Ethanol extract of all the four herbal extracts produced consistently greater zone of inhibition than aqueous extract against all the isolates of all the five bacteria isolated in present investigation, suggesting that ethanol is better solvent than water for extracting active antimicrobial compounds in herbal extracts. Further, minimum inhibitory concentration (MIC) values of all the four ethanol herbal extracts was investigated. *Terminalia bellerica* (baheda) was found to be highly effective against *E. coli* and *Pseudomonas aeruginosa*, producing lowest MIC value (12.5 mg/ml for both). *Terminalia chebula* (harad) also showed significant antibacterial activity against *Klebsiella pneumoniae*, *Proteus vulgaris* and *Staphylococcus aureus* producing MIC value of 25, 12.5 and 6.25 mg/ml, respectively. These results indicated that *Terminalia chebula* possesses potential broad spectrum antimicrobial activity and a search for active compound is needed. Thus, from the present study it is revealed that baheda and harad were the most effective antimicrobial agent among four herbal extracts used against urinary extract infection. Present study also suggested that these herbal preparations may be useful as an effective alternative in treatment of UTI.

Introduction

The experience of human misery in the form of disease is as old as the inception of man on earth. Infectious diseases are the world's leading cause of premature deaths, killing 50,000 people every day (WHO, 2000). Urinary tract infection is prevalent and disruptive disease and is the most common bacterial infection seen by physicians (Winickoff *et al.*, 1981; Tessema *et al.*, 2007). Urinary tract infection is the condition where infection caused by bacteria occurred anywhere

in the urinary system. An infection occurs when microorganisms cling to the opening of urethra and begin to multiply (Schaeffer *et al.,* 2001).

UTI can be caused by different microorganisms. The most common etiological agents of this infection are Gram negative bacteria particularly those which are normally occurring in the gastrointestinal tract. Among the bacteria which are responsible for UTI, *Escherichia coli* are the most common. Many other UTI causing genera are also isolated from patients with variable degree of infection such as *Klebsiella, Enterobacter, Proteus, Serratia and Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ronald, 1989).

Although, many drugs have been introduced for UTI such as norfloxacin, ciprofloxacin, gentamycin, etc., the problem of drug resistance and toxic manifestations of long term use of drugs are common. Unfortunately, decades of antibiotic use has given rise to antibiotic resistance. The increasing drug resistance among these bacteria had made therapy of UTI difficult and has led to greater use of expensive broad spectrum drugs. This resistance problem needs a renewed effort resulting in searching effective antibacterial agents against pathogenic microorganisms resistant to current antibiotics (Soulsby, 2005). For which, we are now facing the threat of superbugs, i.e. pathogenic bacteria resistant to most or all available antibiotics..

This clearly highlights the need for new antibacterial agents with fundamentally different modes of action than that of traditional antibiotics. The enormous demand has triggered worldwide efforts in developing novel antibacterial alternatives, particularly the screening of several medicinal plants for their potential antimicrobial activity. For this reason, researchers are increasingly turning their attention to herbal products and looking for new leads to develop better drugs against MDR microbe strains. Therefore, an effort was made to evaluate the antibacterial potential of herbal ayurvedic products against pathogens causing UTI. In the present investigation, dry fruits of different plants were collected and their extracts were screened for antimicrobial activity against UTI isolates. Thus, this study aims to find out the potential antimicrobial activity of the extracts obtained from the fruits of *Emblica officinalis, Terminalia bellerica, Terminalia chebula,* and that of triphala.

The extracts were obtained in organic as well as aqueous solvents and their antimicrobial activity was compared. The activity of the extracts was finally quantitatively estimated in terms of minimum inhibitory concentration (MIC).

Materials and Methods

Plant material

Dried fruits of amla, baheda and harad were collected from Shantikunj, Hardwar. The mixture of all the three in equal amount constituted the triphala.

Bacterial isolates

Bacterial isolates of UTI were isolated from urine samples obtained from patients suffering from UTI. The samples were collected from Subharti Medical College and Lokpriya Nursing Home, Meerut. Among 200 samples collected, 120 belong to female patients and 80 belong to male patients.

Methods

Collection of samples

The samples were collected in wide mouthed plastic universal containers (350 ml) by clean catch midstream urine method. Once collected, the specimens were transported to the lab (Department of Microbiology, C.C.S. University, Meerut) without any delay and preserved in refrigerator at 4 ^{0}C. Besides the organisms isolated, reference strains which were procured from IMTECH, Chandigarh were used as positive control.

Isolation of pure cultures from urine samples

All specimens received from hospitals may or may not contain urinary tract pathogens. Therefore, they were cultured to test whether the urine sample contained the infectious UTI pathogens. So, the bacterial count was done using plate count method. The serial dilutions of 12-24 h old urine samples were done upto 10^{-6} and that dilution was spread on the nutrient agar plate. The plates were incubated overnight and the colony count was performed next day. The

samples found positive (microbial count = or > 10^5 CFU/ml of urine) were further tested and those found negative were discarded.

Identification of UTI isolates

The cultures were identified on the basis of colony morphology, colour of pigmentation, variations in the colony size, microscopic characters, biochemical tests and other standard characters using standard reference books (Collins *et al.*, 1995; MacCarty *et al.*, 2000).

Collection and processing of herbal samples

A total of 3 herbal samples and their combination were selected and used for the experimental study to determine their antimicrobial activity against UTI isolates. The fruits were washed with clean water and allowed to air dry. This was done to reduce the microbial load of the plant material during handling and transportation. The fruits are dried in a hot air oven at 45 ^{0}C till constant weight is achieved. The dried fruits were grounded in milling machine (Inalsa, Mixer grinder) to obtain the fine dry powder. The powder was weighed using single pan electronic weighing balance (Ohaus model). Equal amount of the three herbal powders were mixed to form triphala.

Preparation of herbal extracts

The solvents used for extraction were distilled water and ethanol. The herbal extracts were prepared at the rate of 1g /5mL of the solvent in a 250 mL Erlenmeyer flasks. The flasks were closed with cotton plug and aluminum foil. The herbal powder was soaked in the desired solvent for 48 h at room temperature with intermittent shaking. The mixture was centrifuged at 3500 × g for 20 min and finally filtered through Whatmann filter paper No.1. The pellet was discarded and the supernatant was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Buchi Type) until semisolid substance was obtained. This was dried inside the crucible under a controlled temperature (45 ^{0}C) to obtain solid powder (Jonathan and Fasidi, 2003). The process of extraction was repeated until it was reduced to half of its original weight. The powder was weighed and reconstituted in 500 mg/mL dimethyl

sulfoxide (DMSO) and stored in the refrigerator at 4 ^{0}C for testing antimicrobial sensitivity.

Screening of herbal extracts for antibacterial activity

The antibacterial activity of herbal extracts in different solvents was determined by agar well diffusion method (Okeke *et al.*, 2001).

The standardized inoculum (10^6 CFU / mL) of each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton agar (MHA) plate (Hi Media) to achieve a confluent growth. Subsequently, 50-µL volume of the test extract prepared in different solvents was poured in a well into MHA cultures. Sterile DMSO served as a negative control. For each test species and for each test substance, three independent replicates were used. The plates were allowed to stand for 1 h or more for diffusion to take place and then incubated at 37 ^{0}C for 24 h. The zone of inhibition was recorded to the nearest size in mm (Norrel and Messely, 1997). Only extracts and solvent exhibiting apparent and maximum zone of inhibition were chosen for further studies.

Determination of MIC of screened herbal extracts

The minimum inhibitory concentration (MIC) was defined as the lowest concentration that completely inhibited the growth (ignoring faint haze or a single colony) for 24 h (Thongson *et al.*, 2004). The MIC for the crude extract was determined only for the 50% ethanol extracts of the test substance(s) by test method agar-well diffusion techniques.

In agar-well diffusion technique, a two-fold serial dilution of the test extracts was prepared by first reconstituting it in DMSO, then diluting it in sterile DMSO only, to achieve a decreasing concentration range of 100 mg/mL to 6.25 mg/mL. 50-µL volume of each dilution was added aseptically into Mueller Hinton agar plates that were already seeded with the standardized inoculums (10^6 CFU/mL) of the test bacterial cells. Sterile DMSO without herbal extract served as negative control. All the experiments were set in triplicate. The

test plates were incubated at 37 ^{0}C for 24 h. The lowest concentration of each extract showing a clear zone of inhibition was considered as the MIC.

	Collected samples	Negative samples	Positive samples
Male	80	55	25
Female	120	45	75
Total	200	100	100

Results and Discussion

In this study, only 100 (50%) out of 200 of the examined urine samples were confirmed as having UTI of which, 75 (75%) were from female patients and 25 (25%) from male patients. This is similar to the findings of Obi *et al.* (1996) who found that *E. coli* was more common in females (69%) as compared to males (37%). Mohammad Tariq (2010), Ravikumar (2010), (Falagas, 2009) and Schaeffer (2001) got similar results. Uzunovic (2006) also found that most UTI isolates were obtained from female patients (77.2%).

Details of positive samples sorted out from total 200 clinical samples of urine.

Prevalence of various bacteria causing UTI

Based on microscopic examination and different biochemical tests, following bacterial isolates were identified from positive urine samples investigated in present study : (i) *Escherichia coli* (70%), (ii) *Klebsiella pneumoniae* (10%), (iii) *Staphylococcus aureus* (10%), (iv) *Pseudomonas aeruginosa* (6%), and (v) *Proteus vulgaris* (4%). These results agree with the observations reported by Manzoor Kadri (2004) who also found that vast majority of UTI isolates were *E. coli* (90%), *Klebsiella* (8%), *and Staphylococcus aureus* (2%). These findings are also in

congruence of reported by S. Ravikumar (2010), Inabo and Obanibi (2006) and Patton (1991). It can also be concluded that in the present study, Gram-negative bacteria (*E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus vulgaris*) were the most common of uropathogens responsible for UTI with 90% in comparison to 10% of Gram-positive bacteria (*Staphylococcus aureus*), and that *E. coli* dominated the group of UTI causing organisms.This observation is supported by similar studies conducted by Newman *et al.* (2006) and Vasquez and Hand (2004).

Frequency of various bacteria isolated and identified from UTI.

S.No.	Bacteria	Frequency	% age
1	*Escherichia coli*	70	70%
2	*Staphylococcus aureus*	10	10%
3	*Klebsiella. pneumoniae*	10	10%
4	*Pseudomonas. aeruginosa*	6	6%
5	*Proteus vulgaris*	4	4%

Antimicrobial profiling of herbal extracts

Antimicrobial profiling of herbal extracts against *E. coli*

Aqueous extract of amla, baheda, harad and triphala produced mean inhibition zones of 5.2 mm, 10.4 mm, 7.3 mm and 6.1 mm diameter, respectively, against *E. coli* isolates whereas ethanol extract of the given herbs produced greater mean inhibition zones of 10.5 mm, 16.2 mm, 10.5 mm, 10.8 mm diameter, respectively, against the same isolates.

Antimicrobial profiling of herbal extracts against *Klebsiella pneumoniae*

Against *Klebsiella pneumoniae*, aqueous extract of amla, baheda, harad and triphala produced mean inhibition zones of 3.4 mm, 7.0 mm, 5.4 mm and 4.9 mm diameter, respectively, whereas ethanol extract of the same herbs produced greater mean inhibition zones of 9.7 mm, 10.2 mm, 10.0 mm, 6.9 mm diameter, respectively, against the same isolates.

Antimicrobial profiling of herbal extracts against *Pseudomonas aeruginosa*

Aqueous extract of amla, baheda, harad and triphala produced mean inhibition zones of 7.3 mm, 12.5 mm, 9.8 mm and 7.2 mm, respectively, against *Pseudomonas aeruginosa* whereas ethanol extract of the same herbs produced greater mean inhibition zones of 10.3 mm, 18.8 mm, 11.8 mm, 11.2 mm diameter, respectively, against *Pseudomonas aeruginosa* .

Antimicrobial profiling of herbal extracts against *Proteus vulgaris*

Aqueous extract of amla, baheda, harad and triphala produced mean inhibition zones of 5.8 mm, 11.5 mm, 9.0 mm and 5.5 mm diameter, respectively, against *Proteus vulgaris* whereas ethanol extract of the same herbs produced greater or equal mean inhibition zones of 9.5 mm, 11.5 mm, 11.5 mm, 11.0 mm diameter, respectively, against *Proteus vulgaris*.

Antimicrobial profiling of herbal extracts against *Staphylococcus aureus*

Aqueous extract of amla, baheda, harad and triphala produced mean inhibition zones of 8.8 mm, 15.3 mm, 13.0 mm and 11.2 mm diameter, respectively against *Staphylococcus aureus* whereas ethanol extract of the same herbs produced greater mean inhibition zones of 4.2 mm, 18.0 mm, 18.6 mm, 15.0 mm diameter, respectively, against *Staphylococcus aureus* .

From the above results, it can be concluded that all herbal extracts (including both aqueous and ethanol) were active against all UTI isolates (including both Gram-positive and Gram-negative bacteria). Thus, all herbal extracts showed a broad spectrum of antibacterial activity. Further, ethanol

extract of all four herbal extracts produced consistently greater zone of inhibition than aqueous extracts against all isolates of the all the five bacteria studied in present investigation. Therefore, results of ethanol extracts are discussed further. This observation clearly indicates greater existence of non-polar residues (active compounds of herbal extracts, such as tannins, saponins, lectins, alkaloids, flavonoids, etc.) in the ethanol extracts, which have higher bactericidal abilities. Our results that ethanol extracts are more effective antimicrobials agree with the observations reported by Ekwenye (2005) in respect of ginger and garlic extracts on *Escherichia coli* and S*almonella typhii.* They found that the ethanol extraction of herbs was better because ethanol is an organic solvent and will dissolve more organic compounds resulting in the liberation of the greater amounts of active antimicrobial components. Furthermore, a high antibacterial activity of ethanol extract may be attributed to two reasons : first, the nature of biological active components (saponins, tannins, alkaloids and anthraquinone), which could be enhanced in the presence of ethanol.

Minimum inhibitory concentration of test herbal extracts

For *E. coli*, MIC values of ethanol extracts were observed to be 50, 12.5, 25, 25 mg/ml for amla, baheda, harad and triphala, respectively. The mean zone of inhibition at given MIC values for the given herbal extracts was 8.4, 7.3, 7.1 and 8.9 mm diameter, respectively. Therefore, based on lowest MIC value and relatively high zone of inhibition, it can be concluded that ethanol extract of baheda is most effective antimicrobial for *E. coli*.

For *Klebsiella pneumonia*, the MIC values of ethanol extract were observed to be 100, 50, 25, 100 mg/ml for amla, baheda, harad and triphala, respectively. The mean zone of inhibition at given MIC values for the given herbal extracts was 9.8, 7.6, 6.7 and 7.7 mm diameter, respectively. Therefore, it can be concluded that ethanol extract of harad is most effective antimicrobial for *Klebsiella pneumonia*.

The MIC values of ethanol extract for *Pseudomonas aeruginosa* were observed to be 50, 12.5, 12.5 and 25 mg/ml for amla, baheda, harad and

triphala, respectively. The mean zone of inhibition at these MIC values for the given herbal extracts was 8.8, 14.7, 5.8 and 6.8 mm diameter, respectively. Thus, based on lowest MIC value and highest inhibition zone, ethanol extract of baheda was the most effective antimicrobial for *Pseudomonas aeruginosa*.

The MIC values of ethanol extract for *Proteus vulgaris* were observed to be 25, 25, 12.5 and 25 mg/ml for amla, baheda, harad and triphala, respectively. The mean zone of inhibition at these MIC values for the given herbal extracts was 6.5, 9.3, 8.0 and 7.3 mm diameter, respectively. Thus, based on lowest MIC value and sufficiently high inhibition zone, ethanol extract of harad was the most effective antimicrobial for *Proteus vulgaris*.

The MIC values of ethanol extract for *Staphylococcus aureus* were observed to be 12.5, 12.5, 6.25 and 12.5 mg/ml for amla, baheda, harad and triphala, respectively. The mean zone of inhibition at these MIC values for the given herbal extracts was 9.0, 13.4, 10.8 and 9.0 mm diameter, respectively. Thus, based on lowest MIC value and sufficiently high inhibition zone, ethanol extract of harad was the most effective antimicrobial for *Staphylococcus aureus*.

From the above results, it is also concluded that harad was the best antimicrobial against three of the five bacteria of UTI, i.e. *Klebsiella pneumonia* (Gram -ve), *Proteus vulgaris* (Gram -ve) and *Staphylococcus aureus* (Gram +ve). However, ethanol extract of baheda was found to be best against two of the five bacteria of UTI, namely *E. coli* and *Pseudomonas aeruginosa*, both of which are Gram –ve. Further, out of these two herbal extracts, harad is considered to be more important as it was most effective against both Gram negative and Gram positive bacteria.

The results of present study are in agreement with the report of Phadke and Kulkarni (1989), Rani and Khullar (2004), Kanna (2009) on *Terminalia chebula*. Present study showed antibacterial activity at a low concentration of herbal extracts whereas Ahmed (1998) reported similar activity at a concentration of 200 mg /ml. Findings of the present study are also similar to those reported by Khanna and Nag (1973) that amla was found to be active against urinary pathogens.

The aforesaid results suggest that the herbal extracts are more effective against Gram-positive bacteria than Gram-negative ones, as the MIC value of all the herbal extracts used was found to be the least for *Staphylococcus aureus* (Gram +ve bacteria).

Conclusion

The obtained results might be considered important for further studies including the isolation and identification of the active principles and to evaluate possible synergism among herbal extract components for their antibacterial activity. Based on the results, it is concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. *Terminalia bellerica* and *Terminalia chebula* showed stronger activity than the other extracts against all the tested bacterial strains. Therefore, *Terminalia bellerica* and *Terminalia chebula* can be selected for further analysis.

References

1. WHO. 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. World Health organization. Geneva.
2. Winickoff RN, SI Wilner, G Gall, et al. February 1981. Urine culture after treatment of uncomplicated cystitis in women. *Southern Medical Journal* 74 (2): 165-9.
3. Tessema B, Kassu A, Mulu A, Yismaw G (2007). Predominant isolates of urinary tract pathogens and their antimicrobial susceptibility patterns in Gondar University Teaching Hospital, Northwest Ethiopia. Ethiop. Med. J., 45(1): 61-67.
4. Schaeffer, A.J., N. Rajan, Q. Cao, B.E. Anderson,. D.L. Pruden, J. Sensibar and J.L. Duncan, 2001. Host pathogenesis in urinary tract infection. *Int J Antimicrob Agents*. 17: 245-251.
5. Ronald A. 1989. The etiology of urinary tract infection: traditional and emerging pathogens. *Dis Mon* . 49(2): 71-82.
6. Soulsby, E.J. 2005. Resistance to antimicrobials in humans and animals. *Br J Med.* 331: 1219-1220.

7. Falagas, M. E., I. K. Kotsantis, E. K. Vouloumanou, and Rafailidis, P. I. 2009. Antibiotics versus placebo in the treatment of women with uncomplicated cystitis: a meta-analysis of randomized controlled trials.
8. Rani, P., Khullar, N. 2004. Antimicrobial Evaluation of some medicinal plants for the antienteric potential against multi drug- resistant *Salmonella typhi*. *Phytother Res.* 18(8): 670-673.
9. Phadke, S.A., Kulkarni, S.D. 1989. Screening of *in vitro* Antibacterial Activity of *Terminalia chebula*, *Eclapta alba* and *Ocimum sanctum*. Ind J Med Sc. 43(4): 113-117.
10. Kannan, P., Ramadevi, S.R., Hopper, W. 2009. Antibacterial Activity of *Terminalia chebula* fruit Extract. Af J Microbiol Res. 3(4): 180-184.