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Assessment of antibacterial potential of trifla churan and its componants (amla, baheda and harad) against UTI pathogens.

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ABSTRACT

Purpose: Urinary tract infection (UTI) is one of the most common infections among all age groups. Objective of present study was to detect the antibacterial activity of trifla churan against isolated UTI pathogens.
Materials and Methods: UTI bacteria were isolated from urine and identified by standard biochemical tests. *Escherichia coli* was found the major pathogen (75% cases studied) while *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* caused infections only in 15%, 5% and 5% cases respectively. Antibacterial activity of aqueous, ethanol, ether and acetone preparations of herbal drug (Trifla Churna) and its components (amla: *Emblica officinalis*, baheda: *Terminalia bellerica* and harad: *Terminalia chebula*) were evaluated against these isolated UTI causing bacteria using agar well diffusion method.
Results: All the extracts are potent antimicrobials against all the pathogens studied. Among the different solvents extracts studied ethanol and ether showed high degree of inhibition followed by acetone and aqueous extracts. Ethanol extracts of trifla churan, baheda and harad were most effective against *E. coli*, but ethanol extract of amla was most sensitive to *Pseudomonas aeruginosa*. In all herbal preparations maximum inhibition zone diameter was obtained in *E. coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* while *Proteus vulgaris* was comparatively less susceptible.
Conclusions: All the isolated pathogens were susceptible to Trifla churna and herbal preparations of all the three components of trifla churan showed almost similar antimicrobial activity (harad showed the maximum inhibition) and showed an enhanced activity when were used in combination in equal. So, herbal drug, trifla churan can be used for the treatment of UTI.

KEY WORDS : UTI, Trifla churan, *E. coli, Klebsiella pneumoniae, Proteus vulgaris,*

INTRODUCTION

Urinary tract infection (UTI) is a general term used to describe an infection of any part of the urinary tract may be kidney, the ureters, the bladder or the urethra [1]. It is one of the most prevalent bacterial infections in women and elderly individuals. This type of infection although can cause less severe life-threatening infections but the patient experienced significant distress [2]. Except among infants and the elderly, the infection occurs more commonly in women than in men and it was estimated that about 40–50% of women experience one episode in their lives and 20–30% of them have other episodes [3]. UTI is caused when microorganisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply [4]. Most infections arise from the Gram negative bacterium *Escherichia coli*, which normally grows and multiplies in the colon. Among the

uropathogen, *Escherichia coli* is the most common bacteria (75–90% of isolates) in both the community and hospital infections, [2,3,4]. Hospitalized patients may develop UTI with the species of *Klebsiella* spp., *Proteus* spp., *Providencia, Pseudomonas* spp., *Enterococcus faecalis, Serratia, Citrobacter, Staphylococcus aureus, Acinetobacter,* occasionally by *Candida* [5].

The occurrence of UTIs in the female is most common as compared to male in India, as well as other developed and underdeveloped countries. The incidences of UTIs were also reported from Uganda (East Africa), Kenya, United States, United Kingdom, etc. [6–7]. The possible reasons for UTIs can be unprotected sex, reduced water intake, and use of infected sanitary clothes (commonly used in small villages). Common symptoms of UTIs include severe back pain; inflammation or burning sensation while urinating; cloudy, dark, bloody, or bad-smelling urine; and fever or chill. If the infections reach the kidney, then it causes pyelonephritis (inflammation of the kidney) [8]. The modern medicine such as broad-spectrum antibiotics are prescribed for the control of uropathogens. However, due to the emergence of drug resistance in bacteria, there is a need for natural antimicrobial molecules.

According to Ayurveda, all urinary tract infections are discussed under a broad term called Mutravaha sroto vikara (difficulty in urination) that includes the conditions of the kidneys as well as the several urinary tract infections. Being a system responsible for homeostasis of fluids in the body it also detoxifies the body by eliminating certain waste products through urine which is an outcome of digestion of food and metabolism. There are many herbs with varied actions specifically aimed at mutravahasrotovikara such as Mutrasangrahaneeya, Mutravirechaneeya output, Mutravirajaneeya Brihatyadiksheerakashyam [9] is a polyherbal formulation consisting of five herbs in which Gokshura is added in double the quantity which is a diuretic and antidysuric [10]. Chandraprabhavati has been mentioned specifically for all types of Mutrakruchra and is sarvarogapranasini [11]. Shweta parpati has three main ingredients in which Suryakshara and Sphatika exhibit tridoshagna, shodhana, ropana properties. Suryakshara and Navasadara is alsokaphavatahara [12]. They maintain an atmosphere (prakritivighata) which helps in preventing the multiplication of growth of bacteria. So the combined effect of these medicines reverse the pathological process in UTI.

Present paper deals with the assessment of antibacterial activity of Trifla churna and its component plants in relation to commonly used antibiotics. Trifla is an important ayurvedic medicinal preparation comprising three fruits: *Phyllanthus emblica* or *Emblica officinalis*, *Terminalia chebula*, and *Terminalia belerica*. It is an antioxidant-rich herbal formulation, and possesses diverse beneficial properties. In ayurvedic practice, Trifla is used to treat gastrointestinal disorders such as dyspepsia, malabsorption, constipation, and ulcerative colitis; it is also a colon cleanser and tonifier. Trifala is also useful in treating ailments such as anemia, asthma, cough, fever, jaundice, leucorrhea, pyorrhea, and obesity [13].

MATERIALS AND METHODS

Isolation and identification of the bacteria from urine samples: The urine samples were freshly collected from midstream from clinically suspected UTI patients. These were centrifuged at 3000 x g for 5 min and sediments were inoculated into nutrient broth. These were incubated at 37^{o}C

temperature for 18-24 h. The growth was used to develop isolated colonies by streak plate, pour plate and spread plate method using nutrient agar medium. Identification was based on colony appearance, cell morphology, Gram's staining, motility and biochemical tests. Size, shape, colour and appearance of the colony were studied on Mac-Conkey, Nutrient, Eosin Methylene Blue and Blood agar media. Biochemical tests included oxidase, peroxidase, catalase, IMViC and sugar fermentation. Pure cultures were maintained on nutrient agar slants at 4^0C for further studies.

Preparation of extracts: The commercial herbal preparation was purchased from local market. This herbal preparation of trifla is prepared from the fruits of three medicinal plants, *Emblica officinalis* (amla), *Terminallia bellerica* (baheda) and *Terminallia chebula* (harad). The aqueous extract was prepared by suspending 1 gm of dry herbal preparation in 100 ml of distilled water. The mixture was homogenized and heated on water bath at 37^0 C temp. It was allowed to stand for 30 min and supernatant was filtered through membrane filter (Milipore size 0.22 μm) and the filtrate was evaporated to dry on sand bath. The dry mass was then sterilized and stored at 4^0C. The organic solvent extract was prepared by adding 1g herbal preparation (powder) in 100ml of organic solvent (acetone, ethanol and methanol) in screw-capped bottles, shaked at 190-220 rpm on a rotary shaker. After 24h of shaking, it was filtrated, evaporated in vacuum and dried by rotary evaporator at 60^0C. Dried extracts were stored in labeled sterile screw capped bottles at 4^0C and later used invitro study.

To examine the antimicrobial activity of the component plants, amla, baheda and harad, aqueous extracts were prepared and used separately as mentioned above. Ethanol, ether and acetone extracts were also prepared as described above.

Test for antibacterial activity: The antibacterial assay was carried out by micro dilution method in order to determine the antibacterial activity of compounds tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 X 10^7 CFU/ml. The inoculums were prepared and stored at 4ºC until use. Dilutions of the inoculums were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculums. All experiments were performed in duplicate and repeated three times.

Agar well diffusion method: Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar plates were swabbed with 24 hr old -broth culture of respective bacteria. Wells (10 mm diameter) were made in each of these plates using sterile cork borer. Stock solution of herbal preparation was prepared at a concentration of 1 mg/ml in different solvents. About 100 µl of different concentrations of herbal solvent extracts were added into the wells and allowed to diffuse at room temperature for 2hr. Control experiments comprising inoculums without herbal extract were set up. The plates were incubated at 37°C for 18-24 h. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Determination of MIC: The minimum inhibitory concentrations (MIC), was performed by a serial dilution technique using 96-well micro titer plates. The different herbal extracts viz. aqueous, ethanol, ether and acetone were taken (1 mg/ml) and serial dilutions of the extract with Luria broth with respective inoculums were used. The microplates were incubated for 72 hours at 28ºC, respectively.

The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

Determination of MBC: The MBCs were determined by serial sub-cultivation of 2 µl into micro titer plates containing 100 µl of broth per well and further incubation for 72 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. All experiments were performed in duplicate and repeated three times.

RESULTS

The aim of the present study was to isolate and identify the causative bacteria of UTI and to evaluate the antimicrobial activity of herbal drug; trifla churan and its constituent medicinal plants (*Emblica officinalis, Terminallia bellerica, Terminallia chebula*) against these isolated pathogens. Only four types of bacteria were isolated from clinically suspected patients. These included *Escherichia coli* (75%), *Klebsiella pneumoniae* (15%), *Proteus vulgaris* (5%) and *Pseudomonas aeruginosa* (5%). Out of these, *E. coli* was found to be most prevalent. The inhibitory effect of different extracts (viz. aqueous, ethanol, ether and acetone) of trifla churan and its componants were evaluated against isolated bacterial strains. The antimicrobial activity was determined using agar well diffusion method and micro dilution method summarized in Table 1-2. The activity was quantitatively assessed on the basis of inhibition zone and their activity index was also calculated along with minimum inhibitory concentration (MIC).

Measurement of antimicrobial activity using Agar well diffusion Method: The antimicrobial potential of herbal preparations were evaluated according to their zone of inhibition against UTI causing isolated pathogens. The results revealed that all the extracts are potent antimicrobials against all the pathogens studied. Among the different solvents extracts studied ethanol and ether showed high degree of inhibition followed by acetone and aqueous extracts.

Ethanol extracts of trifla churan, baheda and harad were most effective against *E. coli* with zone of inhibition of 28 mm, 22 mm and 27 mm respectively, but ethanol extract of amla was most sensitive to *Pseudomonas aeruginosa* (25 mm). For all the tested microorganisms ethanol herbal preparation of Trifla churan and *Terminallia chebula* (harad) showed maximum antibacterial activity.

In all herbal preparations maximum inhibition zone diameter was obtained in *E. coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* while *Proteus vulgaris* was comparatively less susceptible.

All the isolated pathogens were susceptible to Herbal drug (Trifla churna). When components of the test herbal drug preparation were tested, it was found that all three components have almost similar antimicrobial activity (harad showed the maximum inhibition) and showed an enhanced activity when were used in combination in equal amounts (Table 1). *E. officinalis* is rich in vitamin C which is well known to inhibit the growth of *E.coli*. 5000 mg or more vitamin C per day is normally recommended for an acute UTI, while this investigation showed that *E. coli* was more susceptible to harad as compare to amla.

Determination of MIC and MBC values: Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The concentration of plant extract that completely killed the bacteria was taken as MBC. Moreover, it was noted that most of the antimicrobial properties in different plant part extractions shows, MBC value that is almost two fold higher than there corresponding MICs [14].
Ethanol extract of trifla churan showed least MIC values 0.2 µg/ml against all the four pathogens, while ether extract of trifla churan showed 1.3 µg/ml against *E. coli* and *Klebsiella pneumoniae*. Acetone extract of trifla churan showed least MIC value of 1.5 µg/ml against *E. coli* and *Pseudomonas aeruginosa*, while aqueous extract of amla showed the minimum value 1.8 µg/ml against *Proteus vulgaris* (Table 2).

DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [15]. Therefore, medicinal plants are finding their way into pharmaceuticals, nutraceuticals and food supplements.
In the present investigation, different solvent extracts of a herbal drug, Trifla churan and its componants*; Emblica officinalis, Terminallia bellerica* and *Terminallia chebula* were evaluated for exploration of their antimicrobial activity against urinary tract infection causing isolated bacteria, these bacteria includes; *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*. Susceptibility of each herbal preparation was tested by serial microdilution method (MIC) and agar well diffusion method.
Our preliminary investigation showed that all aqueous, ethanol, ether and acetone extracts of trifla churan and its componants were active against the locally isolated human uropathogens like *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa.* This analysis of using several extracts so as to study the efficacy of herbal preparations for antimicrobial activity against UTI causing bacteria have been reported by many scientist in many plant species like *Zingiber officinale Punica granatum Cinnamomum cassia Azadirachta indica Ocimum sanctum* [16].

The alcoholic extracts of herbal preparations showed significant antimicrobial activity against multi-drug resistant isolated uropathogens. Though, the mechanism of the action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of solvent used. This observation clearly indicates that the existence of non-polar residues in the extracts

which have higher both bactericidal and bacteriostatic abilities. Similar results showing that the alcoholic extract having the best antimicrobial activity is also reported by Preethi [17] in *Leucas aspera, Holarrhena antidysenterica*.

Suree also studied the antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria and observed that this difference in the activity between different plant extracts is due to the difference between extract compounds [18].

The study also revealed that ethanolic extract shows minimum antimicrobial activity. Furthermore, aqueous, ether and acetone herbal preparations of trifla churan had been reported to have prominent antimicrobial activity against isolated UTI causing bacteria.

In the present study, the MIC value of the active plant extracts obtained in this study were lower than the MBC values (Table 1-2, Graph 1 (A-B)) suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration [19].

In conclusion, of the present investigation of these herbal preparations contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against urinary tract infections. All the four preparations (aqueous, ethanol, ether and acetone) of trifla churan and its componants possess significant inhibitory effect against isolated pathogens.

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Table 1: Antibacterial activity of herbal preparations against UTI causing bacterial isolates. (Zone of inhibition of growth in mm average of 3 reading)

S.No	Herbal preparations	Isolated pathogens	Aqueous extract	Ethanol extract	Ether extract	Acetone extract
1	Trifla Churan	*E. coli*	24	28	25	25
2	Trifla Churan	*K. pneumoniae*	22	25	23	23
3	Trifla Churan	*P. vulgaris*	18	21	19	20
4	Trifla Churan	*P. aeruginosa*	23	25	24	23
5	Amla	*E. coli*	22	24	23	25
6	Amla	*K. pneumonia*	19	24	22	23

7	Amla	*P. vulgaris*	16	20	18	19
8	Amla	*P. aeruginosa*	21	25	24	23
9	Baheda	*E. coli*	19	22	20	20
10	Baheda	*K. pneumonia*	18	20	19	19
11	Baheda	*P. vulgaris*	14	18	17	16
12	Baheda	*P. aeruginosa*	18	20	20	20
13	Harad	*E. coli*	22	27	24	24
14	Harad	*K. pneumonia*	22	24	23	23
15	Harad	*P. vulgaris*	17	20	21	20
16	Harad	*P. aeruginosa*	23	26	24	25

Table 2: MIC (µg/ml) of different extracts of Trifla churan and its components against isolated pathogenic bacteria.

S.No	Herbal preparations	Isolated pathogens	Aqueous extract	Ethanol extract	Ether extract	Acetone extract
1	Trifla Churan	*E. coli*	2.4	0.2	1.3	1.5
2	Trifla Churan	*K. pneumonia*	2.2	0.2	1.3	1.6
3	Trifla Churan	*P. vulgaris*	1.8	0.2	1.4	1.6
4	Trifla Churan	*P. aeruginosa*	2.3	0.2	1.6	1.5
5	Amla	*E. coli*	2.4	0.3	1.9	2.1
6	Amla	*K. pneumonia*	1.9	0.3	2.0	2.0
7	Amla	*P. vulgaris*	1.8	0.4	2.1	2.0
8	Amla	*P. aeruginosa*	2.1	0.3	1.9	2.2
9	Baheda	*E. coli*	2.0	0.5	1.8	1.9
10	Baheda	*K. pneumoniae*	2.3	0.5	1.7	1.9
11	Baheda	*P. vulgaris*	2.5	0.5	1.8	1.8
12	Baheda	*P. aeruginosa*	2.6	0.4	1.8	1.8
13	Harad	*E. coli*	2.2	0.2	1.6	1.6
14	Harad	*K. pneumoniae*	2.2	0.2	1.4	1.7
15	Harad	*P. vulgaris*	2.1	0.2	1.5	1.7
16	Harad	*P. aeruginosa*	2.3	0.2	1.6	1.7

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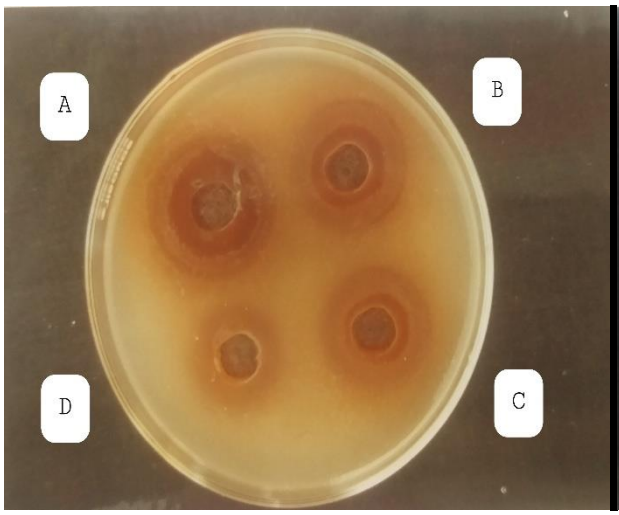

Fig.1

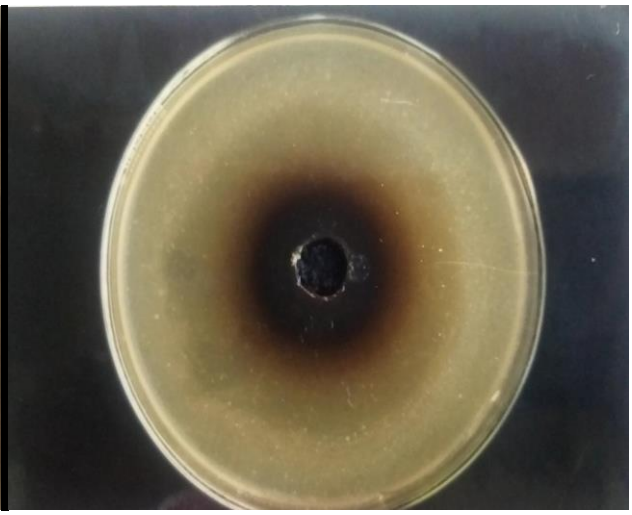

Fig.2

Fig.1: Antibacterial activity of (A) Trifla churan, (B) Harad, (C) Amla and (D) Baheda against *Escherichia coli*.

Fig.2: Antibacterial activity of Trifla churan, against *Escherichia coli.*