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Research Article

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Antimicrobial activity of Ayurvedic herbs against urinary tract infection pathogens

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ABSTRACT

The present study investigated the prevalence of uropathogens and antimicrobial potential of three ayurvedic herbs viz., anantmul (Hemidesmusindicus), gulkhair (Malvasylvestris) and manjishtha (Rubiacordifolia) against the urinary tract infection pathogens.E. coliwas the most prevalent pathogen (42%) while Alcaligeneswas least prevalent (3%). The methanolic extracts were found to more effective as compared to aqueous extracts. The methanolic extract of anantmul exhibited maximum antibacterial property against E. coli(18.3±0.47mm) while aqueous extract of exhibited maximum effect upon Alcaligenes(13.7±0.47 mm). The methanolic extract of gulkhair showed highest potential against E. coli (23.7±0.94 mm) while aqueous extract of gulkhair exhibited maximum antimicrobial effect against E. coli (18.7±0.25 mm). The methanolic extract of manjishtha exhibited maximum antibacterial property against E. coli(29.3±0.47mm) while aqueous extract was most effective against E. coli (18.0±0.47 mm).The ayurvedic herbs exhibited significant antimicrobial potential.

Key words: Uropathogens, Ayurvedic herbs, *Escherichia coli*, Antimicrobial potential, Anantmul, Gulkhair, Manjishtha.

INTRODUCTION

Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. The uropathogens after attaching to the epithelial surface, subsequently colonises and disseminates throughout the mucosa causing tissue damage. After the initial colonisation period, pathogens can ascend into the urinary bladder resulting in symptomatic or asymptomatic bacteriuria. Further progression may lead to pyelonephritis and renal impairment[1]. Specific virulence factors residing on the uropathogen's membrane are responsible for bacterial resistance to the normally effective defense mechanisms of the host.The most common UTIs occur mainly in women and affect the bladder and urethra. Urinary tract infections (UTIs) are among the most common conditions requiring medical treatment with 6-10% of all young females demonstrating bacteriuria[2. 3]. The incidence of UTI's increases with age and 25-50% of females aged 80 or more have bacteriuria[4]. Most of the urinary tract infections are caused by gram-negative bacteria like *Escherichia coli, Klebsiella* sp., *Proteus vulgaris, Pseudomonas aeruginosa, Acinetobacter* and *Serratia*. The treatment mainly involves use of antibiotics but the pathogenic bacteria are becoming increasingly resistant to antibiotics [5, 6]. The indiscriminate use of antibiotics has led to evolution of multi-drug pathogens. This necessitates the search for alternative compounds having antimicrobial property. Therefore emphasis has been laid over medicinal plants[7, 8]. The present study aimed at

characterizing the antimicrobial potential of three ayurvedic herbs, namely anantmul (*Hemides musindicus*), gulkhair (*Malva sylvestris*) and manjishtha (*Rubia cordifolia*) against the urinary tract infection pathogens.

EXPERIMENTAL SECTION

2.1 Isolation of uropathogens

A total of 100 urine samples were collected aseptically from different patients in the hospitals in Dehradoon, Uttarakhand, India. The samples were plated by T-streaking method on CLED agar and blood agar using calibrated loops. The samples in which bacterial count was $>10^5$ cfu/ml were taken for isolation of uropathogens. All samples were plated in triplicates. Isolates were purified by streaking on nutrient agar and pure cultures were maintained.

2.2 Characterization of uropathogens

The morphological and biochemical characterization of recovered uropathogens was carried out. Cell morphology (Gram's reaction, cell shape and arrangement) of isolates were studied. The various biochemical tests viz., Oxidase test, Indole-Methyl Red-Voges-Proskauer-Citrate Utilization test (IMViC), Triple Sugar Iron (TSI) test, Urease test and Nitrate reduction tests were carried out according to [9].

2.3 Acquisition of herbs and preparation of extract

The stem of the plants manjishtha, anantmul and gulkhair were procured from the market. About 100 g of powdered stem (dry) were extracted with methanol (99%) in the ratio 1:5 using soxhlet apparatus for 15 hours in case of anantmul and gulkhair, while that of manjishtha in 56-60 hrs. Alcohol removal carried out under pressure yielded a semi solid mass, which was then distilled for the recovery of ethanol using distillation assembly. It was further kept in orbital shaker at 40^0C for drying the extract. The water extract was prepared in the same way except that distilled water was used instead of methanol.

2.4 Evaluation of antimicrobial activity of extracts

The antimicrobial activity of the crude extracts against pathogenic bacteria was evaluated by using agar well diffusion method. The isolates were inoculated into 10ml of sterile nutrient broth, and incubated at 37 ± 1^0C overnight. The turbidity of culture was compared with Mac Farland standard number II. The cultures were swabbed on the surface of sterile Mueller-Hinton agar plates using a sterile cotton swab and allowed to dry for 3-5 minutes. Agar wells were prepared with the help sterilized borer with 10mm diameter. The extract of spices was diluted to give the final concentration 1000ppm, 2000ppm, 3000ppm and 4000ppm. 100 µl of different dilutions of the extracts was added to the wells of the inoculated plates. The plates were incubated in an upright position at 37 ± 1^0C for 24hrs. The zone of inhibition was measured and expressed in millimetres (mm).

RESULTS AND DISCUSSION

3.1 Prevalence of uropathogens

A total of 150 uropathogens were obtained from positive urine samples which were identified based on morphological and biochemical characteristics (Fig. 1).*E. coli* was the most prevalent uropathogen (42%) followed by *Pseudomonas* (29%), *Proteus* (8%), *Staphylococcus* (7%), *Klebsiella*(6%), Serratia (5%) and *Alcaligenes* (3%).

3.2 Antimicrobial activity of ayurvedic herbs against uropathogens

All extracts of ayurvedic herbs showed good antibacterial property (Table 1 to 3). The methanolic extract of anantmul exhibited maximum antibacterial property against *E. coli*(18.3±0.47mm) and least activity against *Staphyloccus*(14.3±0.34 mm). The aqueous extract of anantmul exhibited maximum antimicrobial activity against *Alcaligenes*(13.7±0.47 mm) and least activity against *Serratia*(10.5±0.45 mm). The methanolic extract of gulkhair showed highest potential against *E. coli* (23.7±0.94 mm) and least potential towards *Klebsiella*(14.7±0.56 mm). The aqueous extract of gulkhair exhibited maximum antimicrobial effect against *E. coli* (18.7±0.25 mm) and minimum activity against *Staphylococcus* (12.3±0.47 mm).The methanolic extract of manjishtha exhibited maximum antibacterial property against *E. coli*(29.3±0.47mm) and least activity against *Serratia*(16.3±0.47 mm). The aqueous extract of manjishtha exhibited maximum antimicrobial activity against *E. coli* (18.±0.47 mm) and least activity against *Staphyloccus* (7.7±0.47 mm).

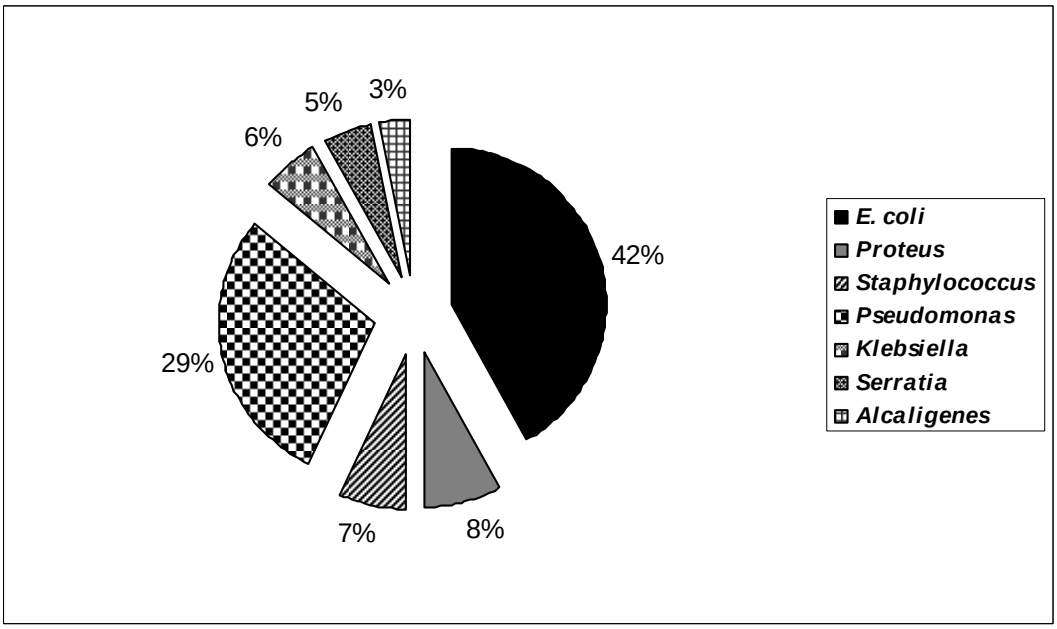

Fig. 1: Prevalence of uropathogens

Table 1a: Antimicrobial activity of methanolic extract of anantmul against urinary tract infection pathogens

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	10.3±0.47	13.3±0.47	15.6±0.47	18.3±0.47
Staphylococcus	9.7±0.23	10.7±0.25	12.3±0.47	14.3±0.34
Pseudomonas	8.3±0.15	10.5±0.21	12.7±0.23	14.7±0.47
Klebsiella	7.3±0.47	10.3±0.23	14.3±0.32	17.5±0.47
Proteus	7.5±0.17	10.3±0.22	13.7±0.23	16.3±0.47
Serratia	8.7±0.47	10.3±0.29	12.4±0.23	14.9±0.47
Alcaligenes	7.3±0.47	10.7±0.47	12.9±0.82	15.3±0.47

Values are mean ± SD of three replicates

Table 1b: Antimicrobial activity of aqueous extract of anantmul against urinary tract infection pathogens

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	7.0±0.41	9.0±0.41	10.3±0.94	11.3±0.47
Staphylococcus	6.3±0.47	7.3±0.38	8.7±0.34	10.7±0.47
Pseudomonas	5.3±0.25	6.0±0.82	8.5±0.47	10.6±0.47
Klebsiella	6.7±0.45	8.7±0.94	10.7±0.94	12.7±0.94
Proteus	5.8±0.36	7.6±0.57	10.7±0.45	13.3±0.47
Serratia	5.2±0.25	6.5±0.45	8.2±0.32	10.5±0.45
Alcaligenes	10.3±0.47	11.3±0.23	12.3±0.34	13.7±0.47

Values are mean ± SD of three replicates

Table 2a: Antimicrobial activity of methanolicextract of gulkhair against urinary tract infection pathogens

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	9.7±0.94	13.0±0.82	18.7±0.47	23.7±0.94
Staphylococcus	8.7±0.24	11.7±0.47	13.3±0.25	16.0±1.63
Pseudomonas	7.6±0.24	9.7±0.47	11.7±0.32	15.7±0.47
Klebsiella	7.7±0.24	9.7±0.23	11.3±0.47	14.7±0.56
Proteus	9.7±0.23	11.5±0.27	14.3±0.47	17.3±0.47
Serratia	10.7±0.22	13.3±0.24	16.3±0.34	20.7±0.47
Alcaligenes	8.6±0.23	11.5±0.33	16.3±0.47	19.7±0.23

Values are mean ± SD of three replicates

Table 2b: Antimicrobial activity of aqueous extract of gulkhair against urinary tract infection pathogens

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	6.3±0.27	10.3±0.45	14.7±0.34	18.7±0.25
Staphylococcus	5.3±0.47	7.6±0.38	9.7±0.34	12.3±0.47
Pseudomonas	6.3±0.47	8.7±0.47	10.7±0.47	13.4±0.47
Klebsiella	6.5±0.47	8.9±0.47	10.8±0.47	13.7±1.25
Proteus	5.7±0.47	7.9±0.47	10.7±0.47	14.3±0.47
Serratia	5.7±0.47	7.3±0.47	10.4±0.47	15.7±0.47
Alcaligenes	8.3±0.47	10.7±0.47	13.3±0.47	16.7±0.47

Values are mean ± SD of three replicates

Table 3a: Antimicrobial activity of methanolic extract of manjishtha against urinary tract infection pathogens

Name of organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	18.3±0.47	22.0±1.41	25.3±3.30	29.3±0.47
Staphylococcus	10.3±0.47	14.3±0.47	17.0±0.82	21.3±0.94
Pseudomonas	15.0±0.82	18.2±1.41	21.7±0.25	24.3±0.47
Klebsiella	12.5±0.47	14.7±0.47	17.3±0.47	20.3±0.47
Proteus	11.7±0.47	13.9±0.82	16.7±0.94	19.5±0.94
Serratia	8.3±0.47	10.3±0.47	13.6±0.47	16.3±0.47
Alcaligenes	9.7±0.47	11.7±0.47	14.3±0.47	17.9±0.47

Values are mean ± SD of three replicates

Table 3b: Antimicrobial activity of aqueous extract of manjishtha against urinary tract infection pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	5.7±0.47	7.7±0.47	10.0±0.82	18.3±0.47
Staphylococcus	2.7±0.47	3.3±0.47	5.7±0.47	7.7±0.47
Pseudomonas	5.7±0.47	7.7±0.47	8.3±0.47	10.3±0.47
Klebsiella	2.5±0.47	3.3±0.47	6.7±0.47	9.7±0.47
Proteus	6.7±0.42	9.7±0.40	12.5±0.46	15.7±0.47
Serratia	6.3±0.47	9.5±0.47	11.7±0.41	13.6±0.47
Alcaligenes	6.7±0.23	9.3±0.32	11.6±0.33	14.3±0.47

Values are mean ± SD of three replicates

DISCUSSION

The emergence of multidrug resistance among bacteria causing several life threatening infections, the increasing failure and spiralling cost of antibiotic therapy has led to screening of several medicinal plants for potential antimicrobial activity[10, 11]. Ayurvedic herbs are used traditionally for treatment of various ailments since ages. However, no emphasis has been laid in characterizing the active compound present in them so that drugs can be developed.

The present study was aimed at determining the antimicrobial potential of ayurvedic herbs against the uropathogens. These herbs are being traditionally used since ages for treatment of various diseases and infections. Anatmul plant enjoys a status as tonic, alterative, demulcent, diaphoretic, diuretic and blood purifier. It is employed in nutritional disorders, syphilis, chronic rheumatism, gravel and other urinary diseases and skin affections. Manjishtha has proven efficacy of fighting a number of diseases. It is a drug of choice for help in various systemic problems like elevated uric acid and gouty arthritis, glandular swellings, recurrent skin infections [12]. The roots have been used internally in the treatment of abnormal uterine bleeding, internal and external hemorrhage. They are also used in the treatment for bronchitis, rheumatism, stones in the kidney, bladder and gall, dysentery etc. Gulkhair has been used as food and medicine in Europe since the time of ancient Greece and Rome. Traditional herbal medicine continues to regard the plant as a useful anti-inflammatory agent for the respiratory tract, the skin, and the gastrointestinal tract[13].

In the present study *E. coli* was observed to be the most prevalent pathogen. Its dominance has been previously reported by various workers [14, 15].The methanolic extract of the three herbs exhibited more antimicrobial potential as compared to the aqueous extract. The solubility of phytochemicals in different solvents decide which

extract will exhibit more antimicrobial potential. The active component of these extracts may exhibit their antimicrobial potential either by degradation of cell wall, disruption of cytoplasmic membrane, leakage of cellular components, damage protein, interfere with the enzymatic activities inside cell, affect synthesis of DNA and RNA, affect electron transport and nutrient uptake, leakage of cellular components, impair the energy production inside cell, change fatty acid and phospholipid constituents [16]. Thus it can be concluded that methanolic extract of these herbs can be used as a potential source of natural antimicrobial compound against pathogenic bacteria. This preliminary study can be further extended in determining the active component so that effective medicinal preparations can be made [17].

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