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Antimicrobial activity of medicinal plants against urinary tract infection pathogens

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Abstract - Urinary tract infections (UTIs) are among the most common infection with an incidence rate of 25-80% of females. The present study aimed at determining the prevalence of uropathogens and antimicrobial activity of four medicinal plants viz., tulsi, amla, neem and henna against the uropathogens. A total of 100 uropathogens were recovered. *E. coli* was the most prevalent uropathogen. *Pseudomonas, Proteus, Staphylococcus, KlebsiellaSerratia*and *Alcaligenes* were also recovered. The aqueous extract of all medicinal plants exhibited maximum antimicrobial activity against all uropathogens. The aqueous extract of henna exhibited maximum activity against *Serratia*(23.7±0.47 mm) while that of amla exhibited maximum antibacterial property against *E. coli*(24.7±0.40mm). The aqueous extract of tulsi showed highest potential against *E. coli* (17.7±0.34 mm) while that of neem was most effective against *Proteus* (23.7±0.47mm). These medicinal plants exhibited effective antimicrobial activity against uropathogens.

Key words: ***Urinary tract infections, Antimicrobial activity, Uropathogens, Tulsi, Amla, Neem, Henna***

I. Introduction

Urinary tract infection is a second most important infection which encompasses the asymptomatic presence of bacteria in urine to severe infection of kidney (Lane and Thakar, 2011). They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and 60% having an infection at some point in their lives (Nicolle *et al.,* 2008). Recurrences are common, with nearly half of people getting a second infection within a year. Urinary tract infections occur four times more frequently in females than males (Oelschlaeger*et al.*, 2006; Dielubanza and Schaeffer, 2011; Salvatore *et al*., 2011.). Pyelonephritis occurs between 20–30 times less frequently (Nicolle *et al*., 2008). They are the most common cause of hospital acquired infections accounting for approximately 40% (KsyckiandNamias, 2009). Age of woman is also an important factor as the rate of infection increases from 2 to 7% in woman in their fertility period to as high as 50% in older women (Dielubanza and Schaeffer, 2011). 7-10% of men over the age of 75 suffer from this infection (Woodford and George,2011). Most of the urinary tract infections are caused by gram-negative bacteria like *Escherichia coli*, *Klebsiella sp.*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Serratia*. 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram-positive bacteria ((Nicolle *et al*., 2008; Lane and Takhar, 2011.).

Treatment involves antibiotics but however the emergence of resistance amongst pathogens against the antibiotics necessitates the need of discovery of new antimicrobial compounds from various species of medicinal plants. Medicinal plants are heavily and worldwide used in folk medicine (Ahmed *et al*., 1998; Bhuvaneswari*et al*., 2002). The active components of medicinal plants need to be identified - which can be used as antimicrobial drug. The present study was aimed at studying the antimicrobial activity of amla (*Emblicaofficinalis)*, neem(*Azadirachtaindica)*, tulsi (*Ocimum sanctum)* and henna(*Lawsoniainermis)* plant extracts against UTI pathogens.

II. Materials and methods

Isolation of uropathogens

A total of 50 urine samples were collected aseptically from different patients in the hospitals in Dehradoon, Uttarakhand, India. The samples were plated 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.

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 Cappucino and Sherman (1992).

Plant samples and extraction procedure

Leaves of neem(*Azadirachtaindica)*, tulsi(*Ocimum sanctum)* and henna (*Lawsoniainermis)* plants and fruit of amla(*Emblicaofficinalis)* were collectedand left to dry at room temperature for 24 hours. The dried leaves and fruit were ground to a powder and were kept in dry containers. Two types of extracts were prepared- The ethanolic extract was prepared by soaking each powder in 100% ethanol in a concentration of 1:4 for 24 hours. This mixture was cooled and filtered by Whatman filter paper No.1. The solvent was dried and concentrated using orbital shaker at 40°C. Water-based plant extracts were prepared in the same way except that distilled water was used instead of ethanol.

III. Results

A total of 100 uropathogens were obtained from positive urine samples which were identified based on morphological and biochemical characteristics.

Prevalence of uropathogens

E. coli was the most prevalent uropathogen (49%) followed by *Pseudomonas* (25%), *Proteus* (10%), *Staphylococcus* (5%), *Klebsiella* (5%),*Serratia*(4% and *Alcaligenes*(2%).

Antimicrobial activity of medicinal plants against uropathogens

All extracts of medicinal plants showed good antibacterial property (Table 3.1 to 3.4). The ethanolic extract of henna showed highest potential against *E. coli* (20.3±0.94 mm), while aqueous against *Serratia*(23.7±0.47 mm). The ethanolic extract of amla exhibited maximum antibacterial property against *Staphylococcus* (20.7±0.94 mm) while aqueous extract against *E. coli*(24.7±0.40mm). The ethanolic extract of tulsi showed highest potential against *Proteus* (13.7±0.32 mm) while aqueous against *E. coli*(17.7±0.34 mm). The ethanolic extract of neem was most effective against *Staphylococcus* (20.7±0.25mm) while aqueous extract was against *Proteus* (23.7±0.47mm).

Table 3.1a: Antimicrobial activity of ethanolic extract of henna against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	10.0±0.82	13.0±0.25	16.7±0.82	20.3±0.94
Staphylococcus	6.7±0.47	10.3±0.94	12.3±0.47	14.7±0.47
Pseudomonas	7.3±0.94	8.7±0.94	9.8±0.82	11.7±0.75
Klebsiella	11.7±0.47	13.7±0.47	15.7±0.45	17.0±0.56
Proteus	6.3±0.47	9.6±0.32	11.7±0.47	12.7±0.47
Serratia	5.3±0.47	7.5±0.35	9.3±0.45	10.3±0.67
Alcaligenes	9.7±0.35	10.7±0.36	11.5±0.45	12.3±0.94

Values are mean ± SD of three replicates

Table 3.1b: Antimicrobial activity of aqueous extract of henna against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	**2000ppm**	**3000ppm**	**4000ppm**
E. coli	8.7±0.47	10.0±0.25	12.3±0.47	18.7±0.43
Staphylococcus	8.3±0.47	11.7±0.35	13.5±0.82	15.7±0.47
Pseudomonas	13.7±0.47	15.6±0.35	16.7±0.56	18.7±0.47
Klebsiella	8.7±0.47	10.7±0.35	14.5±0.43	18.3±0.28
Proteus	10.7±0.47	12.7±0.35	15.3±0.47	17.7±0.47
Serratia	14.7±0.34	16.7±0.32	17.7±0.35	23.7±0.47
Alcaligenes	10.7±0.21	13.7±0.23	17.3±0.24	21.7±0.47

Values are mean ± SD of three replicates

Table 3.2a: Antimicrobial activity of ethanolic extract of amla against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	**2000ppm**	**3000ppm**	**4000ppm**
E. coli	10.3±0.47	12.7±0.94	14.3±0.42	17.3±0.47
Staphylococcus	10.3±0.25	13.0±0.82	16.0±0.63	20.7±0.94
Pseudomonas	7.0±0.82	8.7±0.47	9.4±0.56	10.0±0.81
Klebsiella	7.5±0.25	9.7±0.47	11.7±0.56	15.3±0.65
Proteus	7.3±0.35	9.3±0.32	11.7±0.42	12.7±0.47
Serratia	5.6±0.35	7.3±0.25	9.7±0.34	13.3±0.47
Alcaligenes	11.7±0.47	12.7±0.35	13.7±0.38	15.3±0.94

Values are mean ± SD of three replicates

Table 3.2b: Antimicrobial activity of aqueous extract of amla against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	10.7±0.47	14.7±0.35	19.3±0.47	24.7±0.40
Staphylococcus	7.3±0.47	11.3±0.46	15.3±0.94	22.3±0.47
Pseudomonas	4.3±0.47	8.6±0.35	12.3±0.32	18.3±0.47
Klebsiella	5.7±0.47	8.3±0.42	12.3±0.45	17.7±0.47
Proteus	11.7±0.47	13.7±0.35	17.3±0.34	21.7±0.47
Serratia	13.7±0.47	15.7±0.22	17.7±0.56	24.2±0.47
Alcaligenes	13.7±0.47	15.7±0.35	17.3±0.24	19.7±0.28

Values are mean ± SD of three replicates

Table 3.3a: Antimicrobial activity of ethanolic extract of tulsi against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	7.5±0.28	8.3±0.34	9.7±0.45	10.7±0.32
Staphylococcus	6.5±0.34	6.7±0.47	8.6±0.34	10.3±0.34
Pseudomonas	4.5±0.31	6.7±0.32	8.7±0.40	10.3±0.32
Klebsiella	7.5±0.32	9.3±0.47	11.3±0.25	13.3±0.47
Proteus	6.3±0.94	8.7±0.32	10.5±0.23	13.7±0.32
Serratia	3.7±0.32	5.7±0.42	7.3±0.32	10.7±0.32
Alcaligenes	5.7±0.24	7.7±0.25	9.7±0.23	11.7±0.23

Values are mean ± SD of three replicates

Table 3.3b: Antimicrobial activity of extract aqueous of tulsi against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	9.7±0.45	11.3±0.32	14.7±0.26	17.7±0.34
Staphylococcus	6.7±0.35	8.7±0.34	10.7±0.24	12.3±0.25
Pseudomonas	8.7±0.34	10.7±0.24	12.7±0.25	13.3±0.35
Klebsiella	6.3±0.35	7.7±0.32	8.3±0.47	9.3±0.47
Proteus	9.3±0.28	11.7±0.24	13.7±0.34	16.7±0.37
Serratia	5.7±0.24	9.7±0.28	11.3±0.23	14.7±0.47
Alcaligenes	6.7±0.21	10.7±0.23	13.7±0.47	15.7±0.34

Values are mean ± SD of three replicates

Table 3.4a: Antimicrobial activity of ethanolic extract of neem against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	8.3±0.23	10.6±0.21	12.7±0.23	15.7±0.21
Staphylococcus	10.7±1.25	13.6±0.24	17.3±0.34	20.7±0.25
Pseudomonas	7.5±0.24	9.5±0.23	11.7±0.34	13.7±0.47
Klebsiella	10.3±0.94	11.7±0.47	9.7±0.47	16.0±2.16
Proteus	1.8±0.23	3.6±0.22	5.4±0.23	7.7±0.47
Serratia	6.3±0.47	8.7±0.47	9.3±0.47	12.7±0.47
Alcaligenes	8.3±0.27	10.6±0.25	12.7±0.47	14.7±0.47

Values are mean ± SD of three replicates

Table 3.4b: Antimicrobial activity of extract of aqueous neem against uropathogens

Name of Organism	**Zone of inhibition (mm)**			
	1000 ppm	**2000 ppm**	**3000 ppm**	**4000 ppm**
E. coli	9.3±0.47	13.3±0.47	14.7±0.47	21.7±0.47
Staphylococcus	14.7±0.47	17.7±0.47	19.3±0.94	23.3±0.14
Pseudomonas	8.0±0.82	11.7±0.47	12.7±0.32	19.7±0.22
Klebsiella	8.5±0.21	10.7±0.23	13.7±0.35	15.7±0.24
Proteus	13.7±0.25	15.7±0.32	19.7±0.32	23.7±0.47
Serratia	7.7±0.47	10.7±0.22	13.7±0.15	18.7±0.32
Alcaligenes	10.7±0.29	12.7±0.15	14.7±0.24	17.7±0.47

Values are mean ± SD of three replicates

IV. Discussion

Urinary tract infections (UTIs) are a serious health problem which is most common in females than in males. Incidence in women in the age of 20—40 years ranges from 25 to 30% whereas in older women above 60 years of age it ranges from 4 to 43% (Dielubanza and Schaeffer, 2011). In the present study *E. coli* was the most prevalent uropathogen. The most common etiological agent of uncomplicated UTI is *E. coli*, which is present in about 80%-90% of cases (Al-Jiffri*et al.*, 2011). 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram positive bacteria. Most UTIs in children are monomicrobic, often caused by *Escherichia coli* (Bhat*et al.*, 2011), *Proteus*, *Klebsiella*, *Enterococcus* and coagulase negative *Staphylococci* (Nicolle *et al*., 2008; Lane and Takhar, 2011.).The present study aimed at evaluating the antimicrobial potential of medicinal plants namely, tulsi, henna, amla and neem against the uropathogens as majority of the pathogens have developed multidrug resistance. All extracts of medicinal plants showed good antibacterial property. The aqueous extract of amla exhibited good antimicrobial activity against all isolated uropathogens. The aqueous extracts exhibited more antimicrobial activity than ethanolic extract. Thus the aqueous extract of henna, amla, tulsi and neem can be used as a potential source of natural antimicrobial compound against isolates of urinary tract infection.

V. References

1. Ahmed, J., Mehmood, Z. and Mohammad, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol*., 62: 183-193.

2. Al-Jiffri, O., Zahira, M.F., El-Sayed and Al-Sharif, F.M. 2011. Urinary tract infection with *Escherichia coli* and antibacterial activity of some plant extracts. *Int. J. Microbiol. Res*., 2: 1-7.

3. Bhat, R.G., Katy, T.A. and Place, F.C. 2011. Pediatric urinary tract infections. *Emergency medicine clinics of North America*,29: 637–53.

4. Bhuvaneswari, K., S. GnanaPoongathai, A. Kuruvilla and A. AppalaRaju, 2002. Inhibitory concentrations of Lawsoniainermis dry powder for urinary pathogens. *Indian J. Pharmacol*., 34: 260-3.

5. Cappucino, J. and Sherman, N. 1992. Microbiology: A laboratory manual. Benjamin/Cummings Publishing Company, San Francisco.

6. Dielubanza, E.J. and Schaeffer, A.J. 2011. Urinary tract infections in women. *The Medical clinics of North America*,95: 27–41.

7. Ksycki, M.F. andNamias, N. 2009. Nosocomial urinary tract infection. *Surg. Clin. North Am.*, 89:475-81.

8. Lane, D.R. and Takhar, S.S. 2011. Diagnosis and management of urinary tract infection and pyelonephritis. *Emergency medicine clinics of North America*,29: 539–552.

9. Nicolle, L.E. 2008. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. *Urol. Clin. North Am.*,35: 1–12.

10. Oelschlaeger, T., Fünfstück, R., Oelschlaeger, T. andFünfstück, R. 2006. Recurrent urinary tract infections in women. *Urologea*, 45: 412- 416.

11. Salvatore, S., Salvatore, S., Cattoni, E., Siesto, G., Serati, M., Sorice, P. and Torella, M. 2011. Urinary tract infections in women. *Eur. J. Obs. Gynec. Repr. Biol.*,156: 131–6.

12. Woodford, H.J. and George, J. 2011. Diagnosis and management of urinary infections in older people. *Clinical medicine*,11: 80–3.