

ANTIBACTERIAL ACTIVITY OF *URENA LOBATA* AGAINST UROPATHOGENS

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ABSTRACT

Urinary tract infections (UTI) are one of the most common form of bacterial infections but the treatment becomes cumbersome as the etiological bacteria are developing resistance against antibiotics. This present study evaluated the efficacy of antimicrobial activity of *Urena lobata* against uropathogens. Six urine samples from UTI patients were collected from Pathological Laboratory, G.B. Vaghani Multispecialty Hospital, Surat. Bacteria were isolated from these samples using Nutrient agar, Mac Conkey agar plate, Blood agar, Mannitol salt agar, Eosin Methylene Blue agar and King's agar. The bacterial isolates were identified using cultural characteristics, microscopic features and biochemical characteristics. Leaf extract of *Urena lobata* was prepared using Soxhlet Extraction Method whereby methanol and distilled water were the extractants used. Herbal extract disc was prepared at concentrations of 50, 75, and 100 mg/ml and tested against all the isolates. DMSO and antibiotics (Nitrofurantion, Amikacin, Levofloxacin, Norfloxacin, Ofloxacin and Cephalosporins) were used as negative and positive controls respectively. *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Klebsiella spp.* and *Brevibacillus panacihumi* were isolated from the urine samples. All concentrations of aqueous and methanolic extracts of *U. lobata* leaf displayed highest zone of inhibition against *B. cereus*. No inhibitory effect was observed against the growth of *Klebsiella* except at the highest concentrations. Further study is encouraged on the in-vivo study of efficacy of *U. lobata* on etiological agent of UTI.

INTRODUCTION

Urinary tract infections (UTI) are the most common form of bacterial infection affecting people throughout their life [1,2]. It is one of the common reasons for adults to seek medical help and one of the most frequently occurring nosocomial infections [3]. The treatment of UTI has become more complicated because of the appearance of pathogens with increasing resistance to antimicrobial agents [4]. Such antibiotic resistant organisms are difficult to eliminate during the course of an infection and are lethal to the human population. In addition, antibiotics in these cases are sometimes associated with adverse effects on the host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppressant and allergic reactions [5]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases in general and of UTI in particular.

During the last decade, the use of traditional medicine has expanded globally and is gaining popularity as an alternative medicine. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system [6]. According to the World Health Organization (WHO), herbal medicines serve the health need of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. Hence, pharmacognostic investigation of plants is carried out to find novel drugs or templates for the development of new therapeutic agents. Till now, only 5-10 of more than 250,000 species of higher plants have

been chemically investigated [7]. There is an increasing need for the documentation and development of alternative anti-pathogenic drugs. Accordingly, the antimicrobial properties of certain Indian medicinal plants were reported based on folklore information [8-12] and a few attempts were made to study inhibitory activity against certain pathogenic bacteria and fungi [13-15]. Considering the frequent emergence of antibiotic resistant strains and the immense potential of traditional medicines of plant origin, this work has been designed to study the efficacy of wild medicinal plant (*Urena lobata*) against antibiotic resistant bacterial strains isolated from urinary tract infections. The significance of the present study lies in evaluating the efficiency of *U. lobata* against multiple antibiotic resistant bacterial strains causing nosocomial UTI with significant inhibitory activity.

RESULTS AND DISCUSSION

Isolation of Bacteria from Human Urinary Tract Infection

Six (6) isolates were identified from all the samples. They were identified as follows: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Klebsiella spp.* and *Brevibacillus panacihumi*. This result partly agrees with the findings of Flores-Mireles, 2015 [16] who identified *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus* as the etiological agents of UTIs. The most predominant pathogen in uncomplicated UTIs in

women which associated with more than 80% of cases is *E.coli* while *Klebsiella* sp., *Proteus* sp. and *Enterobacter* sp. are also associated but with mild effect [17]. Several researchers reported that bacteria that usually instigate UTIs are gram negative residing in the intestine [18]. *E.coli* etiologically covers 75-95% and the remaining 5-25% are being caused by *Klebsiella* sp., *Proteus* sp. and *Enterobacter* sp. [19,20].

Antimicrobial Activity of leaf extracts of *Urena lobata*

The results revealed that all the three concentrations of both aqueous and methanolic extracts of *U. lobata* leaf were efficacious against *Bacillus cereus* with highest zone of inhibitions but no inhibitory effects against *Klebsiella* spp. except the highest concentration (Tables 1 and 2). Nitrofurantion, Amikacin and Levofloxacin antibiotics are very effective against the growth of all the uropathogenic bacteria (Table 3). All parts of *U. lobata* are

widely used in herbal medicine as wound healing, antioxidant, antidiabetic, antidiarrheal, antihyperglycemic and antimicrobial agents [21,22]. This work revealed that both aqueous and methanolic extracts of *U. lobata* leaves possessed antimicrobial properties against bacteria of UTI origin at a varying degree. This corroborated the result of Rahmatullah et al., 2009 [23] that the leaf and flower of *U. lobata* are taken orally to cure urinary tract disorder. The aqueous extract of this plant contains flavonoid glycosides (rutin and kaempferol) as useful chemical components with antibacterial activities [24]. Also, both rutin and kaempferol could synergistically augment the performance of other antimicrobial agents [25,26]. Combined therapy of *U. lobata* extract and cefazolin had been reported with an improved effect [27]. The antimicrobial potential of the methanolic extract of *U. lobata* leaves against *E.coli*, *S. aureus* and *Klebsiella* spp. is substantiated by the work of Fagbohun et al., 2012 [28].

Table 1: Diameter of zone of inhibition (mm) shown by methanolic extract of *Urena lobata* leaves against bacterial isolates from UTI

	Methanol (Control)	50mg/ml	75mg/ml	100mg/ml
<i>Staphylococcus aureus</i>	-	23.00	24.00	24.00
<i>Escherichia coli</i>	-	18.00	15.00	15.00
<i>Bacillus cereus</i>	-	30.00	31.00	31.00
<i>Streptococcus pneumoniae</i>	-	-	10.00	10.00
<i>Klebsiella</i> spp	-	-	-	10.00
<i>Brevibacillus panacihumi</i>	-	21.00	20.00	23.00

Table 2: Diameter of zone of inhibition (mm) shown by aqueous extract of *Urena lobata* leaves against bacterial isolates from UTI

	Distilled water (Control)	50mg/ml	75mg/ml	100mg/ml
<i>Staphylococcus aureus</i>	-	20.00	20.00	22.00
<i>Escherichia coli</i>	-	10.00	11.00	11.00
<i>Bacillus cereus</i>	-	23.00	21.00	24.00
<i>Streptococcus pneumoniae</i>	-	14.00	17.00	19.00
<i>Klebsiella</i> spp	-	-	-	11.00
<i>Bacillus cereus</i>	-	21.00	20.00	23.00

Table 3: Diameter of zone of inhibition (mm) shown by antibiotics against bacterial isolates from UTI

Bacterial isolates	Nitrofurantion (300 mcg)	Amikacin (30 mcg)	Levofloxacin (5 mcg)	Norofloxacin (10 mcg)	Ofloxacin (5 mcg)	Cephalothin (30 mcg)
<i>Staphylococcus aureus</i>	24.00	18.00	10.00	10.00	15.00	-
<i>Escherichia coli</i>	20.00	25.00	21.50	29.00	11.50	14.50
<i>Bacillus cereus</i>	22.00	23.00	27.00	25.00	28.00	23.00
<i>Streptococcus pneumoniae</i>	22.00	24.00	10.00	-	14.00	16.00
<i>Klebsiella</i> spp.	22.00	20.00	10.00	-	-	-
<i>Brevibacillus panacihumi</i>	24.00	22.00	36.00	32.00	35.00	22.00

CONCLUSION

The results of this work revealed that the leaves of *Urena lobata* showed better efficacy in controlling bacterial isolates from Urinary Tract Infection at a varying degree. This showed the usefulness of the plant in traditional medicine especially against UTIs. The study of herbal medicine for antimicrobial is an interesting research direction that provides a new way to solve the problem of bacterial drug resistance. Further work is encouraged on isolation, purification and characterization of active chemical components of the *U. lobata* leaf extract and its application in controlling UTI infection as a part of drug discovery.

MATERIALS AND METHODS

Isolation of Uropathogenic Bacteria

Six urine samples from UTI patients were collected from Pathological Laboratory, G.B. Vaghani Multispecialty Hospital, Surat. For the isolation of UTI, loopful of urine sample was streaked onto Nutrient agar, MacConkey agar, Blood agar, Mannitol salt agar, Eosin Methylene Blue agar and King's agar medium and incubated at 37°C for 24 hrs. Pure cultures were obtained after series of sub-culturing. Colonies were identified using both morphological characteristics and biochemical tests.

Collection of Medicinal Plant

The leaves of *Urena lobata* were collected from Bardoli metropolis, Gujarat. The leaves were washed in running tap water to remove the adhering soil particles, sterilized with 0.1% Mercuric Chloride for 1 Min and washed again with distilled water.

Preparation of Plant Extracts

Leaves of *Urena lobata* were air-dried at room temperature for 14 days. The dried leaves were pulverised mechanically into powder using mortar and pestle. Extraction was carried out using Soxhlet Extraction Method whereby methanol and distilled water were the extractants used [29].

After concentrating the extract at 50°C for 6 hrs, it was stored at 4°C until further use. For preparing disc, 1 ml of concentrated extract was incorporated into the pre-weighed watch glass and evaporated by heating at 50-60 °C for 1 hr and weighed again. Thus, the weight of the bioactive component in 1 ml was calculated and hence different concentrations were loaded onto sterile disc at concentrations of 50, 75, and 100 mg/ml [30,31].

Determination of Antimicrobial Activity:

Mueller Hinton Agar (Hi media) plates were prepared and seeded with 16 h broth cultures of bacterial isolates from UTI. Before making the Lawn culture with the inoculum, the turbidity was adjusted to 0.5 % of McFarland solution. Seeded inoculum was treated with different concentrations of herbal extract disc and antibiotics (Nitrofurantoin, Amikacin, Levofloxacin, Norfloxacin, Ofloxacin and Cephalosporins). Discs were also treated with DMSO and plated against isolates as control. The plates were incubated at 37 °C for 24 hrs. The experiment was repeated twice and the average of the zone of inhibition was recorded.

REFERENCES

1. Barnett, B.J. and Stephens, D.S. (1997). Urinary tract infection: an overview. *The American Journal of Medical Sciences*, 314:245-249
2. Foxman, B. (2002). Epidemiology of urinary tract infection: Incidence, morbidity and economic costs. *American Journal of Medicine*, 113 Suppl. 1A:55-135.
3. Gastmeier, P., Kampf, G., Wischniewski, N., Hauer, T., Schulgen, G., Schmacher, M., Daschner, F. and Rude, H., (1998). Prevalence of nosocomial infection in representative German hospitals. *Journal of Hospital Infection*, 38:37-49.
4. Murakami, K., Aoki, Y., Miyaji, Y., Iwaoka, K., Tsuka, H., Saikawa, S., Wada, O., Gohbara, M., Akion, H., Miwa, H., Takamura, M., Ikeda, H., Oyama, N., Fujita, T., Miwa, Y., Moriyama, N., Nakamura, N., Suzuki, Y., Kanamura, H. and Okada, K., (1995). Statistical studies on bacteria isolated from urinary tract infections during ten-year period Nishinohon *Journal of Urology*, 57:1260-1264.
5. Fair, R.J. and Tor, Y., (2014) Antibiotics and bacterial resistance in the 21st century. *Perspect Med Chem* 6: 25–64.
6. Lanfranco, G., (1999). Invited reviewed article on traditional medicine. *Electronic Journal of Biotechnology*, 2: 1-3.
7. Nahrstedt, A., (1996). Is the search for plant constituents as lead structures for pharmaceuticals still relevant? *Medical Research*, Gustav Fischer Verlag, Stuttgart, Germany, 1: 15-41
8. Ashmad, I., Mehamood, Z. and Mohammad, F., (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*, 62:183-193.
9. Dayal, B. and Purohit, R.M. (1971). Screening of some Indian essential oils for their antifungal properties. *Flavours India*, 2: 484-486.
10. Hook, M. and Thomas, S. (1995). Antimicrobial activity of aqueous and starch extracts of *Tinospora cardifolia*. *Current Science*, 67:637
11. Mehmood, Z., Ahmad, I., Mohammad, F. and Ahmad, S. (1999). Indian medicinal plants: a potential source for anticandidal drugs. *Pharmaceutical Biology*, 37:237-242.
12. Suresh, B., Kalyanaraman, V.R., Dharnasekaran, S., Annadurai, K., Dhannaraj, S.A. and Balasubramanian, S., (1995). Evaluation of Santolion oil in search of new drugs against candidiasis. *Indian Journal of Pharmacology*, 27:171-177
13. Taylor, R.S., Manandhar, N.P. and Towers, G.H.N., (1995). Screening of selected medicinal plants of Nepal for antimicrobial Activities. *Journal of Ethnopharmacology*, 46:153-159.
14. Natarajan, B., Paulsen, B.S. and Pushpangadan, P. (1999). An ethnopharmacological study from the Coimbatore district, Tamil Nadu, India: Traditional knowledge compared with modern biological science. *Pharmaceutical Biology*, 37:378-390.
15. Rajan, S., Sethuraman, M. and Mukherjee, P.K., (2002). *Ethnobiology of the Nilgiri Hills, India. Phytotherapy*

16. Flores-Mireless, A.L., Walker J.N., Caparon, M. and Hultgren, S.J. (2015). Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*. 13: 1-16.
17. Gradwohl, S.E., Bettcher, C. M., Chenoweth, C.E., Harrison, R.V. and Zoschnick, L.B. (2016). Urinary Tract Infection: Guidelines for clinical care ambulatory. Michigan Medicine, University of Michigan <http://www.med.umich.edu/1info/fhp/practiceguides/uti/uti.pdf/>.
18. Calderón-Jaimes, E., Casanova-Román, G., Galindo-Fraga, A., Gutiérrez-Escoto, P., Landa-Juárez, S., Moreno-Espinosa, S., Rodríguez-Covarrubias, F., Simón-Pereira, L. and Valdez-Vázquez, R., 2013. Diagnosis and treatment of urinary tract infections: a multidisciplinary approach for uncomplicated cases. *Boletín médico del Hospital Infantil de México*, 70:3-10.
19. Gupta, K., Hooton, T.M., Naber, K.G., Wullt, B., Colgan, R., Miller, L.G, et al. (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis*, 52:103-120
20. Prakash, D., & Saxena, R. S. (2013). Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut city, India. *International Scholarly Research Notices*, 2013, 1-13 <https://doi.org/10.1155/2013/749629>
21. Babu, S. S., Madhuri, D. B., & Ali, S. L. (2016). A pharmacological review of *Urena lobata* plant. *Asian. J. Pharm. Clin. Res*, 9: 20-22.
22. Islam, M. T., & Uddin, M. A. (2017). A revision on *Urena lobata* L. *International Journal of Medicine*, 5: 126-131.
23. Rahmatullah, M., Hossan, S., Hanif, A., Roy, P., Jahan, R., Khan, M., Chowdhury, M.H. and Rahman, T. (2009). Ethnomedicinal applications of plants by the traditional healers of the Marma Tribe of Naikhongchhari, Bandarban District, Bangladesh. *Advances in Natural and Applied Sciences*, 3: 392-401
24. Yang, Y., Huang, Z., Zou, X., Zhong, X., Liang, X. and Zhou, J. (2017). The antibacterial effect of *Urena lobata* L. from v Guangxi on mice with *Staphylococcus aureus pneumonia*. *Afr J Tradit Complement Altern Med.*, 14: 73-88
25. Arima, H., Ashida, H. and Danno G. (2002). Rutin-enhanced antibacterial activities of flavonoids against *Bacillus cereus* and *Salmonella enteritidis*. *Biosci Biotechnol Biochem.*, 66:1009–1014.
26. Lim, Y.H., Kim, I.H. and Seo, J.J. (2007). *In vitro* activity of kaempferol isolated from the *Impatiens balsamina* alone and in combination with erythromycin or clindamycin against *Propionibacterium acnes*. *J Microbiol.*, 45:473–477
27. Qiao, Q., Xiaoqin, Z., Yufang, Y., Runlian, W., Xibin, W., Shasha, Y., Xiaoli, H. and Xinwen, L. (2013). Antibacterial activity of aqueous extract of *Urena lobata* L. combination with antibacterial agents against gram-positive bacteria *in vitro*. *China Pharmacist*, 16:1475–1478.
28. Fagbohun, E.D., Asare, R. R. and Egbebi, A. O. (2012). Chemical composition and antimicrobial activities of *Urena lobata* L. (Malvaceae). *Journal of Medicinal Plants Research*, 12: 2256-2260
29. Patel N, Krishnamurthy R. (2021). In-vitro Phytochemical Screening and Bioactivity of *Moringa oleifera* Accessions. *Biosci. Biotech. Res. Comm.*; 14: 1
30. Zaidan, M. R., Noor Rain, A., Badrul, A. R., Adlin, A., Norazah, A., & Zakiah, I. (2005). In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop biomed*, 22: 165-170.
31. Patel Nikita, Patel Swetal, Soni Aditi, Garuba, Taofeeq, Oyeyinka, Samson, Ahmed, Akeem & Krishnamurthy Ramar. (2020). Antioxidant And Antimicrobial Potentials Of Some Plant-Based Natural Dyes. *International Journal of Biology, Pharmacy and Allied Science* 9: 2090-2101. 10.31032/IJBPA/2020/9.9.5134