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Original Research Article

Antibacterial activity of methanolic root extracts of *Asparagus racemosus* and *Withania somnifera* against uropathogens causing urinary tract infectionKaina Bhonsle^{1,*}, Alka Vyas¹, Harish Vyas², Abhiraj³, Kirti Hemwani⁴¹School of Studies in Microbiology, Vikram University, Ujjain, Madhya Pradesh, India²Govt. Kalidas Girls College, Ujjain, Madhya Pradesh, India³Amaltas Institute of Medical Sciences, Dewas, Madhya Pradesh, India⁴RD Gardi Medical College, Ujjain, Madhya Pradesh, India

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ABSTRACT

Urinary tract infection is one of the most common bacterial disease found in all age groups and has become a major health problem in developing countries. UTI's are treated with various antibiotics but extensive and inappropriate use of antibiotics promotes the development of antibiotic resistance among uropathogenic bacteria and also responsible for emerging multidrug resistant strains. The development of drug resistance among uropathogens made treatment of UTI is difficult which has led to the search of new source of antimicrobial agent that act against these uropathogenic bacteria. The ethnomedicinal plants are natural resource and known as traditional healer, which can use for successful therapy of various infectious diseases. In present study, we observed antibacterial potential of methanolic root extract of two medicinal plants *Asparagus racemosus* and *Withania somnifera* against urinary tract infection causing uropathogens. For this 120 urine samples of suspected patients were collected from different hospitals of Ujjain and processed in SRL laboratory Ujjain Center. It was seen that 72 samples were positive and showing significant bacteriuria and prevalence of UTI was 60%. The major causal bacterial agent was *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *S. aureus* and their frequency of occurrence was 37.5%, 25%, 15.2%, 13.8% and 9.7%. The antibacterial effect of methanolic plant extracts of both *A. racemosus* and *W. somnifera* was determined against isolated uropathogens by disc diffusion method. The effectiveness of both *A. racemosus* and *W. somnifera* was observed and it was found that they have effective antibacterial activity against uropathogens so they can be used as alternative drug against uropathogens.

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1. Introduction

Infectious diseases caused by various types of microorganisms such as bacteria, fungi, viruses and protozoa and it is a major leading cause of death all over the world mainly in developing countries. Bacteria are major causal agent of infection. When the host defense mechanism become impaired then bacteria invade into body and causes infections.¹ Urinary tract infections are the

most common infectious disease found in human urinary tract system and caused by several bacterial uropathogens.² Every year 150 million people suffered with UTI globally.² UTI found in both males and females in all age groups but females are more susceptible for UTI than males and 75% UTI patients were females due to their anatomy and physiology of urinogenital system and risk of infection increases with age.³ About 50% of females suffered with one symptomatic UTI during their lifetime. The risk of infection also observed in postmenopausal women.^{4,5}

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UTI's are most frequently found infectious disease in both hospital and community in developing countries due to lack of awareness, improper sanitation, use of unhygienic public toilets and low economic status. People suffered with hospital acquired UTI during their visit to hospital. They acquired infection by hospital environment and different medical devices. The causal organism associated with hospital acquired UTI are mostly endogenous pathogens but sometimes they originate from the hospital environment.⁶ The causal agent responsible for hospital acquired UTI are *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *Candida* and reported as more resistant strain of uropathogens.^{7,8} The community acquired UTI is an infection that found in the community and mostly observed in rural area. The most common causative agent of community acquired UTI's are *E. coli*, *Klebsiella* and *Enterococcus* among them *E. coli* is the most predominant uropathogen responsible for community acquired UTI.⁹

The treatment of UTI done by various antibiotics, they prevent growth and multiplication of bacteria. UTI's are treated with diverse group of antibiotics such as β -lactamase, aminoglycosides, fluoroquinolones and many others. The unwanted and nonjudicious use of antibiotics, inappropriate dosage, self-medication by direct purchasing of antibiotic from pharmacist without consulting with physician, selling of fake drug are several factors responsible for the development of antibiotics resistance and associated with emergence of MDR strains of bacteria. The emergence of drug resistance among uropathogens create hurdle in treatment and control of diseases. The other reasons of drug resistance among uropathogens are spontaneous mutation or acquisition of resistant genes among organisms by horizontal transfer.^{10,11}

The emergence of drug resistance and costly treatment of UTI has motivated researcher to find out new effective alternative and natural bioactive antimicrobial agent for treatment of infection. From ancient time's large number of tribals, rural and urban community used plants and its parts as a source of medicine to treat different types of infections. Ethnomedicine is still use as a primary and alternative source of medicine in treatment of various infectious diseases because they are cheap and easily available.^{12,13}

The present study conducted to determine the antibacterial activity of *Asparagus racemosus* and *Withania somnifera* against some common bacteria causing urinary tract infection. *Asparagus racemosus* is a woody climbing plant commonly known as 'Shatawari', also called 'queens of herbs' used in Ayurvedic medicine and belongs to family Asparagaceae and found in India, Australia, Asia and Srilanka. The roots and stems are used for making medicine and used in treatment of diarrhea, dysentery and urinary tract infection due to its antimicrobial property. It has antioxidant and antibacterial effect and stimulates the immune system. Its root has a good antidiuretic effect.

It helps control burning sensation in UTI, it increases urine flow and flush out various harmful toxins and pathogens. It is mainly contains β - Sitosterol which have good antibacterial activity against UTI causing bacteria. This drug is safe without any side effects.^{14,15} *Withania somnifera* is annual evergreen shrub belong to Solanaceae family commonly known as 'Ashwagandha' or 'Winter Cherry'. It is native of India, Asia and Africa. It has antibacterial, anti-inflammatory, antioxidant and immunobooster activity. It used to relieve bladder pain, burning sensation during urination and help in removal of kidney stone.^{16,17}

2. Materials and Methods

The study was conducted to evaluated antibacterial effect of methanolic root extracts of two plants *Asparagus racemosus* and *Withania somnifera* on isolated uropathogens. This study was performed in SRL laboratory Ujjain center. The urine samples collected from different hospitals of Ujjain were processed in lab. Total 120 urine samples from suspected patients were collected from November 2018 to October 2019. The collected 0.5 ml of urine sample was inoculated on three different selective and differential media, which were Blood agar, MacConkey agar and Chrome agar with the help of sterilized loop, by streak plate method. The plates were incubated at 37°C for 24 h and after incubation colony forming unit were counted, if colony count is more than 10⁵ colonies forming unit/ml than it indicated significant bacteriuria and was considered as positive urine culture. The single pure colonies were selected and subjected to morphological, microscopic and biochemical examinations as per the standard procedure for confirmation of isolated uropathogenic bacteria.¹⁸ In morphological examination shape, size, color and margin of colony was observed. In microscopic examination gram staining was done for differentiation between gram positive and gram negative bacteria and the shape, color, arrangement of bacteria were also examined. The biochemical tests included Catalase, Oxidase, Coagulase, Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Triple sugar iron, Urease, Mannitol fermentation, Bile Esculine Hydrolysis test and motility tests.

2.1. Antibacterial activity of plant extract

The antibacterial activity of root extract of *Asparagus racemosus* and *Withania somnifera* was determined against isolated uropathogens *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *S. aureus*. For this plant extract of both plants were prepared. The dried powder of plant parts were purchased from Amsar Private Limited, Indore and stored in airtight container. The root part of both plants were taken and their methanolic extract were prepared by

dissolving 5 gram of dried root powder in 9 ml of methanol and volume was made up to 10 ml. The extracts were serially diluted to obtain different concentration of plant extracts. The antibacterial activity of this prepared plant extracts were observed on isolated uropathogens by disc diffusion method.¹⁹ In this method disc of plant extract was prepared by soaking 6 mm filter paper disc in diluted plant extract. After preparing, the disc fresh culture of isolated bacteria was spread on Mueller-Hinton agar plate surface using sterile swab. The inoculated plates were placed in sterile conditions for 1 hour for stability then disc of prepared plant extracts were placed on agar surface with the help of sterile forceps and incubated at 37°C for 24 h. After incubation, the zone of growth inhibition around the plant extract disc was determined and antibacterial activity of both plant extracts against isolated uropathogens was observed.

3. Results

3.1. Prevalence of UTI and frequency of uropathogens

It was seen that out of 120 urine sample, 72 were positive and its prevalence was 60%. The five uropathogens were isolated from patients, among which four were gram negative and one was gram positive uropathogenic bacteria. The *E. coli* was most prevalent uropathogen and its frequency was 37.5% which was followed by *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *S. aureus* and their frequency of occurrence was 25%, 15.2%, 13.8% and 9.7%. The frequency of isolated uropathogens were shown in Figure 1.

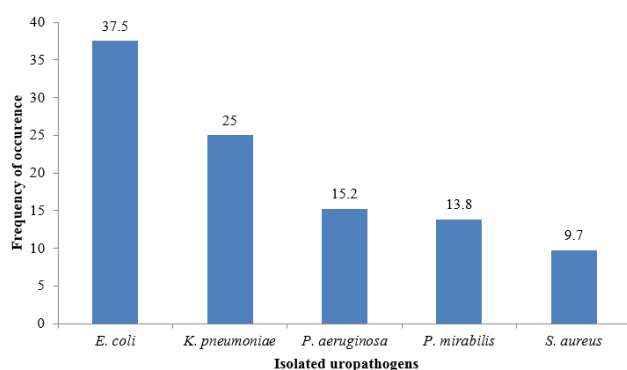


Fig. 1: Frequency of occurrence of isolated uropathogens.

3.2. Effect of plant extracts

In our study plant extracts of both *Asparagus racemosus* and *Withania somnifera* showed antibacterial activity against five isolated uropathogenic bacteria. The antibacterial effects were categorized low, moderate or high based on the size of growth inhibition zone. It was seen that methanolic

root extract of *Asparagus racemosus* had moderate killing activity against *K. pneumoniae*, *P. aeruginosa* and low killing activity against *P. mirabilis* and *S. aureus* and methanolic root extract of *Withania somnifera* was effective for killing *K. pneumoniae* and had moderate killing activity against *E. coli*, *P. aeruginosa*, *P. mirabilis*. The results of antibacterial activity were seen only on sensitive isolates and results are shown in Table 1.

4. Discussion

In our study the overall prevalence of UTI was 60%. Paul *et al.*, (2021) conducted similar study and investigate prevalence of UTI in primary care center of Aasam, India, found percentage of UTI was 59.6%, and Kumar *et al.*, (2019) found that percentage of UTI was 60.6% in Ambala (Haryana), India. In Bangladesh Akhtar *et al.*, (2017) reported 58.9%. These results are almost similar to results of current study, which suggested that the prevalence of UTI is not much different.^{20–22} During the study five uropathogens *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *S. aureus* were isolated and among them *E. coli* was most frequently isolated uropathogens and its The frequency of occurrence was 37.5%. Higher frequency of *E. coli* isolates was also correlates with studies done by Agrawal *et al.*, (2021), Mendem *et al.*, (2020) in India and Odoki *et al.*, (2019), reported frequency of *E. coli* isolates 43.5%, 47.0% and 41.9% respectively.^{23–25}

The antibacterial effect of methanolic root extract of *Asparagus racemosus* and *Withania somnifera* was determined in our study and both have killing effect against uropathogens. The antibacterial effect of *Asparagus racemosus* was also observed by Mukhtair Uddin in 2012, they observed killing effect of methanolic root extract against eight bacterial strains of *K. pneumoniae*, *E. coli*, *P. alkaligenes*, *Proteus spp.*, *Salmonella typhi*, *Shigella*, *V. cholera* and *S. aureus*. In 2014 Chouhan and Pandey studied antibacterial potential of *Asparagus racemosus* extract against bacteria causing UTI. They reported that aqueous and methanolic extracts of *Asparagus racemosus* exhibited broad spectrum antibacterial effect against both gram positive and gram negative bacteria in comparison to ethanolic and ethyl acetate extract. Similarly Arya *et al.*, 2018 also reported efficacy of root powder of *Asparagus racemosus* in reducing UTI, boost the immune system and prevent chances of reinfection.^{26–28}

The antibacterial effect *Withania somnifera* also detected by Bokaeian *et al.*, 2015, they showed that ethanol extract of *Withania somnifera* leaf extract might be used as natural drug for treatment of infectious disease caused by *S. aureus*. Das *et al.*, 2019 investigated root and stem extract of *Withania somnifera* plant have antibacterial activity against some human pathogenic bacteria. Beigomi *et al.*, 2021 evaluated antimicrobial effect of Rosemary and *Withania somnifera* methanol extract prepared by

Table 1: Antibacterial activity of *Asparagus racemosus* and *withania somnifera* against isolated uropathogens.

S. No.	Plant name	Plant part	Solubility	Dilution factor	Antibacterial effect on isolated uropathogens				
					U1	U2	U3	U4	U5
1.	<i>Asparagus racemosus</i>	Root	45% Methanol	10 ⁵	+	++	++	+	++
2.	<i>Withania somnifera</i>	Root	50% Methanol	10 ⁵	++	+++	++	++	++

U1: *E.coli*, U2: *K. pneumoniae*, U3: *P. aeruginosa*, U4: *P. mirabilis*, U5 *S. aureus*.

ultrasound waveform on *E. coli* biofilm isolated from urinary tract infection.^{29–31}

5. Conclusion

The treatment and prevention of UTI can be done by plant and its part because they are cost effective, easily available and safe with no side effects. These plant extracts reduces antimicrobial resistance hazards and help to decrease adverse effects of antibiotic because it contains several secondary metabolites and active phytochemicals responsible for their antibacterial activity. The root extracts of both *Asparagus racemosus* and *Withania somnifera* showed promising antibacterial activity against the isolated uropathogens. Therefore, isolation and identification of secondary metabolites of plants will help in to develop modulator or precursor for synthesis of new antimicrobial agent as alternative of antibiotics.

6. Conflict of Interest

The authors declare no relevant conflicts of interest.

7. Source of Funding

None.

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