Research Article

Phytochemically analyzed Chaste tree leaves extracts involving some catheter-associated urinary tract infection causing bacteria

Aashaq Hussain Allaie[1], Ravi Prakash Mishra[1*], Aijaz Ahmad Ganaie[1], Shoket Ali[1], Ranu Singh[2]

[1]*Environmental Biotechnology Lab. Department of P.G. Studies & Research in Biological Sciences, R. D. University, Jabalpur, India*

[2]*Department of Botany and Microbiology Mata Gujri Mahila Mahavidyalaya (Autonomous), Jabalpur, India*

https://doi.org/10.31024/ajpp.2018.4.2.20

Received: 26 February 2018 Revised: 18 March 2018 Accepted: 27 March 2018

Abstract

Objective: To make scientific validation of the ethanomedicinal *Vitex negundo (Jacq.)* in relation to urinary disorders. **Methods:** Antibacterial study was carried out on clinically isolated Catheter-associated urinary tract infections (CAUTI) infecting bacteria by disc diffusion method. **Results:** Among the two extracts tested against three different Catheter-associated urinary tract infections (CAUTIs) bacteria, methanolic extract was effective against *E. coli* with highest inhibition zone (27 mm) followed by *P. aeruginosa* with inhibition zone (20 mm) and *K. pneumoniae* with inhibition zone (19 mm), while, hexane extract also shows highest effective against *E. coli* with inhibition zone (29 mm) followed by *P. aeruginosa* with inhibition zone (23 mm) and *K. pneumoniae* with inhibition zone (16 mm). Preliminary phytochemical screening shows the presence of phytoconstituents *viz* alkaloids, glycosides, saponins, carbohydrates, proteins, amino acids, flavonoids, steroids, tannins. **Conclusion:** Based on the present study, along with previous studies, the ethanomedicinal use of *Vitex negundo* for the treatment of CAUTI has been scientifically validated.

Keywords: *Vitex negunda*, Phytochemical, Chaste tree, CAUTI

Introduction

The most common healthcare-associated infection is catheter-associated urinary tract infections which occur in patients after insertion of catheter and accounts for 30% to 40% of all the hospital acquired infections (Conway and Larson, 2012; Jaggi and Sissodia, 2012). Catheter associated urinary tract infections (CAUTI) are major concern as one of the nosocomial infections. Insertion of catheter in patients greater than 7 days accounts infection up to 25% and increases to 100% after 30 days (Jaggi and Sissodia, 2012). Catheter-associated urinary tract infections (CAUTIs) are among the most common hospital-acquired infections worldwide, accounting for >1 million cases in the United States and Europe annually (Tambyah and Oon, 2012; Zarb et al., 2012). Catheter associated urinary tract infections (CAUTI) account for more than 80% of all healthcare associated infections (HAIs) as compared to non-catheterized urinary tract infections (Saint and Chenoweth, 2003; Weber et al., 2011). The infection that occurs with an indwelling Foley's urinary catheter within 48 hours is a catheter-associated urinary tract infection (Conway, and Larson, 2012). Most common pathogens associated with CAUTI are *Escherichia coli, Klebsiella pneumoniae, Enterobacter* species*, Proteus* species*, Pseudomonas aeruginosa, S. saprophyticus,* and *Candida* species

The growing antibiotic resistance amongst the uropathogen isolated from CAUTI making difficult for its management. Thus, indwelling urethral catheter use is exceedingly common in health care facilities. Prevention of infections attributable to these devices is an important goal of health-care infection prevention programs.

The plant *Vitex negundo* of Verbenaceae family is commonly known as Nirgundi (Hindi) and five leaved chaste tree (English), and Nirgundi in Sanskrit, is a woody, aromatic shrub growing to a small tree. The plant is found throughout India, tropical Africa, China and Philippines (Kirtikar and Basu, 2008). It is used in all systems of treatment – Ayurveda, Unani, Siddha, Homeopathy and Allopathy since ancient times. It is taken in a variety of ways, both internally and externally. Although all parts of *Vitex negundo* are used as prescription in the indigenous

**Address for Corresponding Author:*
Ravi Prakash Mishra
Environmental Biotechnology Lab. Department of P.G. Studies & Research in Biological Sciences, R. D. University, Jabalpur, (M.P.) India
Email: rpmishra2006@rediffmail.com

arrangement of pharmaceutical, the leaves are the most intense for medicinal use (Kirtikar and Basu, 1984; Samy et al., 1998; Kirtikar and Basu, 2008; Nadkarni, 2002; Sharma et al., 2005). Lagundi tablets prepared from leaves of *V. negundo*, and commercially marketed as Ascof® (Rose Pharmacy, Mandaue, Philippines) are prescribed for the relief of mild to moderate bronchial asthma and cough (Tiwari and Yamin, 2007). The leaves are most potent and numerous therapeutic properties for medicinal use. Hence, the basic aim of the present study was executed to explore the subjective investigation of phytochemicals and antibacterial properties of methanolic and hexane extracts of *Vitex negundo* Linn against uropathogens.

Material and methods

Collection and preparation of the plant material

The plant was selected based on reports of its widespread use among the local communities in Jabalpur region. Identification of the plant was done at the Biological Sciences department of R. D. University Jabalpur. The plant was identified on morphological basis as per description given in the literature (Roy et al., 1992; Moberly, 2008; Singh, 2014). After collection, the leaves were sun dried for 7 days and pounded using pestle and mortar and stored at 35 - 37°C until required.

Extraction procedure

Twenty five grams powdered leaves were taken with 250ml of methanol and hexane solvents for 12h at 30 °C temp. in soxhlet apparatus and then filtered using Whatman No.1 filter paper. The filtrate evaporated to dryness using rotary evaporator and the resultant extract stored in a reagent bottle at 4 –8°C.

Phytochemical analysis

The phytochemical screening for the crude methanolic and hexane extract of *V. negundo* was carried out by standard protocols Raman (2006) and Harbone (2005). The presence of alkaloids, glycosides, saponins, carbohydrates, proteins, aminoacids, flavonoids, steroids, tannins was detected as shown in Table 3.

Isolation and Identification of CAUTI bacteria

The microorganisms present in urine samples of CAUTI infected patients were cultured in the nutrient broth and were differentiated using the gram staining procedure into gram positive and gram negative organisms.

The organisms were transferred to cystine lactose electrolyte deficient (CLED) agar medium for further differentiation of urinary organisms. Urine samples were shake well in their containers for even distribution of bacteria. A calibrated wire loop with internal diameter 3.26mm that hold 0.004 ml of urine was inoculate into the above media. The inoculums were spread with the wire loop on the media plate. Plates were incubated aerobically at 37°C for 24 hours. Six biochemical tests were performed for each organism. Catalase activity, indole production test, citrate utilization test and urease test were done as given in Table 4.

Antibacterial Activity

The antibacterial efficacy of methanolic and dichloromethane leaves extract of *Vitex negunda* was tested by disc diffusion method (Bauer *et al*., 1966). Solution of known different concentration *viz* 25%, 50% and 75% of the test samples in calculated volume of solvents dried and sterilized filter paper discs (6mm diameter) were then impregnated with known amount of the test substances using micropipette. Discs containing the test microorganisms. Standard antibiotic (Ciprofloxacin) disc was used as a positive control. These plates were then kept at low temperature (4 °C) for 24 h to allow maximum diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37 °C for 12 h to allow maximum growth of the microorganisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Results and discussion

A total of 200 patients of all age group clinically diagnosed as catheter associated urinary tract infection (CAUTI) were studied to isolate bacteria from urine. Out of 200 CAUTI 60 (62%) were culture positive.

Table 1. Distribution of culture by CAUTI

Type of UTI	Urine cultured	Culture positive cases	Percentage
NUTI	200	60	30%

Among the samples analyzed and data obtained by the patients of CAUTI it could be assessed that both male and female are equally effected for the above infection i,e the total number of male and female CAUTI Patient was 30 (50 %).

Table 2. Distribution of microorganisms varies in relation to the duration of catheter

Isolated organism	<1 week(Sample 100Patient's)	>1 week(Sample 100 Patient's)
E. coli	09	23
P. aeruginosa	03	08
K. pneumoniae	05	14
Total	**17(17%)**	**43(43%)**

The organism mostly isolated from CAUTI patients was depends on duration of catheterization before 1 week of catheterization *E. coli* 9(9%) followed by *Klebsiella* species 05(5%), and *Pseudomonas aeruginosa* 03(3%) after one week of catheterization isolation of all pathogens increased (Table 4).

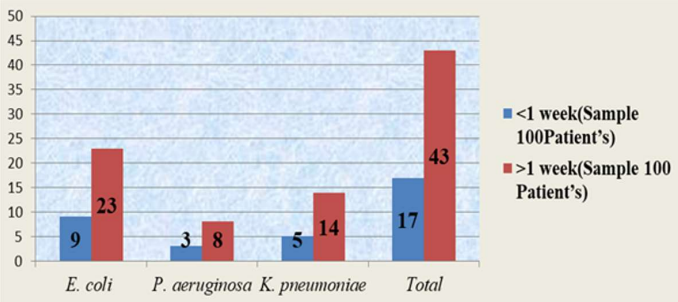

Figure1. Distribution of microorganisms varies in relation to the duration of catheter

The phytochemical screening of the *Vitex negundo* leaf extract in the present study revealed strong to moderate presence of different active constituents (secondary plant metabolites) like alkaloids, amino acids, flavonoids, tannins, glycosides, steroids, carbohydrates and proteins as shown in table 3.

Table 3. Preliminary phytochemical screening of leaves of *Vitex negundo*

Name of the test	Methanol Extract	Hexane Extract
Carbohydrates	+	+
Proteins	+ +	+
Aminoacids	+	+
Steroids	+ +	+
Glycosides	+	+ +
Flavonoids	+	+
Alkaloids	+	+ +
Tannins	+	_

+ = indicates presence, - = indicates absence, ++ = indicates presence of higher concentrations

The bacterial isolates were identified by biochemical tests shown in table 4.

Table 4. Biochemical tests of recovered clinical isolates

Catalase	Indole	Oxidase	Citrate	Urease	Organism confirmed
+	+	_	_	_	*E. coli*
+	_	+	+	_	*P. aeruginosa*
+	_	_	+	±	*K. pneumonia*

+= positive, - = negative

The results of inhibitory effect of methanolic and hexane leaf extract of *V. negunda are* shown in Table 5.

Table 5. Antibacterial activity extract of *Vitex negundo* against selected CAUTI pathogens

Concentration of extract (mg/ml)	*E .coli*		*K. pneumoniae*		*P. aeruginosa*	
	Methanolic extract	Hexane extract	Methanolic extract	Hexane extract	Methanolic extract	Hexane extract
25	13	15	11	10	14	11
50	23	26	13	12	16	17
75	27	29	19	16	20	23
Standard Ciprofloxacin	41		44		40	

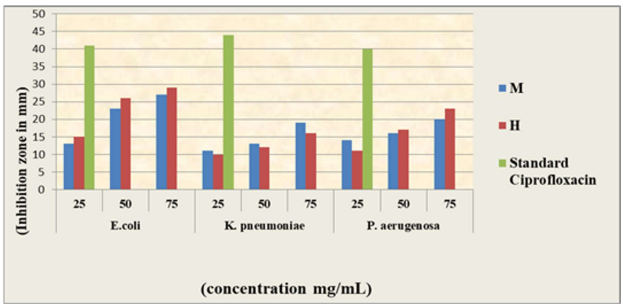

Figure 2. Sensitivity pattern of leaf extract of *V. negundo* against some CAUTI causing Bacteria

The results indicate that three bacterial species exhibit different sensitivities towards the extract. The extract was found to be inhibitory to both bacterial isolates but with variable extent. The order of activity against selected bacteria was *E. coli*> *P. aeruginosa*> *K. pneumoniae*

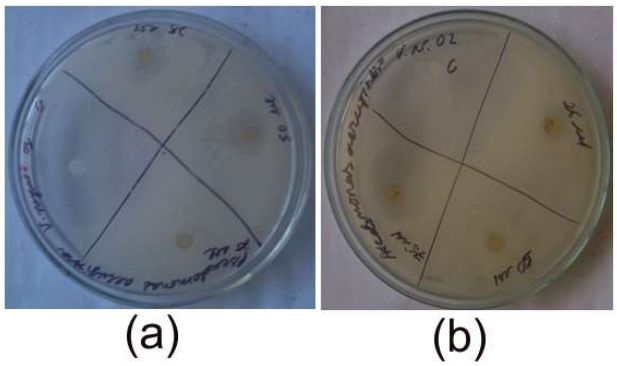

Figure 3. Antibacterial action of (a) methanolic leaf extract and (b) hexane extract of ***V. negundo*** leaves against *P. aeruginosa*

In the present study, the *Vitex negundo* methanolic and hexane leaf extract effectively inhibited both bacteria tested. The results of the sensitivity test are shown graphically in (**Figure 4**).The zone inhibition values of the extract against tested bacteria ranged from 11 to 32 mm. It is clear from **table (vi)** as the concentration increases inhibition zone increases in all cases. All the bacterial species shows highest activity at higher concentration of extracts *E. coli* shows 27 and 29 mm, *P. aeruginosa* 20 and 23 while as *Klebsiella pneumonia* 19 and 16 at 75mg/mL

against methanol and hexane, whereas at minimum concentration shows (13&15mm), (14&11mm) and (11&10 mm) at 25mg/ml respectively. The methanolic extract exhibited maximum activity against *E. coli* (32 mm) followed by *P. aeruginosa* (20 mm). The results in the present study indicate that the antibacterial activity varies according to type of bacteria used for the study. The least activity was exhibited by *Klebsiella pneumonia* with the smallest zone (10mm). The diameters of the zones of inhibition by the addition of *V. negundo* extract is shown in (**Table VI**). The diameters of the zones of inhibition with the standard drug used were 41 mm, 44 mm and 40 mm for the three strains respectively. The studied extracts showed a remarkable antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *and, Klebsiella pneumonia*. Although, the mechanism of action of the extracts has not yet been elucidated in detail, the presented data confirm *in vitro* antibacterial activity of extract of *V. negundo* against UTI causing bacteria. The overall incidence of CAUTI was 4.9 per 1000 catheterized days in another report. The most common uropathogen was *E. coli* followed by *K. pneumoniae*. The development of CAUTIs has been directly linked to the duration of catheterization; nearly 100% of patients undergoing long-term catheterization (≥ 28 days) are found to develop catheter associated bacteriuria (CAB) (Armbruster and Mobley, 2012; Siddiq and Darouiche, 2012; Jacobsen et al., 2008). Medicinal plants have provided copious leads to combat diseases, from the dawn of civilization. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. The extensive survey of literature revealed that *Sinduvara* (*Vitex negundo*) is important medicinal plant with diverse pharmacological spectrum. The presence of secondary metabolites like tannins, saponin, carbohydrates, glycosides, alkaloids, flavonoids, terpenoids, steroids, polyphenols and anthraquinones of *vitex negundo* suggests that the plant might be of medicinal importance and supports the bases for some of the ethno-uses (Raama and Venkataraman, 2010). The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and antibacterial activities and can aid in wound healing and burns (Haslem, 1989).

Conclusion

The results from the current study indicate that *Vitex negundo* (L) leaves contained various types of compounds with potential pharmacological activity against bacterial pathogens associated with CAUTIs. Further research work involving more detailed *in vitro* and *in vivo* investigations is required to establish which components of the extract are biologically active in terms of antibacterial activity versus CAUTI causing pathogens. The isolation of bioactive components from this readily available natural resource and their utilization as potential natural antibacterial agents could be of high economic value.

References

Armbruster CE, Mobley HL. 2012. Merging mythology and morphology: the multifaceted lifestyle of Proteus mirabilis. Nature Reviews Microbiology, 10(11):743-754.

Bauer AW, Kirby WM, Sherries JC, Turckp M. 1996. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45:493-496.

Conway LJ, Larson EL. 2012. Guidelines to prevent catheter-associated urinary tract infection: 1980 to 2010. Heart & Lung. The Journal of Acute and Critical Care, 41:271-283.

Harbone JB. 2005. Phytochemical Methods. New Delhi: Springer (India) Pvt. Ltd, P. **17.**

Haslem E. 1989. Plant polyphenols: Vegetable tannins revisited chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, pp. 169.

Jacobsen S, Stickler D, Mobley H, Shirtliff M. 2008. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis***.** Clinical Microbiology Reviews, 21:26–59.

Jaggi N, Sissodia P. 2012. Multimodal supervision programme to reduce catheter associated urinary tract infections and its analysis to enable focus on labour and cost effective infection control measures in a Tertiary Care Hospital in India. Journal of Clinical and Diagnostic Research, 6**:**1372-1376.

Kirtikar KR, Basu BD. 2008. Indian Medicinal Plants. International Book Distributors, Dehradun, 3, pp 1937-1940.

Kirtikar KR, Basu BD. 1984. Indian Medicinal Plants, Bishen Singh Mahendra Pal Singh, Dehradun. International book distributors, pp 2061- 2062.

Mabberley DJ. 2008. The plant book, a portable dictionary of plants, their classification and uses, Cambridge University Press, Cambridge, pp 227.

Nadkarni KM. 2002. Indian Materia Medica Bombay Popular Prakashan, 1:1278-1280.

Raama MJ, Venkataraman S. 2010. Phytochemical investigation and Antipyretic activity of leaf extract of *Vitex negundo* Linn. International Journal of Pharma Tech Research, 2(2):1068-1073.

Raman N. 2006. Phytochemical Technique. New Indian Publishing Agencies, New Delhi, pp 19.

Roy GP, Shukla BK, Datt B. 1992. Flora of Madhya pradesh. Ashish Publishing House, New Delhi, pp 1-643.

Saint S, Chenoweth CE. 2003. Biofilms and catheter-associated urinary tract infections. Infectious Disease Clinics of North America, 17:411-32.

Samy RP, Ignacimuthu S, Sen A. 1998. Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacology, 62:173-182.

Sharma PC, Yelne MB, Dennis TJ. 2005. Database on Medicinal Plants used in Ayurveda. CCRAS Publication, New Delhi: Reprint Edition, 3:450-471.

Siddiq DM, Darouiche RO. 2012. New strategies to prevent catheter-associated urinary tract infections. Nature Reviews Urology, 9:305–314.

Singh VP. 2014. Flora of Madhya Pradesh (western part). Scientific publisher, 1-558.

Tambyah PA, Oon J. 2012. Catheter-associated urinary tract infection. Current Opinion in Infectious Diseases, 25:365-70.

Tiwari OP, Yamini BT. 2007. Antioxidant properties of different fractions of *Vitex negundo* Linn. Food Chemistry, 100:1170–1176.

Weber DJ, Sickbert-Bennett EE, Gould CV, Brown VM, Huslage K, Rutala WA. 2011. Incidence of catheter-associated and non-catheter-associated urinary tract infections in a healthcare system. Infect Control Hospital Epidemiology, 32:822-3.

Zarb P, Coignard B, Griskeviciene J, Muller A, Vankerckhoven V, Weist K. 2012. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Euro Surveillance, 17:20316.