

In vitro evaluation of anti-bacterial activity of *Cordia dichotoma* Forst on urinary tract pathogens

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Abstract

The aim of present study was to evaluate the antibacterial activity of *Cordia dichotoma* Forst on selected urinary tract pathogens. *Cordia dichotoma* Forst commonly known as Indian cherry belonging to family Boraginaceae is medium sized tree with a short bole and spreading crown. Antibacterial activity of petroleum ether, chloroform, methanol and aqueous extracts against urinary tract pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* and *Proteus vulgaris* using disc diffusion technique was evaluated. The results of this study showed that the methanol extract exhibited better anti-bacterial activity against the bacterial species tested as compared to other extracts. An effort has been made to compare the activity of extracts with standard antibiotics against selected urinary tract pathogens.

Keywords: Anti-bacterial activity, *Cordia dichotoma*, Clinical pathogens, Agar well diffusion technique, Zone of Inhibition.

INTRODUCTION

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. One in five women will develop UTIs in their lifetime in America. Urinary tract infections are the second most common type of infection in the body, accounting for about 8.1 million visits to health care providers each year [1]. UTI is an important cause of childhood morbidity and it has been recommended for the inclusion in integrated management of childhood illness [2]. The most common UTIs include *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella*, *Enterococci* bacteria and *Proteus mirabilis*. Rare bacterial causes of UTIs include *Ureplasma urealyctium* and *Mycoplasma hominis*.

Urinary tract infection (UTI) is a condition where one or more parts of the urinary system (the kidneys, ureters, bladder, and urethra) become infected. UTIs are the most common of all bacterial infections and can occur at any time in the life of an individual. Due to the frequency of antibiotic use for UTI's bacteria develops resistance to the antibiotics, making them less effective over a period of time. Certain herbal remedies relieve

urinary tract infections by combating the bacteria, decreasing irritation and healing urinary tract tissues. Some herbs also help prevent future occurrences [3].

In several Asian and African countries, 80% of the population depends on traditional medicine for primary health care including UTI. Worldwide there are several reports for herbal treatment of UTIs. The most commonly used herbs are *Vaccinium oxycoccus* and *Arctostaphylos uva-ursi* [4]. Sahoo et al [5] have investigated the antibacterial activity *Hybanthus enneaspermus* Muell. against six selected UTIs pathogens. Other important herbs used in UTIs are Goldenseal (*Hydrastis canadensis*) [6], Marshmallow root (*Althea officinalis*) [7], Buchu (*Barosma betulina*) [8], Corn silk (*Zea mays*) [9] and the pteridophyte Horsetail – *Equisetum arvense* [10, 11].

Cordia dichotoma Forst belonging to family Boraginaceae is medium sized tree with a short, usually crooked trunk (90-100 cm in girth) and bearing globose, grows in India, Srilanka and other warmer countries [12-14]. The medicinal attributes of *Cordia dichotoma* have

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been known since a long time. The fruits of the plant are used as cooling, astringent, emollient, expectorant, anthelmintic, purgative and diuretic [15]. A number of pharmacological properties such as analgesic, anti-inflammatory and hepatoprotective have been reported [16-18].



Figure 1: Fruits of Cordia dichotoma Forst

Although Cordia dichotoma is used ethno medicinally in Urinary tract infection, there is no specific study on antibacterial activity against the pathogens causing urinary tract infection. The aim of the present study is to determine the antibacterial activity of the plant Cordia dichotoma against selected urinary tract pathogens

MATERIALS AND METHODS

Plant material: Fresh ripe fruits of Cordia dichotoma Forst were collected from the local area of Pune District of Maharashtra, India in February 2011. The plant specimen was identified and authenticated as Cordia dichotoma Forst by Botanical Survey of India, Pune, India. The voucher specimen (No. MUGCOD2) is preserved in the herbarium of Department of Pharmacognosy. Successive solvent extraction was carried out with Soxhlet using different solvents like petroleum ether (60° – 80° C), chloroform, methanol and water as per increasing order of polarity. The extract was dried using rotary evaporator and was kept in a dessicator till experimentation.

Test microorganisms: Bacterial strains used were Escherichia coli (NCIM No 2065), Klebsiella pneumonia (NCIM No 2957), Proteus mirabilis (NCIM No 2387), Pseudomonas aeruginosa (NCIM No 2036), Staphylococcus aureus (NCIM No 2079) and Proteus vulgaris (NCIM No 2857). These organisms were obtained from NCL, Pune, India.

Methodology: The extracts were prepared in 5mg/ml and 10mg/ml and 20mg/ml concentrations in sterile water. About 50µl of extract of these concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 250µg, 500 µg and 1000 µg respectively. The discs were dried and kept aseptically.

Screening of antibacterial activity [disc diffusion technique]

Agar Well Diffusion Method: The screening of antibacterial activity of plant extracts was carried out using the agar well diffusion method. The bacterial strains were inoculated into tubes of Brain heart infusion agar and incubated at 37°C overnight. Each of the cultures was then adjusted to 0.5 [19-22]. Mc Farland turbidity standard. Culture of the test organisms were made on the Brain heart infusion agar [BHI-Hi media M211] plates using sterile cotton swab and the plates were dried for 15 minutes. A sterile cork borer was then used to make wells (6mm diameter) for different concentrations of the extracts on each of the plates containing cultures of the different bacterial strains. 50µl of the varying concentrations (250 µg, 500µg and 1000 µg) of the extracts were introduced into the wells with the help of micropipette and were then incubated in upright position at 37°C for 24 h. After 24 hrs, antibacterial activity was determined by measurement of diameter of zones of inhibition (mm) [23]. Standard antibiotic discs of Amoxicillin (30mcg/disc) and Ciprofloxacin (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

RESULTS AND DISCUSSION

The antibacterial activity of the extracts at different concentrations was screened by disc diffusion technique and by measuring the zone of inhibition. The results are given in the Table 1.

Table 1: Antibacterial activity of different extracts of *Cordia dichotoma* F. fruit

Extract	Conc (µg)	Zone of inhibition (mm)					
		B1	B2	B3	B4	B5	B6
Pet ether	250	09	-	07	09	08	08
	500	13	08	09	11	13	10
	1000	15	10	13	14	16	15
Chloroform	250	08	07	08	-	07	09
	500	10	09	11	08	09	11
	1000	16	11	13	11	12	14
Methanol	250	14	13	10	-	09	10
	500	19	16	14	07	10	16
	1000	22	21	22	12	12	21
Aqueous	250	09	09	07	-	09	10
	500	13	14	10	07	11	15
	1000	15	18	14	10	14	17
Ciprofloxacin	30 mcg/disc	23	20	22	23	20	23
Amoxycillin	30 mcg/disc	24	22	23	25	22	23

- No significant zone of inhibition, B1 *Escherichia coli*, B2 *Klebsiella pneumoniae*, B3 *Proteus mirabilis*, B4 *Pseudomonas aeruginosa*, B5 *Staphylococcus aureus* B6 *Proteus vulgaris*.

The methanolic extract was more effective against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Proteus vulgaris* with a zone of inhibition of 22 mm,

21 mm, 22 mm and 21 mm diameter (at concentration 1000 µg) respectively and was least effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with zone of inhibition of 12 mm and 11 mm (at concentration 1000 µg) respectively. The aqueous extract also showed activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris* with a zone of inhibition of 15mm, 18mm and 17mm diameter (at concentration 1000 µg). With other tested bacterial strains, the aqueous extract was not found to be effective. While no zone of inhibition was observed for pet. ether and chloroform extracts.

CONCLUSION

The use of herbs in folk medicine suggests that they represent a cost-effective and safe alternative to treat infectious diseases. It is clear from the results that, the fruit extracts of *Cordia dichotoma* act as a good source of antimicrobial agent against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage is determined for proper administration.

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