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Antimicrobial Activity of the Leaf Extracts of Two Medicinal Plants Against MRSA (Methicilin Resistant *Staphylococcus aureus*) from Human Urinary Tract Pathogens

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ABSTRACT

The objective of this study was to assess the antimicrobial activity of the methanolic leaf extracts of two Indian medicinal plants *Clitoria ternatea* and *Achyranthes aspera* on urinary pathogens. Urinary pathogens were isolated from 127 urine samples taken from subjects diagnosed with Urinary Tract Infection (UTI). Microorganisms were plated on Muller-Hinton agar. Plant extracts were tested by disc diffusion method and the zones of inhibition against pathogenic strains were measured. *Clitoria ternatea* and *Achyranthes aspera* showed antimicrobial activity against seven and five strains of urinary pathogens, respectively. Interestingly both plants showed antimicrobial activity against MRSA (Methicilin Resistant *Staphylococcus aureus*). These results showed that crude plant extracts has wide range of antibacterial activity against UTI pathogen particularly against MRSA.

Key words: *Clitoria ternatea, Achyranthes aspera,* zone of inhibition, disc diffusion method, MRSA

INTRODUCTION

Majority of medicinal plant species are rich in bio-molecule contents which can cope with health hazard and recently, antibacterial activity of many plant species have been reported by Pandey and Mishra (2010). Over several years, natural materials have been investigated as sources of antimicrobial agents. The different parts of the plant are traditionally used for the treatment of various disorders and as an antidote for snake bites and scorpion stings (Uma Devi, 2001). The medicinal properties of several herbal plants and their preparation have been documented in ancient Indian literature and found to be effective in the treatment of numerous diseases (Sampathkumar *et al*., 2008). Development of medication-induced antibiotic resistance, has been evident in *E. coli* and other urinary tract bacteria. As occurrence of multidrug resistant bacteria is increasing, it is necessary to probe new sources for identifying antimicrobial compounds (Bonjar and Nik, 2004).

Plants exhibit protective mechanism against pathogens as both evolved at same period and survive in same niche. Therefore, it is reasonable to expect a variety of plant-compounds to have specific as well as general antimicrobial activity. The bioactive substances in plants are their secondary metabolites. Most promising targets in search for such biologically active compounds are

plants used in folk medicine. Medicinal plants, with their wide variety of chemical constituents, offer a promising source of new antimicrobial principle. Efforts are needed to establish and validate evidence regarding safety and practice of Ayurvedic medicines (Cooper, 2004; Patwardhan *et al.*, 2005). India is one of the richest with vast resource medicinal and aromatic plants. It constitutes of 11% of total known world flora having medical property (Sati *et al.*, 2010). Plants proved to be a good source of antimicrobial substances which pave the way to identify and isolate new pharmaceutical compounds (Khanna and Kannabiran, 2008). Also the exploration of traditional herbal remedies is a viable research initiative for new pharmaceuticals, as consumers have become more conscious about the side effects of synthetic drugs (Parekh and Chanda, 2007). Judicious use of medicinal herbs is presumed to cure even deadly diseases that have long defied synthetic drugs (Lokhande *et al.*, 2007).

Several plant extracts, like those of *Ocimum*, *Cymbopogom* have been tested for inhibitory activity against urinary tract pathogens (Pereira *et al.*, 2004). *Lawsonia innermis* leaves have shown definite antimicrobial activity against the common urinary pathogens (Bhuvaneswari *et al.*, 2002). Methanolic extracts of *Bridelia crenulata*, roots and stems of *Pinus brutia* also exhibit inhibitory activity against urinary tract pathogens (Ramesh *et al.*, 2001; Kizil *et al.*, 2002).

Clitoria ternatea Linn. (Fabaceae) is commonly used in Indian folk medicine. It is a twining climber found abundant in tropical and subtropical regions, mostly as terrestrial mesophytes. The plant parts are used for various ailments. Juice of leaves is used to check fever and cure ulcers; bark and root promote urine flow, soothe and protect alimentary tract. The dry root powder is given to cure irritation of bladder and urethra. *Achyranthes aspera* Linn. (Amaranthaceae) is an erect herb. Leaves of the plant, seeds and roots are used as a laxative, for enhanced urine flow and for asthma and cough (Bhattacharjee, 2004).

Traditional herbalists use plant extracts to treat ailment but with no knowledge of scientific base of their activities (Andy *et al.*, 2008). The present investigation is yet another search for specific medicinal property in plants. In this study, the medicinal plants *Clitoria ternatea* and *Achyranthes aspera* were tested for their antimicrobial property against uropathogenic bacteria.

MATERIALS AND METHODS

The samples were collected from CSI, Mission General Hospital, Woraiyur, Tiruchirappalli, Tamilnadu, India, during December-2004 to February-2005. Midstream urine samples were collected from 85 female patients and 42 male patient in aseptic condition.

Preparation of plant extracts: Leaves of both the species were collected from mature plants and identified by comparing with herbarium specimens. The leaves were air- dried and powdered. The dry leaf powder was extracted by reflexed in 100 mL methanol for 24 h, using a Soxhlet apparatus (Khan *et al.*, 1988). The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator and dried at 55°C. Dried extract was stored at 20°C in labeled, sterile screw-capped bottles.

Microorganisms: The bacterial colonies were isolated from hospital samples, their pure cultures were maintained in nutrient agar and stored at 4°C. Ten bacterial species were isolated, namely *E. coli. Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi A, Staphylococcus aureus,* Methicilin Resistant *Staphylococcus aureus* (MRSA), *Edwardsiela tarda, Pseudomonas aeruginosa, Citrobacter diverses, Serratia liquefaciens* and the fungus *Candida albicans*.

Antimicrobial assay: Sensitivity tests were performed by disc diffusion with standard antibiotics, following Kirby-Bauer method (Bauer *et al.*, 1966). The air-dried extracts at different concentrations such as 10, 20, 30, 40 µL were dissolved in DMSO and loaded in empty sterile discs. The assessment of antibacterial activity was based on measurements of the diameter of inhibition zones (NCCLS, 1998).

RESULTS

Antimicrobial activity of the methanolic extracts of *Clitoria ternatea* was tested against eleven urinary tract pathogens. Table 1 shows the inhibitory effect on seven urinary pathogens: *Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi, Staphylococcus aureus,* Methicilin Resistant *Staphylococcus aureus* (MRSA), *Edwardsiela tarda* and a *fungus Candida albicans*. The extracts had no inhibitory effect against *E. coli, Pseudomonas aeruginosa, Citrobacter diverses* and *Serratia liquifaciens*. Among the different concentrations used, 40 µg concentration showed maximum activity against the susceptible pathogens. Maximum inhibitory zone was formed against *Edwardsiella tarda* and *Candida albicans*. The two microbes were highly susceptible and showed 14 mm zone of inhibition. A zone of inhibition of 12 mm was observed for *Klebsiella pneumoniae* and MRSA.

Methanolic extract of *Achyranthes aspera* showed effective antimicrobial activity against four urinary pathogens: *Klebsiella pneumonia, staphylococcus aureus, Salmonella paratyphi* and Methicilin resistant *Staphylococcus aureus*.

Among the ten microbes tested, two gram positive, five gram negative bacteria and a fungus were susceptible to the methanolic extract of *Clitoria ternatea*. Methanolic extract of *Achyranthes aspera* also showed similar antimicrobial effect, however to a lesser extent against the UTI pathogens. It showed antimicrobial activity against four pathogens, namely *Klebsiella pneumonia, Salmonella paratyphi*-A, *Statphylococcus aureus* and MRSA. Antimicrobial activity of *Clitoria ternatea* and *Achyranthes aspera* were comparable to those by standard antibiotics, although at a lesser degree in overall inhibitory activity. The UTI bacterial pathogens were resistant against nystatin, only *Candida albicans* showed zone of inhibition. Amikacin showed maximum inhibition against all pathogen near 20 mm, whereas vancomycin and penicilin recorded maximum resistance from all pathogen and recorded lowest zone of inhibiton of 8 mm. Even methanolic extracts of the two plants exhibited more zone of inhibition against MRSA, *Salmonella typhimurium, Salmonella paratyphi*-A when compared to standard discs of Amikacin, Penicilin and Vancomycin (Table 2).

Table 1: Survey of antimicrobial activity of Selected plants

		Micro organisms/inhibition zone (mm)										
Plant species	µg mL^{-1}	1	2	3	4	5	6	7	8	9	10	11
Clitoria ternatea	20	-	-	-	8	-	-	-	-	-	-	11
	30	-	8	8	9	11	10	13	-	-	-	12
	40	-	12	8	12	12	12	14	-	-	-	14
Achyranthes aspera	20	-	-	-	10	-	-	-	-	-	-	-
	30	-	10	-	11	11	9	-	-	-	-	-
	40	-	12	-	12	12	12	-	-	-	-	-
Methanol control		-	-	-	-	-	-	-	-	-	-	-

1: *E.coli*, 2: *Klebsiella pneumoniae*, 3: *Salmonella typhimurium*, 4: *Salmonella paratyphi*- A, 5: *Staphylococcus aureus*, 6:Methicilin Resistant *Staphylococcus aureus*, 7: *Edwardsiella tarda*, 8: *Pseudomonas aeruginosa*, 9: *Citrobacter diverses*, 10: *Serratia liquifaciens*, 11: *Candida albicans*

Table 2: Antimicrobial activities of Standard antibiotics (Inhibition zones in mm)

Microbes	P_{10}	A_{10}	Ctx	VA_{30}	Ofx_5	Te_{30}	Ak_{30}	NY_{100}
E. coli	14	12	22	8	22	20	19	-
Klebsiella pneumoniae	17	28	25	8	20	28	18	-
Salmonella typhimurium	10	8	22	8	23	12	24	-
Salmonella paratyphi- A	8	12	21	10	27	17	26	-
Staphylococcus aureus	8	25	28	12	26	21	24	-
MRSA	10	28	26	10	24	23	27	-
Edwardsiella tarda	10	23	24	12	28	20	25	-
Pseudomonas aeruginosa	28	28	29	17	21	16	18	-
Citrobacter diverses	12	14	21	15	13	10	20	-
Candida albicans	-	-	-	-	-	-	-	18

A: Ampicillin, Ctx: Cefataxime, Va: Vancomycin, Ofx: Ofloxacin, P: Penicillin, Te:Teicoplanin, Ak-Amikacin, NY: Nystatin

DISCUSSION

Medicinal plants are used by large proportion of Indian population. The reasons for this include true improvement, absence of harmful side effects and the high cost of other forms of treatment. Urinary Tract Infections (UTIs) are a leading cause of morbidity and involve high health care expenditure in persons of all ages. Sexually active young women are disproportionately affected, but several other populations including elderly persons and those undergoing genito-urinary instrumentation or catheterization, are also at risk. An estimated 40 percent of women reports having had a UTI at some point in their lives. UTIs are the leading cause of gram-negative bacteremia. Several plants have been used in traditional medicines to combat UTI.

From the earlier studies it is obvious that most of the plants differ significantly in their antimicrobial property. These differences may be attributed to the differences in the cell wall constituents of bacteria which vary among the gram positive and gram negative ones (Yao and Moellering, 1995; Ozcelik, 1998). Inhibitory effect of medicinal plant extract exhibited against UTI pathogens in this study is similar to the results obtained in the following studies. In general, alcoholic extracts exhibit highest degree of antimicrobial activity as compared to aqueous and hexane extract fractions. The inhibitory activity was found to be maximum in the methanolic extracts of Euphorbiaceae plants against urinary tract pathogens such as *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Ramesh *et al*., 2001). Antimicrobial activity against *Klebsiella* was observed in oils extracted from *Ocimum* and *Cymbopogom* (Pereira *et al*., 2004). Antimicrobial activity was also documented against *Klebsiella pneumoniae* and *Staphylococcus aureus* in the root extract of *Pholidata articulata* (Mehmood *et al*., 1999). Antibacterial activity against *E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was exhibited by *Chlorophytum borivilianum* (Sundaram *et al*., 2011). Similar zones of inhibition could be obtained in this study with the methanolic extracts *Clitoria ternatea* and *Achyranthes aspera*. Antimicrobial activity of these plants was thus evident.

Earlier studies have established that traditional medicinal plant extracts also produce effective inhibitory zones against *Salmonella typhi* and *Salmonella paratyphi* (Ahmad and Beg, 2001; Rani and Khullar, 2004). Similar results could also be obtained in the present experiment against those urinary pathogens by the extract of *Clitoria ternatea*. Antifungal activity exhibited was also compared to earlier reports with other plant extracts (Ahmad and Beg, 2001). Methanolic extracts of African medicinal plants also show similar results against all common pathogens similar to that observed in this study (Mariita *et al*., 2011).

Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasingly resistant isolates during antibacterial therapy are rising throughout the world (Obrien *et al*., 1999; Cookson, 2000; Archibald *et al*., 1997). To combat multidrug resistant strains like MRSA, the development of new antibacterial compounds for substituting the ineffective ones is the immediate task. As medicinal plants as well as microorganisms are the appropriate candidates, they should receive continuous research attention. Further development of antibiotic resistant strains and the side effects caused by the overuse of antibiotics is a serious concern. Hence much attention is being paid recently towards extracts and biologically active compounds isolated from plants used in herbal medicine (Essawi and Srour, 2000; Cos *et al*., 2002; Shahidi *et al*., 2002). At this juncture it is interesting to note that both the plant extracts used, showed effective inhibitory zones against Methicillin Resistant *Staphylococcus aureus* (MRSA). Similar results was observed in the earlier study of Anam *et al*. (2010) using leaf extract of *Terminalia muelleri*, which showed antibacterial activity against MRSA closer to range of standard antibiotics discs of Vancomycin and Penicilin.

From the present study it is evident that the crude methanolic extracts of *Clitoria ternatea* and *Achyranthes aspera* possess antibacterial and antifungal property. Further identification and isolation of specific antimicrobial compounds and their purification can pave better ways to control these kinds of urinary tract infections, and also to find novel approaches to tackle the emergence of drug resistant strains of pathogens.

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