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Full Length Research Paper

Antimicrobial activity of *Mirabilis Jalapa* and *Dichrotachys cinerea* against biofilm and extended spectrum of beta lactamase (ESBL) producing uropathogenic *Escherichia coli*

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The aim of this study was to evaluate the antimicrobial efficacy of *Mirabilis jalapa* and *Dichrotachys cinerea* against biofilm and extended spectrum of beta lactamase (ESBL) producing uropathogenic *Escherichia coli* (UPEC). *M. jalapa* and *D. cinerea* are widespread medicinal plants traditionally used to treat infectious diseases. Aqueous, acetone and ethanol extracts of leaves of *M. jalapa* and *D. cinerea* were tested for antimicrobial activity *invitro* by the agar well diffusion method. Ethanol extract of *M. jalapa* leaves exhibited antimicrobial activity against all tested biofilm producing UPEC strains, whereas it inhibited only the ESBL producing UPEC strains 42 and 96. Similarly, the acetone extract of *M. jalapa* leaves inhibited the growth of biofilm producing UPEC strains 1, 17 and 82, whereas it inhibited only the ESBL producing UPEC strains 42 and 96. Ethanol extract of *D. cinerea* leaves exhibited inhibitory activity against all tested biofilm producing UPEC strains, whereas it inhibited only the ESBL producing UPEC strain 87. Similarly, the acetone extract inhibited only the growth of biofilm producing UPEC strain 82, whereas it inhibited the growth of ESBL producing UPEC strains 87 and 96. The aqueous extracts of *M. jalapa* and *D. cinerea* leaves failed to show any inhibitory effect against both biofilm and ESBL producing UPEC strains. These antimicrobial properties seem to be related to the presence of alkaloids, tri-terpenoids and tannin contents in *M. jalapa* and *D. cinerea*. The present study shows that crude extracts of *M. jalapa* and *D. cinerea* especially the acetone and ethanol extracts exhibited significant activity against biofilm and ESBL producing Uropathogenic *E. coli* strains.

Key words: Biofilm, extended spectrum of beta lactamase (ESBL), *Escherichia coli*, *Mirabilis jalapa* and *Dichrotachys cinerea*, antimicrobial activity.

INTRODUCTION

In the recent past, the rapid development of multi-drug resistant bacterial strains of clinically important pathogens fetches the interest of scientists to develop newer broad spectrum antimicrobial agents (Weisser et al., 1966). The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in different traditional literatures (Jones et al., 1996; Satish et al., 1999).

Mirabilis jalapa Linn belongs to the family *Nyctaginaceae*, known as four o'clock, Dondiego, maravilla, buenas tardes, jalap, de nuit, noche buena, etc is extensively used for treatment of dysentery and as a laxative (purgative) by Mexican people (Encarnaci´on et

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Abbreviations: UTI's, Urinary tract infections; **UPEC,** uropathogenic *Escherichia coli.*

al., 1998; M´arquez et al., 1999) and for treatment of diarrhea, muscular pain and abdominal colic by people from other different countries (Holdsworth, 1992; Comerford, 1996; Moreira, 1996). Furthermore, *M. jalapa* extracts have also been reported to have biological activities like antibacterial, antiviral, antifungal and protein synthesis inhibition (De Bolle et al., 1996; Vivanco et al., 1999; Oska and Sari, 2007). *M. jalapa* leaves are used in traditional folk medicine in the South of Brazil to treat inflammatory and painful diseases (Siddiqui et al., 1990; Somavilla and Canto-Dorow, 1996).

Dichrotachys cinerea is commonly called “dundu” among the Hausa speaking people of Northern Nigeria and “Kora” among the Yoruba speaking people of Western Nigeria (Gill, 1992). The plant is a shrub, usually attaining a height of up to 5 – 10 m found in tropical and sub tropical conditions. The leaves are compound and pinnate (Mann et al., 2005). Traditionally, the plants were used as vermifuge and also in leprosy, syphilis, dysentery, headache, toothache (Kirtikar and Basu, 1998). Ethanolic extract of roots, fruits, leaves and seeds of *D. cinerea* were reported to have antibacterial activity (Eisa et al., 1999; Bansu and Adeyemo, 2007). The Haikum Bushmen of Namibia applied directly to treat snakebites. Extracts of the leaves and bark, as well as powdered bark are used for wound healing (Van Wyk et al., 2005). In Sri Lanka, it is commonly used for traditional medicinal purposes as an aphrodisiac and for eye diseases (Wijesundara, 2003). In Sudan, it is used for the treatment of wounds (Eisa et al., 1999) and in Zimbabwe; it is frequently used for the treatment of sexually transmitted diseases (Kambizi and Afolayan, 2001).

Urinary tract infections (UTI’s) pose a serious health threat with respect to antibiotic resistance and high recurrence rates. Generally, there is an agreement among the authors in the literature that the predominant uropathogens acquired from any source are gram negative bacteria with *Escherichia coli* accounting for the highest prevalence in most instances (Moges et al., 2002). Microorganisms responsible for urinary tract infection (UTI) such as *E.coli* have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat (Bal, 2000).

Uropathogenic *E. coli* (UPEC) form intracellular bacterial communities with biofilm like properties within the bladder epithelium (Anderson et al., 2003). According to National Institutes of Health, “more than 60% of all microbial infections are caused by biofilm. A biofilm is a population of cells growing on a surface and enclosed within an exopolymer matrix that can restrict the diffusion of substances and bind antimicrobials. It is well documented that biofilm are notoriously difficult to eradicate and are often resistant to systemic antibiotic therapy and removal of infected device becomes necessary (Lewis, 2001). The mechanism of resistance of biofilm bacteria is not conclusively established, but it has been suggested that the resistance may be related to beta-lactamase production by the biofilm bacteria (Goto et al., 1999).

Since no previous attempts have been made to examine the antimicrobial effects of *M. jalapa* and *D. cinerea* against uropathogenic *E. coli* strains, we focused on *M. jalapa* and *D. cinerea.* The objective of this research was to substantiate the antimicrobial sensitivity of different extracts of *M. jalapa* and *D. cinerea* leaves against uropathogenic ESBL and Biofilm producing *E. coli* strains to lengthen the queue of antimicrobial herbs.

MATERIALS AND METHODS

Collection of plant materials

Leaves of *M. jalapa* and *D. cinerea* were collected from villages in and around Coimbatore district, South India. Plant leaves were dried under the shadow. The dried leaves were fine powdered and stored in polythene bags at room temperature (30±2ºC) until use.

Chemicals

All chemicals used were of analytical grade and purchased from typical chemical companies.

Extract preparations

Aqueous extract

To obtain the aqueous extracts, dried and finely powdered leaves of *M. jalapa* and *D. cinerea* were weighed about 10 g each and homogenized using 100 ml of water. They were added to Soxhlet apparatus and the boiling point of water was set up at 100ºC. The water evaporates continuously and was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solution loses the colour.

Acetone extract

To obtain the solvent extracts, dried and finely powdered leaves of *M. jalapa* and *D. cinerea* were weighed about 10 g each and homogenized using 100 ml of 70% acetone. They were added to Soxhlet apparatus and the boiling point of acetone was set up at 56.6ºC. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its colour. The extract was then transferred to a sterile petridish and kept for evaporation of acetone at room temperature. Residues of extracts were collected and stored in the refrigerator.

Ethanol extract

To obtain the solvent extracts, dried and finely powdered leaves of *M. jalapa* and *D. cinerea* were weighed about 10 grams each and homogenized using 100 ml of 70% ethanol. They were added to Soxhlet apparatus and the boiling point of ethanol was set up at 56.6ºC. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its colour. The extract w as then

Table 1. Antimicrobial activity of three different extract of *M. jalapa* and *D. cinerea* leaves by well diffusion method against Biofilm and ESBL producing uropathogenic *E. coli.*

Plant extract 1 mg/ml	Solvent	Uropathogenic *E. coli* (zone of inhibition in mm)							
		Biofilm				ESBL			
		UPEC 1	UPEC 17	UPEC57	UPEC 82	UPEC 42	UPEC 85	UPEC 87	UPEC 96
M. jalapa	Aqueous	-	-	-	-	-	-	-	-
	Acetone	22	20	-	18	18	-	-	20
	Ethanol	24	22	22	22	24	-	-	22
D. cinerea	Aqueous	-	-	-	-	-	-	-	-
	Acetone	-	-	-	18	-	-	20	18
	Ethanol	24	32	18	24	-	-	18	-

UPEC= Uropathogenic *E. coli*, '-'Indicates no significant zone of inhibition

transferred to a sterile petridish and kept for evaporation of ethanol at room temperature. Residues of extracts were collected and stored in the refrigerator.

Antibacterial activity of plant extracts: Agar well diffusion method

Antibacterial activity of the aqueous, acetone and ethanol extracts of leaves of *M. jalapa* and *D. cinerea* were tested using agar well diffusion method. A loop full of culture was inoculated into peptone broth and incubated for 2 to 6 h at 35°C until it achieved the turbidity of 0.5 McFarland's standard. The test cultures were swabbed on nutrient agar plates, within 15 min after adjusting the turbidity of the inoculum suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removed the excess inoculum from the swab. The dried surface of a nutrient agar plate was inoculated by streaking the swab and the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed and wells were made using the sterile well puncture. Different concentrations (200 to 1000 µg) of the sterile aqueous, acetone and ethanol extracts were added to each well. The plates were incubated in an upright position at 37°C for 24 h. The diameter of inhibition zones were measured in mm and the results are recorded.

RESULTS

In vitro antibacterial activities of leaves of *M. jalapa* and *D. cinerea* have been investigated against biofilm and ESBL producing Uropathogenic *E. coli* (UPEC) and the results are shown in Table 1. The biofilm producing strains employed for the antimicrobial activity of aqueous, acetone and ethanol extracts of leaves of *M. jalapa* and *D. cinerea* includes UPEC 1, UPEC 17, UPEC 57 and UPEC 82. Similarly, the ESBL producing strains include UPEC 42, UPEC 85, UPEC 87 and UPEC 96. The organisms are selected based on the resistant pattern exhibited against the antibiotics used to treat UTI caused by *E. coli.*

The aqueous extract of *M. jalapa* and *D. cinerea* leaves failed to exhibit any inhibitory action against both biofilm and ESBL producing UPEC strains even at the highest concentration (1000 µg/ml). The acetone extract of *M. jalapa* exhibited a zone of inhibition of 22, 20 and 18 mm respectively against biofilm producing strains UPEC 1,UPEC 17 and UPEC 82. Similarly, the acetone extract of *D. cinerea* exhibited a zone of inhibition of 18mm only against biofilm producing strain UPEC 82. The acetone extract of *M. jalapa* exhibited antimicrobial activity against ESBL producing strains UPEC 42 and UPEC 96 (18 and 20 mm) and no zone of inhibition was observed for the other ESBL strains. The acetone extracts of *D. cinerea* exhibited antimicrobial activity against ESBL producing strains UPEC 87 and 96.

But in the case of ethanolic extracts of *M. jalapa* and *D. cinerea*, all the tested biofilm strains were greatly inhibited showing a different zone of inhibition (18-32 mm). Among the tested biofilm strains, UPEC 1 was greatly inhibited showing a zone of inhibition of 24 mm by ethanolic extract of *M. jalapa* and UPEC 17 was greatly inhibited showing a zone of inhibition of 32 mm by ethanolic extract of *D. cinerea*. But in the case of ESBL producing strains, the ethanol extract of *M. jalapa* exhibited enhanced activity against UPEC 42 and UPEC 96 (24 and 22 mm) at 1000 µg/ml. Similarly, the ethanolic extract of *D.cinerea* showed inhibitory action only against UPEC 87 (18 mm) at 1000 µg/ml and failed to show any inhibitory activity against other tested ESBL strains.

DISCUSSION

The presence of antibacterial substances in the higher plants is well established (Srinivasan et al., 2001). The inhibitory activities exhibited by the tested plants tends to agree with the report that antibacterial properties of these plants are due to the presence of tannins, alkaloids, flavonoids, terpenoids or essential oils (Bassole et al., 2003; Viljoen et al., 2003; Erasto et al., 2004). The increase in antibacterial effectiveness observed with increase in concentration of tannins is in agreement with the work of Kurosoki and Nishi (1983), who reported that higher concentrations of antimicrobial substances

showed appreciable growth inhibition to microorganisms.

Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phyto-medicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Jigna and Sumitra, 2007). Banso and Adeyemo (2007) have reported the presence of tannins, alkaloids and glycosides in the leaf extracts of *D. cinerea*.

There does not appear to be any previous study on the comparison of aqueous, acetone and ethanol extracts of *M. jalapa* and *D. cinerea* leaves. In this study, the aqueous extract did not exhibit any inhibitory action but observed a moderate to higher activity with acetone and ethanol extracts. This is in agreement with earlier reports that use of organic solvents is always better for extraction of antibacterial compounds (Varadarajan et al., 2007). Eisa et al. (1999) have reported that water extract of *D. cinerea* leaves failed to exhibit any inhibitory action against *E. coli,* whereas the methanol extract showed moderate activity. Similarly, even in the present study aqueous extract failed to exhibit growth inhibition against biofilm and ESBL producing uropathogenic *E. coli*.

Conclusion

The results of this study showed that the *M. jalapa* and *D. cinerea* leaves have exhibited varied antimicrobial activities against the biofilm and ESBL producing uropathogenic *E. coli*. These findings on antibacterial activity support the claim of the traditional healers that *M. jalapa* and *D. cinerea* would be used against uropathogenic *E. coli*. Thus in search of novel broad spectrum antimicrobial agent, the formulation comprising different proportions of these extracts may be proven good.

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