

Antimicrobial Activity of *Coccinia grandis* Against Biofilm and ESBL Producing Uropathogenic *E. coli*

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Abstract: Uropathogenic organisms have evolved numerous defense mechanisms against antimicrobial agents, hence resistance to old and newly available drugs are increasing at an unprecedented level. The events of antibiotic resistance have lead for screening of several medicinal plants for their potential antimicrobial activity. The aim of this study was to evaluate the antimicrobial efficacy of *Coccinia grandis* against biofilm and Extended Spectrum of Beta Lactamase (ESBL) producing Uropathogenic *Escherichia coli* (UPEC). *C. grandis* is a widespread medicinal plant traditionally used in India to treat infectious diseases. Aqueous, acetone and ethanol extracts of leaves of *C. grandis* were tested for antimicrobial activity *in vitro* by the agar well diffusion method. Ethanol extract of leaves exhibited antimicrobial activity against biofilm producing strains UPEC 17 and 82, whereas the aqueous and acetone extracts showed antibacterial activity only against UPEC 57. Ethanol extract of leaves exhibited inhibitory action against ESBL producing UPEC 87 and 96, whereas the aqueous extract inhibited the growth of only UPEC 85. Similarly, the acetone extract inhibited the growth of UPEC 42 and 96. These antimicrobial properties seem to be related to the presence of tannin, alkaloids and tri-terpenoids in *C. grandis*. It can be concluded that *C. grandis* can be used to discover natural products that may serve as lead for the development of new pharmaceuticals, addressing the major therapeutic needs especially for biofilm and ESBL producing uropathogenic strains.

Key words: Biofilm • ESBL • *Escherichia coli* • *C. grandis* • Antimicrobial activity

INTRODUCTION

In the recent past, the rapid development of multi-resistant bacterial strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents [1]. 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 literature [2, 3]. *Coccinia grandis* (L.) belongs to family *Cucurbitaceae*, commonly known as Kundru in Hindi and Ivy Gourd in English, is a vegetable grown wildly throughout India [4]. Every part of this plant is valuable in medicine for ring worm, psoriasis, small pox, scabies [5] and other itchy skin eruptions and ulcers [6]. The plant can also be used to

treat cough [7]. The leaves of the plant possess antimicrobial, anti-diabetic, antipyretic, anti-inflammatory, antispasmodic, cathartic and expectorant activities [8, 9]. The leaves of this plant contain tri-terpenoids, alkaloids and tannins [10].

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 [11]. 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 [12].

Uropathogenic *E. coli* form intracellular bacterial communities with biofilm like properties within the bladder epithelium [13]. 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 [14]. It is well documented that biofilm are notoriously difficult to eradicate and are often resistant to systemic antibiotic therapy [15]. 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 [16].

Since no previous attempts have been made to examine the antimicrobial effects of *C. grandis*, against uropathogenic *E. coli* strains we focused on *C. grandis*. The aim of this paper was to substantiate the antimicrobial sensitivity of different extracts of *C. grandis* leaves against uropathogenic Biofilm and ESBL producer *E. coli* strains to lengthen the queue of antimicrobial herbs.

MATERIALS AND METHODS

Collection of Plant Materials: Leaves of *C. grandis* 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 ($30\pm 2^\circ\text{C}$) 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 *C. grandis* were weighed about 10 grams each and homogenized using 100ml of water. They were added to Soxhlet apparatus and the boiling point of water was set up at 100°C . 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 *C. grandis* were weighed about 10 grams 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 56.6°C . 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 *C. grandis* were weighed about 10 grams each and homogenized using 100ml of 70% ethanol. They were added to Soxhlet apparatus and the boiling point of ethanol was set up at 56.6°C . 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 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 *C. grandis* was tested using agar well diffusion method. A loop full of culture was inoculated into peptone broth and incubated for 2 to 6 hours 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 minutes 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 ($200\mu\text{g}$ to $1000\mu\text{g}$) 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 hours. The diameter of inhibition zones were measured in mm and the results are recorded.

RESULTS AND DISCUSSION

In vitro antibacterial activities of leaves of *C. grandis* have been investigated against biofilm and ESBL producing uropathogenic *E. coli* (UPEC) and results are shown in table 1. The biofilm producing strains employed for the antimicrobial activity of aqueous, acetone and

Table 1: Antimicrobial activity of three different extract of *C. grandis* leaves by well diffusion method against Biofilm and ESBL producing Uropathogenic *E. coli*

Plant Extract (1000µg/ml)	Solvent	Uropathogenic <i>E. coli</i>							
		Biofilm				ESBL			
		UPEC 1	UPEC 17	UPEC57	UPEC 82	UPEC 42	UPEC 85	UPEC87	UPEC 96
<i>C. grandis</i>	Aqueous	-	-	22	-	-	18	-	-
	Acetone	-	-	26	-	20	-	-	20
	Ethanol	-	22	-	24	-	-	18	24

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

ethanol extracts of leaves of *C. grandis* 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 test organisms were selected based on the resistant pattern exhibited against the antibiotics used to treat UTI caused by *E. coli*.

When the aqueous extract of (200-1000µl) *C. grandis* leaves tested against the biofilm producing UPEC strains, no significant zone of inhibition was observed against the test strains except UPEC 57, where 22mm zone of inhibition was observed at a concentration of 1000 µg/ ml. Similarly, when the same extract tested against ESBL producing UPEC strains, it was found that only UPEC 85 strain was greatly inhibited showing a maximum zone of inhibition of 18mm at 1000 µg/ ml, whereas it failed to exhibit antimicrobial activity against the other UPEC strains.

When the acetone extract (200-1000µg/ml) of *C. grandis* leaves tested against biofilm producing UPEC strains, no significant zone of inhibition was observed against the test strains except UPEC 57, where 26 mm zone of inhibition was observed at the highest concentration (1000 µg/ml) studied. Similarly, when the same extract tested against ESBL producing UPEC strains, it was found that UPEC 42 and UPEC 96 were greatly inhibited showing a maximum zone of inhibition of 20mm each at 1000 µg/ml, whereas it failed to exhibit inhibitory action against other tested strains.

It is clear from the table 1 that the ethanol extract of *C. grandis* leaves have exhibited antimicrobial activity against biofilm producing UPEC17 and UPEC 82 with a maximum zone of inhibition of 22mm and 24mm respectively, but failed to exhibit inhibitory action against UPEC 1 and UPEC 57. Similarly, the ethanol extract of *C. grandis* exhibited antimicrobial activity against ESBL producing strains UPEC 87 and 96 (18mm each), whereas it failed to show any inhibitory action against UPEC 42 and 85.

The antimicrobial activities of various plants have been reported by many researchers [17, 18,]. Umbreen *et al.* [19] have reported the significant activity of methanol and ethyl acetate extracts of leaves and stem of *C. indica* against different bacteria providing a support to the fact that methanol is a better solvent for extraction and isolation of phytochemicals having antimicrobial activity. The present study revealed that the ethanol extract was found to be active against two biofilm producing UPEC strains namely UPEC 17 and UPEC 82. Among the biofilm strains, UPEC 1 was found to be resistant because none of the extracts inhibited their growth even at the highest concentration (1000 µg/ml) studied. Similarly, the ethanol extracts were found to be active against two ESBL producing UPEC strains namely UPEC 87 and UPEC 96. Dewanjee *et al* [20] have also reported that methanol extract of *C. grandis* leaves exhibited significant antimicrobial activity. There does not appear to be any previous study on the comparison of aqueous, acetone and ethanol extracts of *C. grandis* leaves. In this study, the water 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 [21]. Furthermore, the effectiveness of the extracts was not due to one main constituent, but to the combined action of other chemical compounds involved in it [22]. Some of them include alkaloids, flavonoids, terpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds [23].

In conclusion results of this study showed that the *C. grandis* 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 *C. grandis* would be used against uropathogens.

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