African Journal of Basic & Applied Sciences 3 (5): 239-242, 2011
ISSN 2079-2034

Antimicrobial Activity of *Coccinia grandis* Against Methicillin Resistant *Staphylococcus aureus*

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Abstract: Methicillin-resistant *staphylococcus aureus* (MRSA) have emerged in the 1960s as a cause of infection among patients exposed to the bacteria in health care settings. MRSA organisms have evolved numerous defense mechanisms against antimicrobial agents. 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 methicillin resistant *Staphylococcus aureus* (MRSA). 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 methicillin resistant producing strains MRSA 2 and 4, whereas the aqueous and acetone extracts showed antibacterial activity only against MRSA 3. 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 methicillin resistant producing *S. aureus* strains.

Key words: MRSA · Coccinia grandis · 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 [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 the herbs with significant antimicrobial activity have been reported in different traditional literature [2, 3]. *Coccinia grandis* (L.) belongs to the 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 eruptions [5] 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].

Staphylococcus aureus is a Gram-positive bacterium frequently implicated in a variety of infectious processes ranging from relatively benign skin infections to life-threatening systemic illnesses. The organism possesses several properties that contribute to its ability to cause serious diseases, including the production of toxins [11]. Since *S. aureus* is a significant cause of nosocomial and community-acquired infections, the rising rates of antibiotic resistance that have been noted globally are of concern.

Methicillin-resistant *Staphylococcus aureus* (MRSA) as a cause of nosocomial sepsis has become an increasing concern with more than 50% of the hospital *S. aureus* isolates resistant to methicillin [12]. As these nosocomial MRSA are resistant to multiple antibiotics; infections caused by them are difficult to treat, resulting in high morbidity and mortality. Although community-acquired *S. aureus* infections are common, recent reports suggest that they may increasingly be caused by MRSA strains [13-16].

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Community-acquired MRSA appears to be a new emerging pathogen. There is a considerable concern that community-acquired MRSA would cause infections difficult to treat in the outpatient setting and would markedly increase the need for vancomycin therapy [17]. Interestingly, community-acquired MRSA isolates uniquely differ from nosocomial MRSA isolates by being generally susceptible to multiple antimicrobial agents other than â lactams [18]. Clindamycin susceptibility has been shown to have a very significant correlation with community-acquired MRSA and has been used as a surrogate marker for its detection.

The aim of this study was to substantiate the antimicrobial sensitivity of different extracts of *C. grandis* leaves against clinical isolates of methicillin resistant *Staphylococcus aureus* 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°C) until use.

Extract Preparations

Aqueous Extract: To obtain the aqueous extracts, about 10 grams of the dried and finely powdered leaves of *C. grandis* were 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 color [19].

Acetone Extract: Ten grams of the dried and finely powdered leaves of *C. grandis* were homogenized using 100 ml of 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 color. The extract was then transferred to a sterile Petri dish and kept for evaporation of acetone at room temperature. Residues of extracts were collected and stored in the refrigerator.

Ethanol Extract: Ten grams of the dried and finely powdered leaves of *C. grandis* were homogenized using 100ml of 70% ethanol. They were added to Soxhlet apparatus and the boiling point of ethanol was set up at 78°C. The solvent was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the solvent loses its color. The extract was then transferred to a sterile Petri dish and kept for evaporation of ethanol at room temperature. Residues of extracts were collected and stored in the refrigerator.

Antibacterial Activity of Plant Extracts: Antibacterial activity of the aqueous, acetone and ethanol extracts of leaves of *C. grandis* was tested using the agar well diffusion method. Four methicillin resistance producing strains were employed for testing the antimicrobial activity of the aqueous, acetone and ethanol extracts of leaves of *C. grandis*; MRSA 1, MRSA 2, MRSA 3 and MRSA 4.The selection of the test organism was based on the resistant pattern exhibited against the antibiotics used to treat methicillin resistant *S. aureus*. A loop full of culture of each test strain was inoculated into peptone broth and incubated for 2 to 6 hours at 37°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's suspension. Wells were made using the sterile well puncture. Different concentrations (200µg to 1000µg) of the sterile aqueous, acetone and ethanol extracts were added to each well. The plates were incubated at 37°C for 24 hours. The diameter of inhibition zones was measured in millimeter (mm) and the results were recorded.

RESULTS AND DISCUSSION

In vitro antibacterial activities of leaves of *C. grandis* against *Staphylococcus aureus* are shown in Table 1. The aqueous and acetone extracts of (200-1000µl) *C. grandis* leaves showed no significant zone of inhibition against the tested strains except MRSA 3 with inhibition zone diameter about 22 and 26 mm, respectively achieved at the highest extracts concentration (1000 µg/ml).

It is clear from the Table 1 that the ethanol extract of *C. grandis* leaves have exhibited antimicrobial activity against MRSA 2 and MRSA 4 with maximum zones of inhibition of 22 and 24 mm respectively, but failed to exhibit inhibitory action against MRSA 1 and MRSA 3.

Table 1: Antimicrobial activity of the different extracts of *C. grandis* leaves by well diffusion method against *Staphylococcus aureus*

		Methicillin resistant *Staphylococcus aureus*			
Plant Extract (1000µg/ml)	Solvent	MRSA1	MRSA 2	MRSA 3	MRSA 4
C. grandis	Aqueous	-	-	22	-
	Acetone	-	-	26	-
	Ethanol	-	22	-	24

MRSA= Methicillin resistant *Staphylococcus aureus*, '-'Indicates no significant zone of inhibition

The antimicrobial activities of various plants have been reported by many researchers [20, 21]. Umbreen *et al.* [22] 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. Similarly Poovendran *et al.* [19] have reported that the significant activity of ethanol extract of leaves *C. grandis* was found to be active against Uropathogenic *E. coli* strains. The present study revealed that the ethanol extract was found to be active against two methicillin resistant producing strains namely MRSA 2 and MRSA 4 and resistant against MRSA 1 and MRSA 3. Dewanjee *et al.* [23] have reported that methanol extract of *C. grandis* leaves exhibited significant antimicrobial activity. In this study, the water extract displayed lower antibacterial activity than acetone and ethanol extracts. This is in agreement with earlier studies which reported that use of organic solvents is always better for extraction of antibacterial compounds [24]. Furthermore, the effectiveness of the extracts are not due to one main constituent, but to the combined action of other chemical compounds involved in it [25]. Some of them include alkaloids, flavonoids, terpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds [26]. The results of this study showed that the *C. grandis* leaves have exhibited varied antimicrobial activities against the methicillin resistant *Staphylococcus aureus*. These findings support the claim of the traditional healers that *C. grandis* would be used against methicillin resistant *S. aureus*.

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