IJPSR (2013), Vol. 4, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 December, 2012; received in revised form, 30 January, 2013; accepted, 22 March, 2013

PRELIMINARY ANTIMICROBIAL ACTIVITY AND CYTOTOXICITY OF PLANT EXTRACTS (ROOTS) OF *COCCINIA GRANDIS* (FAMILY: CUCURBITACEAE)

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Keywords:
Coccinia grandis, Antimicrobial activity, Cytotoxic activity, Lethal Concentration 50%.

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ABSTRACT: In the present study, the *in vitro* antimicrobial and cytotoxic activity of root extract of *Coccinia grandis* were evaluated. Ethanolic crude extract with its various solvent soluble fractions of *C. grandis* showed significant antimicrobial activity by disc diffusion method on some gram positive and gram negative bacteria. The extracts exhibited 9-12 mm (approximately) of zone of inhibition comparing with standard ciprofloxacin (15-19 mm). In the brine shrimp lethality bioassay, the crude ethanolic extract showed significant cytotoxicity with LC_{50} of 2.496 µg/ml in comparison to vincristine sulphate (LC_{50} value 0.451 µg /ml).

INTRODUCTION: *Coccinia grandis* (L.) is a wild cucurbitaceous medicinal plant with many pharmaceutical applications. As an ethnic tribal plant, it has potential therapeutic values as anti diabetic, anti-ulcer, anti-inflammatory, anti-oxidant and anti-tumor properties[1-4]. In traditional medicine fruits have been used to treat leprosy, fever, asthma, bronchitis and jaundice. The fruit possesses mast cell stabilizing; anti anaphylactic and antihistaminic potential[5]. It is also called tindora' (tindori, tindoori), tondli in Marathi, toroda (oriya), ghiloda, kundri, kundru, kowai, kovai, dondakaya , manoli, tindla, tendli, thendli, thainli, ivy gourd or little gourd[6]. They may be eaten immature and green, or mature and deep red. The young shoots and leaves may also be eaten as greens[7].

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Article can be accessed online on:
www.ijpsr.com

The present study is intended to evaluate the antimicrobial and cytotoxic activity of plant extracts (roots) of *Coccinia grandis*.

MATERIALS AND METHOD:

Collection of Plant Materials: The roots of *Coccinia grandis* were collected from Botanical garden, Dhaka, Bangladesh in January 2009 and confirmed about the species by the taxonomist of the National Herbarium of Bangladesh. The root (after cutting into small pieces) was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature (40°C) for better grinding.

The dried samples were then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka. The coarse powder was then stored in air-tight container with marking for identification and kept in cool, dark and dry place for future use.

Preparation of Crude Extract: The powder material (300 gm) was taken in separate clean, round bottomed flask (4 liters) and soaked in 3 liters of ethanol. The container with its content was sealed by cotton plug and aluminium foil and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton plug followed by Whatman No.1 filter paper. The filtrate then treated with n-hexane, Carbon tetrachloride, Dichloromethane to isolate n-hexane soluble fraction, carbon tetrachloride soluble fraction, dichloromethane soluble fraction, aqueous soluble fraction and methanolic crude extract. The filtrate thus obtained was concentrated at 40° C with a Heidolph rotary evaporator. The concentrated extract was then air dried to solid residue.

Evaluation of Antimicrobial Activity: For the evaluation of antimicrobial activity, widely acceptable disc diffusion method [8] was used. In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples (Ethanolic crude extract, n-hexane fraction, dichloromethane fraction, carbon tetrachloride fraction and aqueous fraction) of known amounts (300 µg/disc) are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (ciprofloxacin) discs and blank discs were used as positive and negative control. These plates were kept at low temperature (4° C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media [9]. The plates were then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent was then determined by measuring the diameter of zone of inhibition expressed in millimeter [8].

Cytotoxicity by Brine Shrimp Lethality Bioassay: Brine shrimp lethality bioassay [10, 11] technique was applied for the determination of general toxic property of the plant extract. Dimethyl sulfoxide (DMSO) solutions of the samples were applied against *Artemia salina* in a one day *in-vivo* assay. Four mg of test sample was taken and dissolved in pure DMSO and solution of varying concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) were obtained by serial dilution technique using DMSO. Vincristine sulphate (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078125 and 0.0390 µg/ml) was used as positive control.

RESULTS AND DISCUSSION: The antimicrobial activities of root extracts from *Coccinia grandis* were examined in the present study. The results are given in **table 1**. The zones of inhibition produced by the crude ethanolic extract, *n*-hexane, carbon tetrachloride, dichloromethane and aqueous soluble partitionates of the ethanolic extract [12] of *C. grandis* (root) were microorganism specific and ranged from 9.00-12.00 mm (approximately) compared with standard ciprofloxacin (15.00-19.00 mm) at a concentration of 300 µg/disc.

TABLE 1: ANTIMICROBIAL ACTIVITY OF TEST SAMPLES OF *COCCINIA GRANDIS*

Test microorganisms	Diameter of zone of inhibition (mm)					
	CEE	HSF	CTSF	DCMSF	AQSF	Ciprofloxacin
Gram positive bacteria						
Staphylococcus aureus	12	10	11	9	8	17
Bacillus cereus	10	-	9	8.5	8.5	17
Sarcina lutea	9	-	-	9	-	18
Gram negative bacteria						
Escherichia coli	9.5	8.5	-	10	-	15
Pseudomonas aeruginosa	10	-	10	9.5	-	18
Salmonella typhi	10	9.5	-	9.5	-	19

*__CEE__ = Crude ethanolic extract (300 µg/disc), **HSF** = Hexane soluble fractions of the Ethanolic extract (300 µg/disc), **CTSF =** Carbon tetrachloride soluble fractions of ethanolic extract (300 µg/disc), **DCMSF** = Dichloromethane soluble fractions of ethanolic extract (300 µg/disc), **AQSF =** Aqueous soluble fractions of ethanolic extract (300µg/disc).

Following the procedure of Meyer [11] the lethality of the ethanolic crude extract of *C. grandis* (root) to brine shrimp was determined and the summary is expressed in **table 2**. The lethal concentration LC_{50} of the test samples after 24 hour was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data (**figure 1**) by means of regression analysis.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF ROOT OF *COCCINIA GRANDIS* ON BRINE SHRIMP NAUPLII

Concentration (µg/ml)	log C	% mortality	LC_{50} (µg/ml)
1000	3	55%	2.496
500	2.69897	50%	
250	2.39794	45%	
125	2.09691	40%	
62.5	1.79588	30%	
31.25	1.49485	25%	

The LC_{50} values of the ethanolic crude extract of root was found 2.496. However, varying degree of lethality to *C. grandis* extract was observed with exposure to different dose levels of the test samples. The degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration (31.25 µg/ml) to highly significant with the highest concentration (1000 µg/ml). Maximum mortalities took place at concentration 1000 µg/ml, whereas least mortalities were at 31.25 µg/ml. From the results of the brine shrimp lethality bioassays it can be well predicted that the ethanolic crude extracts possess cytotoxic principles and have considerable cytotoxic potency.

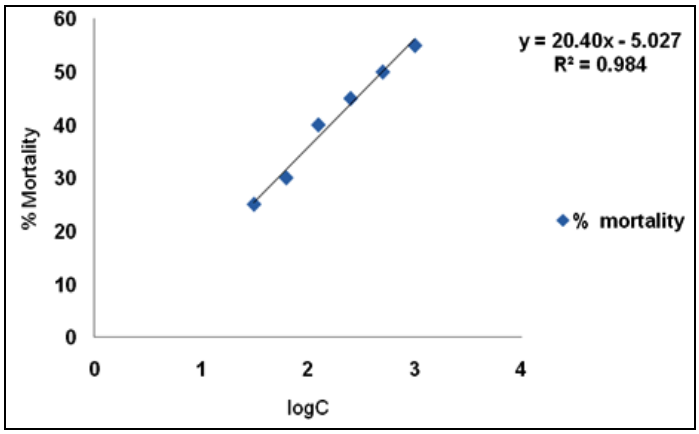

FIGURE 1: EFFECT OF CRUDE ETHANOLIC EXTRACT OF *C. GRANDIS* ON BRINE SHRIMP NAUPLII

CONCLUSION: In conclusion, our observations confirm that in case of antimicrobial activity the ethanolic extract with its various solvent soluble fractions of *C. grandis* showed significant activity. In case of cytotoxic activity crude ethanolic extract revealed potent cytotoxic action compared with vincristine sulphate. This study is a substantial step and further studies are needed to establish the mechanism of action for antimicrobial and cytotoxic action of the plant extract.

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How to cite this article:
Md. Hasanuzzaman, Sayeed MSB, Islam MS, Md. Sarwar S, Moghal MMR, Ahmed JU and Islam MS: Preliminary Antimicrobial activity and Cytotoxicity of plant extracts (roots) of *Coccinia grandis* (Family: Cucurbitaceae). *Int J Pharm Sci Res* 2013; 4(4); 1466-1468.