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ANTIBACTERIAL AND PRELIMINARY CYTOTOXIC ACTIVITY OF THE ROOTS OF *VETIVERIA ZIZANIOIDES*

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ABSTRACT

Vetiveria zizanioides (Family- *Germaine*) is growing in the shallow water of a Lake or pond. The volatile oil isolated from the plant is used as perfume and as an insecticide. The aim of the present study was to investigate the antibacterial and cytotoxic activity of roots of *Vetiveria zizanioides* (*Graminae*). Preliminary studies in our laboratory indicated that the chloroform extract of the roots of *Vetiveria zizanioides* which was collected from Timbi Lake at Bakrol, Tal. Waghodiya, Dist. Baroda inhibited the growth of *Streptococcus aureus* using cup borer method on the solid agar media. The preliminary cytotoxic activity of crude extracts was observed by brine shrimp bioassay which showed significant cytotoxic action as compared to reference standard, Caffeine. We are further planning to work on the antioxidant activity using different in vitro models.

Key words: *Vetiveira zizaniodes*, Anti bacterial activity, Cytotoxic activity.

INTRODUCTION

Vetiver Plant contains fresh as well as dried leaves and roots of the plant Vetiveria zizanioides belongs to family Germaine [1]. *Vetiveria zizanioides* is a tall, tufted, perennial, scented grass, with a straight stem, long narrow leaves and an abundant network of roots that is abundant, complex and extensive. It is considered sterile outside its natural habitat. It offers an inexpensive yet effective and eco-friendly tool to combat soil erosion. The roots have been used in Asia for centuries for their fragrance, and are woven into aromatic matting and screens. The roots of some cultivars and ecotypes possess essential oil that has been utilized as fragrant material since ancient times [2]. The plant also contains active ingredients used in traditional medicine and as botanical pesticide. Vetiver has traditionally been utilized as medical and aromatic plant since ancient times, particularly in India, Indonesia, Pakistan, Senegal, Sri Lanka and a few other countries as well as in Thailand.

MATERIALS AND METHODS

COLLECTION OF PLANT

The plant material of *Vetiver zizanioides* (*Graminae*) was collected from Timbi Lake, Bakrol, Baroda in November 2008. The plant material was cleaned thoroughly with water and made free from soil. Then the plant material was dried under shade for two weeks and powdered using pulvelizer by passing through sieve no. 40 #.

EXTRACTION OF PLANT DRUG

The dried, powdered material (100g) was extracted with chloroform (200 ml) by cold maceration method. Then the extract was weighted and its extractive value was calculated.

EVALUATION OF ANTIBACTERIAL ACTIVITY

PREPARATION OF TEST ORGANISM

The antimicrobial activity was performed using two gram positive strains *Staphylococcus aureus, Bacillus subtilis,* two gram negative strains *Proteus vulgaris, Escherichia coli* [3,4]

PREPARATION OF STOCK SOLUTION (SAMPLE)

The stock solution (1000 µg/ml) was prepared by dissolving 100 mg of chloroform extract in 1ml of DMSO and diluted up to 100 ml with distilled water. From above stock solution, 250µg/ml, 300 µg/ml, 350 µg/ml, 400

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400 µg/ml, 450 µg/ml and 500 µg/ml were prepared in distilled water. The concentration of DMSO should not be exceeding than 1 %.
DMSO = DIMETHYL SLUFOXIDE

ANTIBACTERIAL ASSAY

The antibiotic assay medium was sterilized (Table 1) by autoclaving and prepared Petri plate in laminar air flow. The test micro-organism was spread on prepared agar Petri plate by toping agar. By using flame sterilized cork borer, 2 cups were prepared in each plate. Prepared different conc. of extracts was added in each cavity of plate. All the plates were incubated in incubator at 32^0c - 35^0c for 24 hrs [5,6].

Table 1. Requirements: Nutrient Broth, Incubator and Autoclave

S. No.	Composition	gm/liter
1	Peptic digest of animal tissue	5.00
2	Beef extract	1.50
3	Yeast extract	1.50
4	Sodium chloride	5.00

Final pH 4 ± 0.2, take 13 gm/liter

EVALUATION OF CYTOTOXIC ACTIVIY
PREPARATION OF STOCK SOLUTION (SAMPLE)

The stock solution (100 µg/ml) was prepared by dissolving 10 mg of chloroform extract in 1ml of DMSO and diluted up to 100 ml with saline, made in distilled water (40 gm/l sea salt) [7]. From above stock solution, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 30 µg/ml dilutions were prepared in saline. The conc. of DMSO should not be exceed than 1 %.

BRINE SHRIMP ASSAY FOR CYTOTOXIC ACTIVITY

Brine shrimp eggs were hatched in artificial sea water (40 gm/l sea salt). After 48 hrs incubation, 15 brine shrimps were transferred to each sample vial using pipette in different beaker. Dilution were made in DMSO (1%) and dilutions were as follows 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 30 µg/ml. Brine shrimps were added in each different conc. vial and made volume up to 10 ml by salt solution [8]. Survivors were counted after 24 hrs and LC_{50} values determined using prohibit analysis. Control vial was prepared using DMSO only. The readings were taken in triplicates [8].

EVALUATION OF ANTIOXIDANT ACTIVITY
PREPARATION OF STOCK SOLUTION (SAMPLE)

The stock solution (200 µg/ml) was prepared by dissolving 20 mg of extract in 100 ml of methanol and from this 10 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml dilutions were prepared in distilled water.

PREPARATION OF STOCK SOLUTION OF STANDARD (ASCORBIC ACID)

The stock solution (100 µg/ml) was prepared by dissolving 10 mg of standard (ascorbic acid) in 100 ml of distilled water. From this 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml were prepared [9].

DETERMINATION OF REDUCING POWER ASSAY

To 1 ml of different conc. of extract (10 – 100 µg/ml), 2.5 ml of phosphate buffer (0.25 M) and 2.5 ml of potassium ferricyanide (1%w/v) were added. The mixture was incubated at 50^0c for 20 min. After incubation, 2.5 ml of TCA was added to the reaction mixture, which was then centrifuged at 3000 rpm for 10 min. Then 2.5 ml of supernatant was taken and to it add 2.5 ml of distilled water and 0.5 ml of ferric chloride solution (0.1%w/v) and the absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

It has been reported that the plant Vetiver (*Vetiveria zizanioides*) contains volatile oil and this study also resulted the presence of steroids, terpenes, glycosides and fats in the plant. The chloroform extract showed good antibacterial activity (Table 2) against *P. vulgaris*, better cytotoxic activity (Table 3, Table 4) than that of the reference standard caffeine and antioxidant activity (Table 5) as compared to reference standard ascorbic acid as a standard reducing agent or antioxidant.

Table 2. Zone of Inhibition in Antibacterial activity by Agar cub borer method

S. No.	Sample	Conc. (µg/ml)	Zone of inhibition (mm)			
			S. aureus	***B. subtilis***	***P. vulgaris***	***E. coli***
1	Gentamycin	20	16.1	18	20	19
2	Extract	250	5	6	7	6.5
		300	5.5	6.3	7.4	6.5
		350	6	6.5	8	7
		400	10	11.2	12.5	12
		450	11.1	11.8	13	12.5
		500	14.5	16	17.5	17

Table 3. % Inhibition in Cytotoxic activity by Brine shrimp bioassay

S. No.	Sample	Conc. (µg/ml)	No. of survivors			Means ± SEM	% Inhibition
			I.	I.	I.		
1	Control	-	15	15	15	15 ± 0.000	-
2	Caffeine	50	10	11	11	10.667 ± 0.333**	15.78
		100	9	10	8	9.00 ± 0.577***	28.95
		200	8	9	8	8.333 ± 0.333***	34.21
		300	7	7	7	7.00 ± 0.000***	44.68
3	Extract	5	13	10	12	11.667 ± 0.862*	22.22
		10	6	9	10	8.333 ± 1.202***	44.44
		25	3	5	6	4.667 ± 0.882***	68.89
		50	1	4	2	2.333 ± 0.882***	84.45

Values are expressed as Mean ± SEM, n=15; Data analyzed by One-way ANOVA followed by Dunnette's test; **p<0.01, ***p<0.001, *Vetiveria zizanioides* chloroform extract

Table 4. LC_{50} value in Cytotoxic activity by Brine shrimp bioassay

S. No.	Sample	LC_{50}
1	Caffeine	335.72
2	Extract	29.60

Table 5. Absorbance obtained for different concentrations in Antioxidant activity by Reducing power assay

S. No.	Sample	Conc. (µg/ml)	Absorbance			Mean Abs. ± SEM
			I	II	III	
1	Ascorbic acid	10	0.2336	0.2367	0.2396	0.2366 ± 0.002
		20	0.3154	0.3154	0.3136	0.3136 ± 0.001
		40	0.3774	0.3756	0.3738	0.3756 ± 0.001
		60	0.4120	0.4107	0.4084	0.4102 ± 0.001
		80	0.4829	0.4815	0.4803	0.4816 ± 0.001
		100	0.5450	0.5437	0.5423	0.5437 ± 0.001
2	Chloroform extract	10	0.1444	0.1431	0.1420	0.1432 ± 0.001
		25	0.1679	0.1665	0.1653	0.1666 ± 0.001
		50	0.2035	0.2058	0.2083	0.2059 ± 0.001
		100	0.2441	0.2460	0.2479	0.2460± 0.001

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