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Research Article

ANTIBACTERIAL AND ANALGESIC EFFECTS OF THE LEAVES OF *DICHROSTACHYS CINEREA*

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ABSTRACT

In the present study the chloroform extract of the dried leaves of *Dichrostachys cinerea* was prepared and evaluated for the antibacterial and analgesic activities. The antibacterial activity was evaluated against different bacterial strains by detecting minimum inhibitory concentration and zone of inhibition. The minimum inhibitory concentration values were compared with control and zone of inhibitions were compared with standard ciprofloxacin. The analgesic activity was studied by acetic acid induced writhing test.

Key words: *Dichrostachys cinerea*, Antibacterial, Analgesic.

INTRODUCTION

Dichrostachys cinerea belongs to the family Fabaceae is a deciduous thorny shrub or small rounded tree found in tropical and subtropical condition. Traditionally the plants are used as vermifuge and in leprosy, syphilis, dysentery, headache, toothache etc[1]. All these traditional uses indicate that there must be some antibacterial and analgesic properties. In the present investigation the chloroform extract was subjected for antimicrobial and analgesic activity studies.

MATERIAL AND METHODS

The plant was identified by the taxonomists of Botanical Survey of India, Shibpur, Howrah. After authentication, fresh leaves were collected in bulk from young matured plants at the rural belt of Mayurbhanj district in the month of august 2007.The leaves were washed, shade dried and milled in to coarse powder by a mechanical grinder. The powder materials were passed through sieve number 40 and used for further studies.

Preparation of extract

The dried powdered materials (100 g) were extracted with chloroform as solvent for 45 hr. by soxhlet extractor. The extract was filtered while hot and concentrated in vacuum under reduced pressure using rotary flask evaporator and dried in a desiccator. The yield was 2.52 % (w/w). The concentrated chloroform extracts were further subjected for its antibacterial and analgesic activity studies.

Determination of minimum inhibitory concentration

The molten nutrient agar media containing various concentrations of the extract (0,100,200,300,400 and 500 µg/ml) were poured and solidified onto sterile 100mm Petridishes to give sterile nutrient agar plates with varying dilutions of the extract. Then these plates were kept in the refrigerator (4ºC) for 24 hours for uniform diffusion of the extract into nutrient agar media. The plate was then dried at 37ºC for 2 hours. One loopful (diameter-3mm) of an overnight grown peptone water culture of each test organism was placed in petidish marked by the checker board technique. The spot inoculated plate was incubated at 37ºC for 24 hours and MIC value obtained[2-3]. The experiment was repeated in triplicate and average values were written in the Table 1.

Table 1 : Determination of MIC values of *Dichrostachys cinera* extract against different bacteria

Name of bacteria	Growth in nutrient agar containing different concentration of chloroform extract in µg/ml					
	0	**100**	**200**	**300**	**400**	**500**
S.aureus, NCTC- 6751	+	-	-	-	-	-
S.aureus, ML-6	+	-	-	-	-	-
S.aureus, ML-59	+	-	-	-	-	-
S.aureus, ML-276	+	+	-	-	-	-
Shigella flexneri	+	+	-	-	-	-
Pseudomonas 1006	+	-	-	-	-	-
Salmonella typhi NCTC-74	+	-	-	-	-	-
Bacillus brevis-7096	+	+	-	-	-	-
Bacillus licheniformis-10341	+	+	-	-	-	-
V. cholerae-1311	+	+	+	-	-	-
V. cholerae-792	+	+	+	+	+	-
V. cholerae, PN-7						

'0'Control (without extract), '+' Growth, '-' No growth

Determination of zone of inhibition

For the determination of ZOI pure ciprofloxacin were taken as a standard antibiotic for comparison of the results. Two sets of two dilutions (100 and 200 µg/ml) of *D.cinera* extract and ciprofloxacin (100 and 200 µg/ml) were prepared in double distilled water in Mc Cartney bottles. Sterile nutrient agar plates were prepared and incubated at 37ºC for 24hrs to check any sort of contamination. Two sterile filter paper discs (Whatmann no.1) of 6mm diameter were soaked in two different dilutions of crude extract and placed in appropriate position on the surface of the flooded plate, marked as quadrants at the back of the petridishes. The petridishes were incubated at 37 ºC for 24 hrs and the diameter of the zone of inhibition were measured in mm. Similar procedure were adopted for the pure ciprofloxacin and the corresponding zone diameter were compared accordingly[4]. The experiment was repeated in triplicate and average values were written in the Table 2.

Table 2 : Determination of zone of inhibition of *Dichrostachys cinerea* extract.

Name of bacteria	Leaves extract (µg/ml)		Ciprofloxacin (µg/ml)	
	100	200	100	200
S.aureus, NCTC- 6751	15	18	20	24
S.aureus, ML-66.3	14	18	19	22
S.aureus, ML-59	12	15	21	24
S.aureus, ML-276	13	16	20	23
Shigella flexneri	12	15	20	24
Pseudomonas 1006	12	16	18	20
Salmonella typhi NCTC-74	11	14	16	20
Bacillus brevis-7096	10	12	19	23
Bacillus licheniformis-10341	13	15	19	24
V. cholerae-1311	09	11	21	24
V. cholerae-792	08	10	20	24
V. cholerae, PN-7	13	15	19	23

Tests were done in triplicate and values were expressed as mean in mm.

Determination of analgesic activity of the plant extract

Swiss albino mice weighing between 20 to 25 gm of either sex were used and were maintained at 25± 3ºC they were kept in a well ventilated animal house under the natural photo periodic condition in large polypropylene cage and were fed standard rat chow and water *ad libitum*. The animal experiment was approved by animal ethical committee of institution.

The antinociceptive activity of *Dichrostachys cinera* was assessed using the writhing test. Acetic acid solution 0.6% v/v (10mg/kg) was injected intraperetoneal and abdominal muscles constriction together with stretching of hind limbs was counted over a period of 20min stating immediately after acetic acid injection. *D.cinerea* extract (15mg/kg, 30mg/kg) and Aspirin (100mg/kg) were administered 30min before the acid injection. Antinociceptive activity was expressed as the percentage of inhibition of writhing compared with the control animal [5- 6] as shown in table 3.

Table 3 : Effect of *Dichrostachys cinerea* chloroform extract on acetic acid induced writhing in mice

Groups	Number of wriths	Percentage of inhibition
Control	72.9±3.2	……
Aspirin (100mg/kg)	40.6±1.2**	44.3
D. cinerea.extract		
15 mg/kg	48.9±2.9*	32.9
30 mg/kg	42.1±3.6**	42.2

Values are mean ± SEM n= number of animal in each group (6) *p<0.005 compared with control **p<0.001 compared with control value.

RESULTS AND DISCUSSION

The observations of the MIC study of *Dichrostachy cinerea* has been tabulated in table-1 and it was found that the chloroform extract was active against both gram positive and gram negative bacteria, but more active against gram positive at low concentration. *S.aureus* NCTC6751, *S.aureus* ML6, *S.aureus* ML59, *Pseudomonas* 1006, *Salmonella typhi NCTC 74* and *V.cholerae* PN7 were inhibited at concentration 100µg/ml and the strains *Shigella flexanari* , *Bacillus brevis* 7096 and *Bacillus lichiformis* 10341 were inhibited at 200 µg/ml , where as *V.cholerae* 1311 was inhibited at concentration 300µg/ml. The results of zone of inhibition of choloroform leave extract and its comparison with standard antibiotic Ciprofloxacin was recorded in table-2. The antibacterial efficacy of the leave extract was found to decrease in the following order against different bacterial strains *Staphylococcus aureus, Vibrio cholerae, Bacillus brevis, Pseudomonas, Shigella flexneri, Salmonella typhi* respectively.

Effect of *Dichrostachys cinerea* chloroform extract as acetic acid induced writhing was shown in the table 3. Dose of 30mg/kg body weight was injected intra peritoneal route showed the significant reduction in the number of writhes induced by acetic acid in dose dependent manner when compared to standard aspirin. Acetic acid produced nociception by liberating endogenous substances including serotonin, bradykinin, histamine and prostaglandin, which may stimulate sensory nerve ending. Therefore, *Dichrostachys cinerea* extract might be inhibit the synthesis and /or release of these endogenous substances. This concluded that, chloroform extract of *Dichrostachy cinerea* leaves could be beneficial in the management of pain. The new findings in the present investigation offer a scientific support to the ethno medicinal use of the plant by the traditional people.

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