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Antioxidant and Antiulcer Activities of Methanolic extract of *Alternenthra pungens*

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In this paper, *in vitro* antioxidant and *in vivo* antiulcer activites of methanolic extract of *Alternenthra pungens*. Antioxidant activity was determined by two *in vitro* methods-DPPH and H_2O_2 radical scavenging. To study the antiulcer activity of methanolic extract of *Alternenthra pungens* (100 and 200 mg/Kg) using different models of ulceration in rats *viz.*, aspirin induced ulcer and cold restraint stress induced gastric lesions in rats. While in aspirin and cold restraint stress induced models, ulcer index and % inhibition of gastric ulcers was determined. Famotidine (20 mg/Kg) was used as a reference drug. Methanolic extract of *Alternenthra pungens* treated animals exhibited protective effect on ulceration induced by aspirin induced ulcer and cold restraint stress in rats. Control animals had ulcers, while animal treated with methanolic extract of *Alternenthra pungens* showed reduction in ulcers in the models in a dose dependent manner; it significantly ($p < 0.001$) decreased the volume of gastric acid secretion and also reduced with respect to control and comparable to the standard drug.

Key Words: Antiulcer, Antioxidant, *Alternenthra pungens*.

INTRODUCTION

Alternenthra pungens (*Amaranthaceae*) is a very common plant found through out India[1]. It is a low growing herb, upto 80 cm tall, found in dense mat, particularly around houses, lawns, camp sites, stockyards or overgrazed areas. The prostrate herb with perennial root system and annual above ground growth. Taproot often large and woody. Stems shortly silky hairy. Leaves ovate to circular, hairless to sparsely hairy, margins entire; leaf stalk 0.2-1.0 cm long. Infloresence ovoid, 6-10 mm wide. Fruit 1-1.15 mm long. Seeds about 1 mm wide brownish, globe shaped[2]. The native place of this plant are Brazil, Ecuador, Peru and Venezuela. According to traditional use the different parts like leaves, roots are used as stimulant and carminative. The antioxidant activity was found in flavone c-biosides from the aerial parts of *Alternanthera pungens*[3-5]. In the present study we report the *in vitro* antioxidant and *in vivo* antiulcer activites of methanolic extract of *Alternenthra pungens*. A review of the literature revealed that antiulcer activity of the plant has not been subjected to scientific evaluation, therefore this study was undertaken to investigate the antiulcer potential of methanolic extract of *Alternenthra pungens* using rats.

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EXPERIMENTAL

The plant materials were collected in the month of April from Erode district of Tamil Nadu. The plant materials were taxonomically identified and the voucher specimens (No. AP I and APII, respectively) have been preserved in our laboratory for future reference.

Extraction: The fresh plant material was washed under running tap water to remove dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The dried powdered plant material (500 g) was extracted successively with methanol by Soxhlet for 72 h at a temperature not exceeding the boiling point of the solvent. Standard methods[6,7] were used for preliminary phytochemical screening of the extracts to know the nature of phyto constituents present in it.

Experimental animal: Inbred male, Wister Albino rats (100-150 g) were selected for these studies. Six rats were taken for each group. The rats were used after an acclimatization period of 7 d to the laboratory environment. They were provided with food and water *ad libitum*. All animal experiments carried out according to the guidelines and approval of the Animal Ethics Committee.

Evaluation of *in vitro* antioxidant activity: Diphenylpicrylhydrazyl (DPPH) radical scavenging activity, hydrogen peroxide (H_2O_2) radical scavenging activity was determined by following procedures[8-13].

DPPH Radical scavenging activity: 0.1 mm solution of DPPH in ethanol was prepared and 1 mL of this solution was added to 0.3 mL of extract solution in water at different concentrations (10-100 µg/mL). After 0.5 h, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical and the standard BHT was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

H_2O_2 Radical scavenging activity: A solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). H_2O_2 concentration was determined spectroscopically measuring absorption with extinct coefficient for H_2O_2. Different concentrations of the extracts in distilled water were added to a H_2O_2 solution (0.6 mL, 40 mM). Absorbance of H_2O_2 at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without H_2O_2. Ascorbic acid was used as the standard. The % of H_2O_2 scavenging was calculated by the equation:

$$H_2O_2 \text{ scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Evaluation of antiulcer activity: In present study the effect of methanolic extract of *Alternenthra pungens* on aspirin induced and cold-restraint stress-induced gastric lesions in rats was evaluated according to methods described earlier[14-18].

Aspirin (ASP)-induced ulcers: The animals were treated with control or drug samples for 7 d. At the end of the 7th day, rats were fasted for 24 h. ASP in dose of 200 mg/kg (20 mg/mL) was administered to the animals on the day of the experiment. After 4 h of aspirin induction animals were sacrificed and the stomach was then excised and cut along the greater curvature. The ulcer index was then determined as described above.

Cold-restraint stress (CRS)-Induced ulcers: Methanolic extract of *Alternenthra pungens* (100 and 200 mg/kg) and test drug (Famotidine 20 mg/kg) was administered to rats for 7 d. The control group was fed normal saline solution. Thereafter the rats were deprived of food, but not water, for *ca.* 18 h before the experiment. On day 8, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4-6 °C. After 2 h, the animals were sacrificed by cervical dislocation and ulcers wee examined on the dissected stomachs as described above.

Statistical analysis[19]: The results of the experiments are expressed as mean ± SEM. After confirming the variances homogeneity of results by Barlett's test, the differences were estimated by one-way ANOVA followed by Tukey's test for the single dose studies or by means of Dunnet's test for individual comparison of groups with control. When the probability (p) was < 0.05, the results were considered to be significant.

RESULTS AND DISCUSSION

Antioxidant activity: The extract exhibited scavenging potential with IC_{50} value of 31.24 and 217.73 µg/mL for DPPH and H_2O_2 radicals, respectively. The value was found to be lesser than that of BHT (17.12 µg/mL) and ascorbic acid (125.64 µg/mL), used as standards in respective assays (Tables 1 and 2).The extract showed dose dependant increase in reducing power that was comparable to standard BHT.

TABLE-1
DPPH RADICAL SCAVENGING ACTIVITY

Drug concentration (µg/mL)	DPPH Scavenged (%)	
	BHT	*A. pungens*
10	26.00 ± 0.21	18.4 ± 0.17***
20	59.60 ± 0.67	49.8 ± 0.68***
50	78.20 ± 1.10	67.8 ± 1.06***
100	93.08 ± 1.54	85.46 ± 1.54**
IC_{50}	17.12 µg/mL	31.24 µg/mL

Values are mean ± SE, n = 6; data analyzed by one way ANOVA and student's "t" test;
**p < 0.001, *** p < 0.0001 *vs.* control.

TABLE-2
HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Drug concentration (µg/mL)	DPPH Scavenged (%	
	BHT	*A. pungens*
50	29.17 ± 0.45	13.14 ± 0.29***
100	44.24 ± 0.82	28.78 ± 0.65**
200	65.56 ± 1.21	51.26 ± 1.14***
300	77.72 ± 1.54	60.21 ± 1.28**
350	86.64 ± 2.03	68.54 ± 1.60***
IC_{50}	125.64 µg/mL	217.73 µg/mL

Values are mean ± SE, n = 6; data analyzed by one way ANOVA and student's "t" test; **p < 0.001, *** p < 0.0001 *vs.* control.

Antiulcer activity: Methanolic extract of *Alternenthra pungens* exhibited a significant protective effect on ulceration induced by aspirin induced and cold restraint stress in rats (Tables 3 and 4). The control animals had ulcers and hemorrhagic straks, whereas in animals administered with the extract there was reduction in ulcer index in all the models in a dose dependant manner. The ulcer index significantly ($p < 0.001$) decreased after treatment with methanolic extract of *Alternenthra pungens*, at both the tested doses (100 and 200 mg/kg) and was comparable to the standard drug famotidine (20 mg/kg). The percent inhibitions of gastric lesions were recorded more than 50 % in all the models. In aspirin model the inhibition was 53.39 and 64.21 %, respectively (Table-3), while in case of cold restraint stress induced ulcers, the inhibition was marginally higher (70.73 %) with methanolic extract of *Alternenthra pungens* at dose of 200 mg/Kg body weight compared to famotidine at 20 mg/Kg dose (69.94 %) (Table-4).

TABLE-3
EFFECT ON ASPIRIN INDUCED GASTRIC LESIONS

Treatment	Ulcer index (mm^2)	Inhibition (%)
Control (saline solution)	37.5 ± 0.84	–
A. pungens 100 (mg/kg)	15.58 ± 0.20**	53.39
A. pungens 200 (mg/kg)	11.33 ± 0.58**	64.21
Famotidine (20 mg/kg)	11.83 ± 0.29	62.83

Values are mean ± SE, n = 6; *p < 0.01 *vs.* control by, student's "t" test.

TABLE-4
EFFECT ON COLD RESTRAINT STRESS INDUCED ULCERS

Groups	Ulcer index (mm^2)	Inhibition (%)
Control (saline solution)	26.59 ± 0.68	–
A. pungens 100 (mg/kg)	7.54 ± 0.02**	64.58
A. pungens 200 (mg/kg)	5.82 ± 0.08**	70.73
Famotidine (20 mg/kg)	7.05 ± 0.05***	69.94

Values are mean ± SE, n = 6; **p < 0.001 and ***p < 0.0001 *vs.* control by student's "t" test;

Present results imply that *A. pungens* possesses significant antiulcer property either due to cytoprotective action of the drug or by strengthening of gastric and duodenal mucosa or due to its antioxidant activity. There is also possibility that all of these mechanisms may be acting simultaneously and thus enhancing mucosal defense. These activities may also be due to the presence of various phytochemicals present in it and detected during phytochemical analysis.

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