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PHYTOCHEMICAL SCREENING AND INVESTIGATION OF ANTIULCER ACTIVITY OF *TRIDAX PROCUMBENS*

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Abstract

Tridax procumbens {Asteraceae} is a plant currently used for the treatment of stomach ailments. The present study was performed to evaluate the anti-ulcerogenic activity of the hydroalcoholic, chloroform and petroleum ether combined extracts of the plant *Tridax procumbens* against gastric ulceration induced by fasting and administration of Indomethacin in rats. Gastric ulceration was induced in 48 hrs starved rats. The rats were given the plant extract at the doses of 250 mg/kg and 500 mg/kg orally for group III and IV respectively. The positive controls (group II) received Ranitidine at the dose of 30 mg /kg, while negative control (group I) received distilled water. Five minutes after drug treatment, 1 ml of the ulcerative agent Indomethacin (30 mg/kg b.wt.) was given to each rat orally. Rats were sacrificed and the ulcer areas of the gastric walls were determined. The results show that the whole plant extract of *Tridax procumbens* has protective properties against Indomethacin induced ulcers in rats. The gastric mucosal protection against irritant substances can be mediated by a number of mechanisms that include enhanced gastric mucosal defence through increased mucus production, reducing the volume of gastric acidity. *Tridax procumbens* (250 mg/kg) significantly inhibited gastric ulceration when compared to control group and *Tridax procumbens* (500 mg/kg) caused significant reduction in ulcer when compared to standard.

Keywords: *Tridax procumbens*; Antiulcer; Rats.

Introduction: Natural products research continues to be an important part of the drug discovery process. The main advantage of natural products as a source of lead compounds is the tremendous molecular diversity found in nature. Advances in the methodologies used to evaluate extracts, fractions and pure compounds for biological activity have

enabled the miniaturization and automation of extremely specific biochemical tests. The net result of these advances is that screening has again become a cost-effective way of discovering new lead compounds. When coupled with new chemical methods, it is now possible to isolate and identify interesting biologically active compounds on a submilligram scale. Natural products must be able to compete effectively with these sources for the limited amount of money available for screening, in order for the field to remain of interest.

Tridax procumbens is a semi-prostate annual or short-lived perennial, with stems up to 50cm long. It is a weed of pastures and a wide range of perennial crop types [1-5]. The persistent pappus enables the achenes to be carried by wind over a wind range. The plant bears daisy like yellow-centered white or yellow flowers with three-toothed ray florets [6]. The leaves are toothed and generally arrowhead-shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. This weed can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semi-tropical climates.

In traditional Ayurvedic system of medicine, several medicinal properties have been attributed to this plant. Recent pharmacological studies have established the Hepatoprotective activity, Immunomodulatory activity, wound healing activity, Ant diabetic activity, and Antimicrobial activity [7-10].

The present study was under taken to determine the anti ulcer potential of the combined hydroalcoholic, chloroform and petroleum ether extracts of whole plant *Tridax procumbens* using an experimental gastric ulcer model namely fasting and Indomethacin induction [11-15].

Materials and methods:

Experimental Animals:

Albino rats of Wistar strain of male sex weighing 250 gms were used. They were housed in polypropylene cages at room temperature. In this experiment the animals were divided into four groups each group containing 4 animals.

Preparation of the plant extract:

Tridax procumbens plants were collected from HITS COLLEGE OF PHARMACY, Bogaram. They were dried under shade and powdered. The powdered plant material was subjected for extraction using three solvents viz. petroleum ether, chloroform, hydroalcholic mixture (50:50). About 300 gms of plant material was used for the extraction process [16-19]. The yield of total extract was found to be 36 gms. The plant was first macerated in these solvents separately for 36 hours

and the marc was subjected to further extraction using Soxhlet apparatus for 48 hours at a temperature of 50-60°C the extracts were concentrated at a temperature of 45-50°C and were subjected to Preliminary phytochemical screening (Figure 1).

Figure 1: Soxhlet Extraction of Plant extract.

Phytochemical screening:

The use of the test of Shinoda help to determine the presence of flavonoids, Liebermann-Buchard`s test revealed the existence of sterols. While presence of saponins, alkaloids, carbohydrates, tannins, proteins were revealed by respective tests (Table 1) [20-29].

Table: 1 Qualitative Chemical Examination.

S. no	Test	Hydroalcholic extract	Chloroform extract	Petroleum ether extract	Inference
1.	Detection of **alkaloids**: To the extract dilute HCl was added and filtered.				Alkaloids are present in hydro alcoholic and chloroform extract and absent in petroleum ether extract.
A.	To the filtrate Mayer's reagent was added.	Cream color precipitate was observed	Cream color precipitate was observed	Cream color precipitate was not observed	
B.	To the filtrate Dragendroff's reagent was added.	Reddish brown precipitate was observed	Reddish brown precipitate was observed	Reddish brown precipitate was not observed	Alkaloids are present in hydroalcoholic and chloroform extract and absent in petroleum ether extract.
C.	To the filtrate Wagner's reagent was added	Brown color precipitate was observed	Brown color precipitate was observed	Brown color precipitate was not observed	Alkaloids are present in hydroalcoholic and chloroform extract and absent in petroleum ether.

D.	To the filtrate Hager's reagent was added	Yellow color precipitate was observed	Yellow color precipitate was observed	Yellow color precipitate was not observed	Alkaloids are present in hydroalcoholic and chloroform extract and absent in petroleum ether extract.
2.	Detection of **carbohydrates**:				
A.	Molich's test: To the extract α-napthol in sodium hydroxide was added and few drops of concentric sulphuric acid were added from the sides of the test tube.	A violet color ring was observed at the junction of two layers	A violet color ring was not observed at the junction of two layers	A violet color ring was not observed at the junction of two layers	Carbohydrate are present in hydroalcoholic extract and absent in chloroform extract and petroleum ether extract
B.	Detection of reducing sugars: Fehling`s test: To the extract Fehling`s solution A and B were added and warmed on water bath for 5 min.	Brick red precipitate was observed.	Brick red precipitate was not observed	Brick red precipitate was not observed	Reducing sugars are present in hydroalcoholic extract and absent in chloroform and petroleum ether extract
3	Tests for **Flavonoids**				
A.	Shinoda test: to the extract Magnesium ribbon and HCl solution were added and heated.	Magenta color was observed	Magenta color was observed	Magenta color was observed	Flavonoids are present in hydroalcoholic, chloroform and petroleum ether extract
B.	Ferric chloride test: to the extract few drops of neutral Ferric chloride was added	Blackish red color was observed.	Blackish red color was observed	Blackish red color was observed	Flavonoids are present in hydroalcoholic, chloroform and petroleum ether extract
4.	Detection of **Saponins**: The extract was shaken with distilled water.	Foaming was observed	Foaming was observed	Foaming was not observed	Saponins are present in hydroalcohol and chloroform extract. And absent in petroleum ether extract.
5.	Detection of **Tannins**:				
A.	Lead acetate test: To the extract 10% Lead acetate solution was added.	White precipitate was observed	White precipitate was not observed	White precipitate was not observed	Tannins are present in hydroalcoholic extract and absent in chloroform and petroleum ether extract
B.	Ferric chloride test: To the extract ferric chloride solution was added.	Green color precipitate was observed	Green color precipitate was not observed	Green color precipitate was not observed	Tannins are present in hydroalcoholic extract and Absent in chlororm and petroleum ether extract

6. A.	Detection of **proteins**: Million's test: To the extract Million's reagent was added.	Pink color was observed	Pink color was observed	Pink color was observed	Proteins are present in hydroalcoholic, chloroform and petroleum ether extract
B.	Biuret's test: to the extract copper sulphate solution was added	Violet color was observed	Violet color was observed	Violet color was observed	Proteins are present in hydroalcoholic, chloroform and petroleum ether extract
7.	followed by the addition of sodium hydroxide solution.	Green color was not observed	Green color was observed	Green color was observed	sterols are present in chloroform and petroleum ether extract and absent in hydroalcholic extract
	Detection of **sterols**: Liebermann-Burchard test: To the extract concentric Sulphuric acid, few drops of glacial acetic acid, followed by addition of acetic anhydride.				

Plant extract: *Tridax procumbens*

The three extracts- hydroalcoholic, chloroform and petroleum ether of *Tridax procumbens* were combined and used as test drug for detection of anti ulcer activity on experimental animals.

Preparation of the Test drug (plant extract):

Preparation of the test drug $\mathbf{T_1}$: *Tridax procumbens* 250 mg/kg b. wt: About 3.125 g of combined plant extract was prepared as a suspension in distilled water which gives a dose of 62.50 mg/ml.

Preparation of the test drug $\mathbf{T_2}$: *Tridax procumbens* 500 mg/kg b. wt: About 6.250 g of combined plant extract was prepared as a suspension in distilled water which gives a dose of 125 mg/ml.

Preparation of the standard drug: Ranitidine 30 mg/kg b. wt: Ranitidine (150mg) tablet powder quantity equivalent to 375 mg was weighed and prepared as a suspension in distilled water which gives a dose of 7.5mg/ml, used as a standard drug in the experiment.

Control: Distilled water

Induction of ulcers in experimental animals

Fasting and Indomethacin: Gastric ulceration was induced in 48 hrs starved (deprived of food and water) rats using Indomethacin at a dose of 30 mg/kg body weight.

Preparation of Indomethacin: About 750 mg of Indomethacin was dissolved in 100 ml of 1% Tween 80 prepared using distilled water which gives a dose of 7.5 mg/ml.

Experimental protocol

Group I: Rats served as normal-control and received the vehicle (1 ml distilled water/rat) orally.

Group II: Rats served as standard and received Ranitidine 30 mg/kg b. wt in distilled water as a fine aqueous suspension orally.

Group III: Rats served as test (T1) and received a dose 250 mg/kg b. wt. in distilled water as fine aqueous suspension orally.

Group IV: Rats served as test (T2) and received a dose 500 mg/kg b. wt. in distilled water as fine aqueous suspension orally.

Fasting-Indomethacin induced ulcer:

Gastric ulceration was induced in 48 hrs starved (deprived of food and water) rats. The rats were given the plant extract at the doses of 250 mg/kg and 500 mg/kg orally for group III and IV respectively. The positive controls (group II) received Ranitidine at the dose of 30 mg/kg, while negative control (group I) received distilled water. Five minutes after drug treatment, 1 ml of the ulcerative agent Indomethacin (30 mg/kg b. wt.) was given to each rat orally. The rats were killed after 24 hrs using chloroform, and the stomach removed and observed for ulcers in the glandular region (Figures 2-5). The surface area of each lesion was measured and scored. The ulcer index of each rat was taken as the mean ulcer score. The percentage ulcerated surface was calculated as the total area covered by all lesions expressed as a proportion of the total corpus mucosal surface area. The percentage of inhibition (%I) was calculated (Figure 6). $\%I = \frac{(USc-USt).100}{USc}$

Ulcer Index:

The stomach was removed and fixed on a cork plate and the number and severity of the ulcers was registered with a stereo-microscope using the following scores

Score:

0 = Normal colored stomach.

0.5 = Red coloration

1 = Spot ulcer

1.5 = Hemorrhagic streaks.

2 = Ulcer >3 but < 5,

3 = Ulcer >5

Results and discussion:

Oral administration of Indomethacin produced characteristic lesions in the glandular portion of the rat stomach, with a total surface 76.66 mm^2. The whole plant extract of *Tridax procumbens* produced dose-dependent inhibition of gastric ulceration ranging from 89 % at the dose of 250 mg/kg to 94% at the dose of 500mg /kg with a respective ulcer surface area of 10 and 4.5 mm^2 (**Table 2**).

Table 2: Effect of *Tridax procumbens* (ulcer index) in Fasting- Indomethacin induced ulceration.

Group	Treatment	Dose (mg/kg)	Ulcer index	U S area (mm^2)	% I
I	Control	--	7.5	76.66	0
II	Ranitidine	30	1	6.0	92.17
III	Extract	250	1.5	10	86.95
IV	Extract	500	0.75	4.5	94.12

Number of rats per group=4

I=Inhibition

U S= Ulcerated Surface

These results show that the whole plant extract of *Tridax procumbens* has protective properties against Indomethacin induced ulcers in rats. The gastric mucosal protection against irritant substances can be mediated by a number of mechanisms that include enhanced gastric mucosal defense through increased mucus production, reducing the volume of gastric acidity. *Tridax procumbens* (Test 1) significantly inhibited gastric ulceration when compared to control group and *Tridax procumbens* (Test 2) caused significant reduction in ulcer when compared to standard.

Conclusion:

Tridax procumbens is known for several potential therapeutic activities like antiviral, anti oxidant, antibiotic efficacies, wound healing activity and insecticidal. The powdered plant material was subjected for extraction using three solvents viz. petroleum ether, chloroform, hydroalcholic mixture (50:50) and was subjected to Preliminary phytochemical

screening. The hydro alcoholic, chloroform, and petroleum ether extracts gave the positive test for flavonoids, proteins. The hydro alcoholic, chloroform extracts gave the positive test for alkaloids, saponins. The chloroform, petroleum ether extracts gave the positive test for tannins, sterols.

The rats were given the plant extract at the doses of 250 mg/kg and 500 mg/kg orally for group III and IV respectively. The whole plant extract of *Tridax procumbens* produced dose-dependent inhibition of gastric ulceration ranging from 89 % at the dose of 250 mg/kg to 94% at the dose of 500mg /kg with a respective ulcer surface area of 10 and 4.5 mm^2. Significant anti ulcer activity was found by the combined extract (hydro alcohol, petroleum ether, chloroform) of *Tridax procumbens.*

Figures:

Effect of *Tridax procumbens* Extract on Fasting Ulceration with Indomethacin.

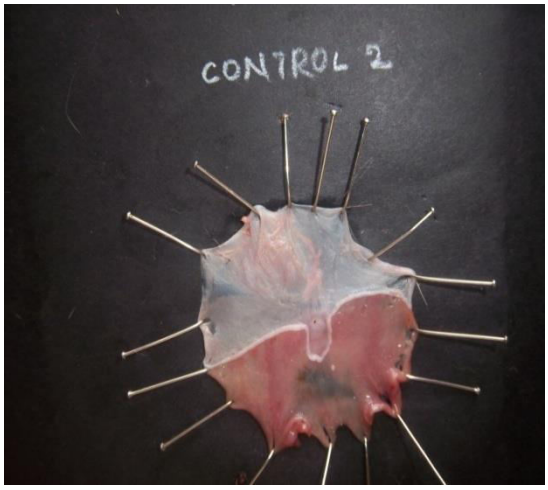


Figure 2A: Control group

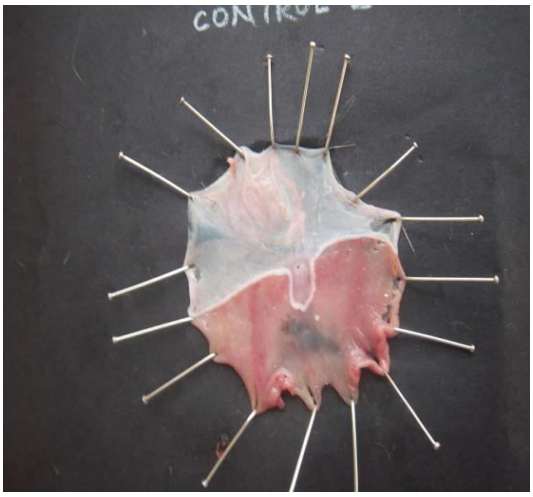


Figure 2B: Control group

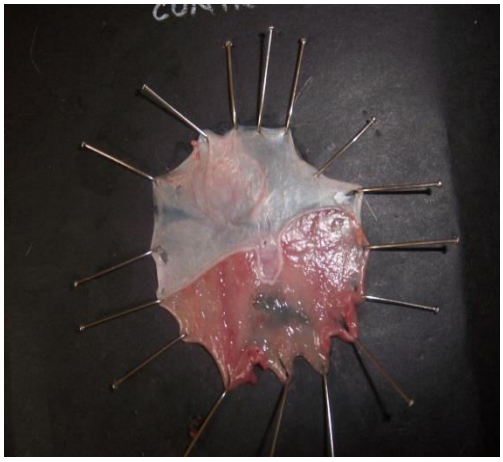


Figure 2C: Control group

Figure 2: Exposed stomach region of Control group rats

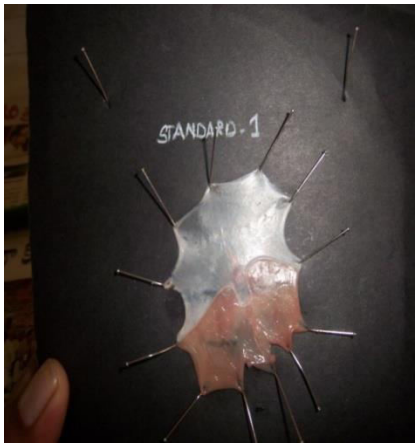
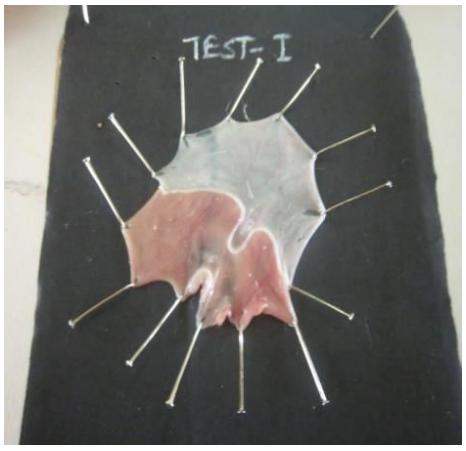


Figure 3A: Ranitidine (30 mg/kg) Figure 3B: Ranitidine (30 mg/kg)

Figure 3: Exposed stomach region of Standard group rats



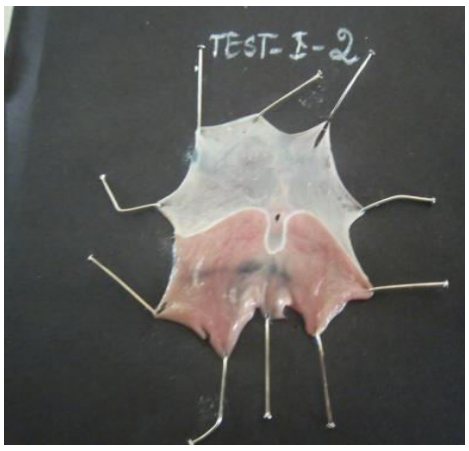
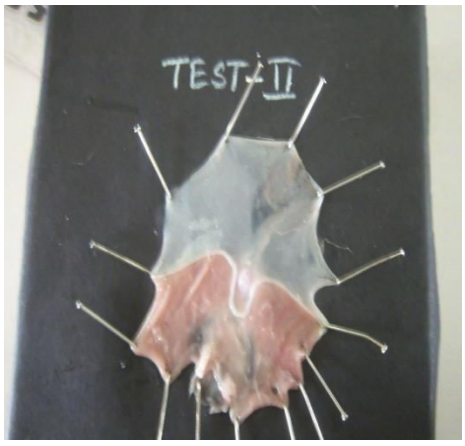


Figure 4A: Plant Extract T_1 (250 mg/kg) Figure 4B: Plant Extract T_1 (250 mg/kg)

Figure 4: Exposed stomach region of Test -1 group rats



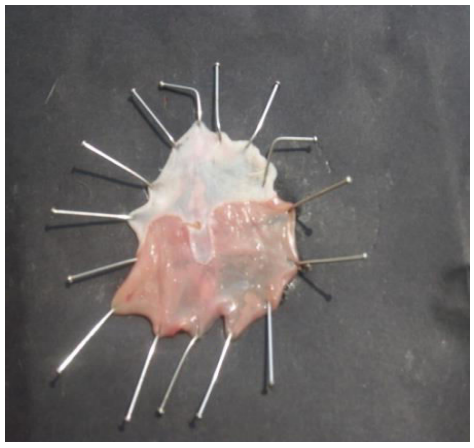


Figure 5A: Plant Extract T_2 (500 mg/kg) Figure 5B: Plant Extract T_2 (500 mg/kg)

Figure 5: Exposed stomach region of Test-2 group rats.

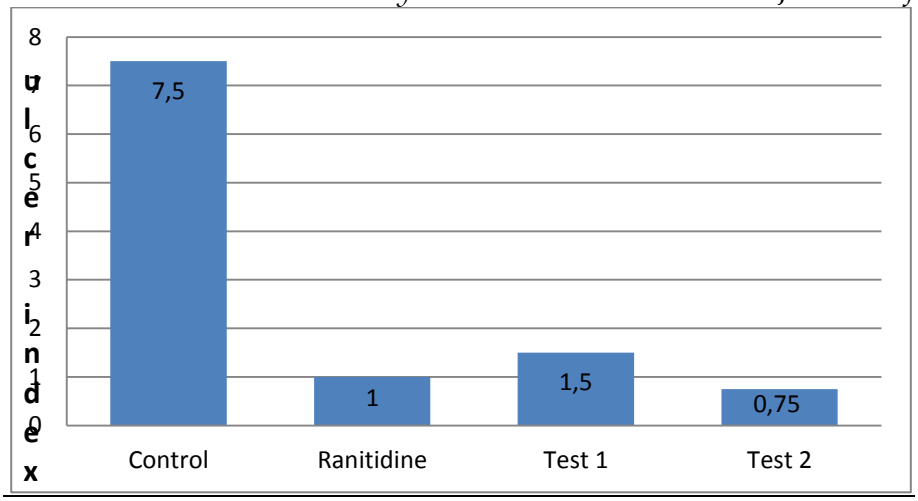


Figure 6: Effect of Tridax procumbens extract on indomethacin induced ulcers.

Group 1: Control

Group 2: Ranitidine (30 mg/kg)

Group 3: Plant Extract (250 mg/kg)

Group 4: Plant Extract (500 mg/kg)

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