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Influence of Various Solvent Extracts of *Tridax procumbens* for its Antidiarrhoeal Potential

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Abstract

Background and objective: Diarrhoea is one of the serious health problems especially for infants and children in the developing countries and it cause severe dehydration that some time may lead to death. WHO have promoted studies which help in the prevention and treatment of diarrhoea using traditional medicinal practices. So, it is vital to identify and screen universally existing natural drugs that can act against any type of diarrhoea. The present study has been designed to investigate the influence of the petroleum ether, dichloromethane and ethanolic extract of the whole plant of *Tridax procumbens* against diarrhoea.

Methods: The anti-diarrhoeal activity was assessed by castor oil-induced diarrhoea, gastrointestinal motility test and prostaglandin-E2 induced enteropooling in experimental animals.

Result: In the anti-diarrhoeal studies, all the extracts showed significant anti-diarrhoeal activity against the incident and severity of diarrhoea produced in experimental animals by all the three models which were comparable to the control and standard drug-treated animals.

Conclusion: The results of the investigations revealed that all the extracts of *Tridax procumbens* have a significant antidiarrheal activity and this may prove the use of the plant in traditional medicine. Extended pharmacological investigations are needed to explore the exact mechanism to exhibit anti-diarrhoeal activity.

Keywords: Anti-diarrhoeal activity, Castor oil, Charcoal meal, Gastrointestinal motility, *Tridax procumbens*, Prostaglandin E_2.

INTRODUCTION

Diarrhoea has been considered as one of the foremost ill health in the developing countries [1]. Worldwide, diarrhoea accounts for over 5 - 8 million deaths annually in infants and tiny kids of less than 5 years [2, 3]. In the United States elders are more susceptible to diarrhoeal illness [4]. Hence, attention has been paid to elucidate the medicament potential of the whole plant of *Tridax procumbens* on different experimental models.

The phytochemical screening of *Tridax procumbens* unconcealed the presence of saponins, alkaloids, tannins, flavonoids (catechins and flavones) and carotenoids. This plant is suggested to have a potential source of protein and vitamin like carotenoids [5]. Variety of chemical constituents viz: n-hexane, β-sitisterol, fumaric acid, luteolin, oxoester, quercitin, lauric acid, palmitic, myristic, arachidic and linoleic acid were also reported [6].

Tridax procumbens has been extensively utilized in the Ayurvedic system of medication and is dispensed as "Bhringraj" by some practitioners of Ayurveda which is well-accepted medicine for a liver disorder. It's been found to possess significant medicinal properties against bronchial catarrh, blood pressure, malaria, diarrhoea, dysentery, stomach ache, wound healing, headache, it also prevents hair fall and checks haemorrhage from cuts and bruises [7]. Its leaves and flowers have insecticidal, parasiticidal and antiseptic properties [8, 9]. The plant also shows many pharmacological activities like anti-diabetic, immunomodulatory, anti-hepatotoxic, anti-inflammatory, analgesic, anti-oxidant, and respiratory depression. However, there is no systematic experimental report for the entire plant of *Tridax procumbens* to exhibit its anti-diarrhoeal activity. The current study was chosen to investigate the antidiarrhoeal properties of the petroleum ether, dichloromethane and ethanolic extract of the whole plant of *Tridax procumbens.*

MATERIALS AND METHODS

Extraction

Whole plants of *Tridax procumbens* were collected locally from in and around BG Nagara, Mandya district. The parts were separated, washed and shade dried under sunlight. Coarse powder of the parts were prepared and were subjected to extraction using soxhlet apparatus with different solvents of increasing polarity i.e. petroleum ether (60-80^{o}c), dichloromethane (39.6^{o}c) and ethanol (64.5-65.5^{o}c). The extract was concentrated using a rotary evaporator.

Animals

Albino rats and mice of either sex weighing about 140-160 g and 20-25 g respectively were procured from Sri Raghavendra Enterprises, Bangalore. Animals were maintained at standard environmental conditions (Temperature: 27.0±1.0 and Humidity 55±1%) at the central animal facility. Animals were provided with free access to water given *ad libitum* and food. All the conducted studies were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Adichunchanagiri College of pharmacy BG Nagara, with ethical clearance No. SACCP-IAEC/30/2015.

Acute toxicity studies

The acute toxicity studies of petroleum ether, dichloromethane and ethanolic extracts of the *Tridax procumbens* were carried out using albino mice (20-30g) of either sex. After administration with different doses of petroleum ether, dichloromethane and ethanolic extracts,

the number of animals survived with every extract was noted for 48 h and 14 days. The LD_{50} Value was calculated as per OECD guidelines number 425.

Phytochemical Screening

Preliminary phytochemical investigations were carried out using standard procedures stated by Khandelwal and Kokate [10, 11].

Castor oil induced diarrhoea

The method described by Awouters F. *et.al.* [12]. Albino rats were divided into 6 groups of 6 rats each. Overnight fasted animals with free access to water were taken for the study. Group 1 served as control (0.2 ml of vehicle p. o.), Group 2 received 3mg/kg of loperamide orally and served as standard and group 3-6 were treated with petroleum ether, dichloromethane and ethanolic extracts of *Tridax procumbens* at the dose of 200 and 400 mg/kg body weight respectively.

1 ml castor oil was administered to each rat orally after 30 minutes of treatment. Each and every rat was then housed separately in the metabolic cages with special provision to separate urine and faeces. The weight and number of dropping of diarrhoea were observed and noted for the duration of 4 h. The mean stool weight was taken to calculate diarrhoeal percentage and its inhibition. Anti-diarrhoeal activity was calculated with respect to the percentage of protection using a formula:

Percentage of protection (%) = A-B/A

Where 'A' is the total weight of stools of control animals.

'B' is the total weight of stools of extracts treated animals.

The data of stools weight are expressed as mean ± SEM.

Gastrointestinal Motility test

This method was described by Pazhani G.P. *et.al.* [13]. Albino rats were divided into 6 groups of 6 rats each. 24 h fasted animals with free access to water at all the time were taken for the study. Group 1 served as control (0.2 ml of vehicle p. o.), Group 2 served as standard and treated with Atropine sulphate (5mg/kg p.o.) and group 3-6 were treated with petroleum ether, dichloromethane and ethanolic extracts of *Tridax procumbens* at the dose of 200mg/kg and 400mg/kg body weight respectively.

After 30 minutes, all the rats were orally administered with one ml of a charcoal meal (i.e. 3% deactivated charcoal in 10% of normal saline). Again after 30 minutes of this treatment, each rat was sacrificed and the distance from which the charcoal meal was travelled in the region of pylorus to caecum of the intestine was measured and noted. Comparisons were made among control, extracts and standard drug treated animals. Travelled percentage and the inhibition percentage was calculated using formulae,

% travelled = (A/B)*100.

% of inhibition = {(B-A)/B}*100.

Where 'A' is the distance travelled by the charcoal meal,

'B' is the total length of the small intestine.

Prostaglandin-E_2 induced enteropooling

The method described by Murugesan T. *et. al.* [14]. Seven groups of albino rats comprising of six animals were used. Food and water were destituted to all the rats for 18 h before the study. Group 1 served as control and administered orally with 1 ml 5% ethanol in normal saline and then with 0.2 ml vehicle. Group 2 served as positive control and administered orally with 1 ml 5% ethanol in normal saline. Group 3 received 3 mg/kg Loperamide orally and served as standard and group 3-7 were treated with petroleum ether, dichloromethane and ethanolic extracts of *Tridax procumbens* at the dose of 200 and 400 mg/kg body weight respectively.

Soon after the treatment above, administered prostaglandin - E_2 (100 µg/kg in 5% ethanol in normal saline) to each animal orally. Group I served as control so received only vehicle not prostaglandin-E_2.

30 minutes later each and every animals were sacrificed and the intestine (from pylorus to caecum) is isolated and its fluid or contents were collected. Total fluid in each animal of each group was noted. Percentage reduction or inhibition in oedema volume was calculated by following formula:

Percentage inhibition = 100 – (A/B)*100

Where "A" is the volume of intestinal fluid in the treated animals

"B" is the volume of intestinal fluid in the prostaglandin E_2 treated group animals

Statistical Analysis

The results were expressed as Mean ± SEM and subjected to statistical analysis using ANOVA followed by Dunnett 't' test.

RESULTS

The qualitative phytochemical investigation publicized the presence of tannins and flavanoids in petroleum ether extract; carbohydrates, proteins, flavanoids, phytosterols, glycosides and alkaloids in dichloromethane extract; carbohydrates, proteins, saponins, triterpenoids, flavonoids, phytosterols, glycosides and alkaloids in ethanolic extract. In acute toxicity studies, up to the dose of 2000mg/kg body weight no signs and symptoms of toxicity or mortality were shown in all the extracts. Hence $1/5^{th}$ and $1/10^{th}$ of the maximum tested dose was chosen for anti-diarrhoeal activity. The anti-diarrhoeal activity of petroleum ether, dichloromethane and ethanolic extract of *Tridax procumbens* on different models were given in table 1, 2 and 3. In the model of castor oil prompted diarrhoea, all the extracts of *Tridax procumbens* showed dose-dependent anti-diarrhoeal activity by reducing the incidence of defecation and total weight of wet feces. In gastrointestinal motility test all the extracts of *Tridax procumbens* showed dose-dependent anti-diarrhoeal activity by decreasing the propulsion of charcoal meal as compared to control. In prostaglandin-E_2 encouraged diarrhoeal model, the lower dose of petroleum ether extract exhibit better anti-diarrhoeal activity by reducing the volume of intestinal fluid compared to the higher dose. *Tridax procumbens* extract of ethanol and dichloromethane showed dose-dependent activity.

Table 1: Anti-diarrhoeal activity of *Tridax procumbens* in castor oil induced diarrhoeal model

Group	Treatment	Mean weight of stools± SEM after 4 h (g)	Percentage Inhibition
1	Control	3.82 ± 0.27	-
2	Standard	0.2 ± 0.02**	94.76
3	PETP 400	0.53 ± 0.02**	86.12
4	PETP 200	0.82 ± 0.03**	78.53
5	DCMTP 400	1.11 ± 0.09**	70.94
6	DCMTP 200	1.76 ± 0.16**	53.92
7	EETP 400	0.38 ± 0.01**	90.05
8	EETP 200	0.89 ± 0.11**	76.70

Values are expressed as mean ± SEM (n = 6). ***P<0.001, **P< 0.01 and *P< 0.05
PETP 400: Petroleum ether extracts of *Tridax procumbens* (400 mg), PETP 200: Petroleum ether extracts of *Tridax procumbens* (200 mg), DCMTP 400: Dichloromethane extracts of *Tridax procumbens* (400 mg), DCMTP 200: Dichloromethane extracts of *Tridax procumbens* (200 mg), EETP 400: Ethanolic extracts of *Tridax procumbens* (400 mg), EETP 200: Ethanolic extracts of *Tridax procumbens* (200 mg).

Table 2: Anti-diarrhoeal activity of *Tridax procumbens* in gastrointestinal motility test model

Group	Treatment	Mean total length ± SEM (cm)	Mean distance traveled ± SEM (cm)	Mean percentage movement of charcoal ± SEM (cm)	Percentage Inhibition
1	Control	87.5	79.83	91.22 ± 1.75	-
2	Standard	81.66	24.833	30.66 ± 1.96**	69.59
3	PETP 400	80	50	62.75 ± 1.78**	37.5
4	PETP 200	94.16	69	73.21 ± 2.17**	26.72
5	DCMTP 400	89.16	60.5	67.94 ± 1.82**	32.14
6	DCMTP 200	85.66	68	79.72 ± 1.77**	20.61
7	EETP 400	88.16	64.83	73.5 ± 1.09**	26.46
8	EETP 200	85.16	71.16	83.56 ± 1.75*	16.43

Values are expressed as mean ± SEM (n = 6). **P< 0.01 and *P< 0.05
PETP 400: Petroleum ether extracts of *Tridax procumbens* (400 mg), PETP 200: Petroleum ether extracts of *Tridax procumbens* (200 mg), DCMTP 400: Dichloromethane extracts of *Tridax procumbens* (400 mg), DCMTP 200: Dichloromethane extracts of *Tridax procumbens* (200 mg), EETP 400: Ethanolic extracts of *Tridax procumbens* (400 mg), EETP 200: Ethanolic extracts of *Tridax procumbens* (200 mg)

Table 3: Anti-diarrheal activity of *Tridax procumbens* in prostaglandin E2 induced enteropooling

Group	Treatment	Mean volume of intestinal fluid ± SEM (ml)	Percentage Inhibition
1	Control	0.95 ± 0.15**	-
2	Positive control	1.45 ± 0.08**	-
3	Standard	0.58 ± 0.10**	60
4	PETP 400	1.81 ± 0.03**	24.82
5	PETP 200	0.98 ± 0.08**	32.41
6	DCMTP 400	0.8 ± 0.08**	44.82
7	DCMTP 200	1.06 ± 0.04**	26.89
7	EETP 400	0.81 ± 0.07**	44.13
8	EETP 200	1.00 ± 0.06**	31.03

Values are expressed as mean ± SEM (n = 6). **P< 0.01 and *P< 0.05
PETP 400: Petroleum ether extracts of *Tridax procumbens* (400 mg), PETP 200: Petroleum ether extracts of *Tridax procumbens* (200 mg), DCMTP 400: Dichloromethane extracts of *Tridax procumbens* (400 mg), DCMTP 200: Dichloromethane extracts of *Tridax procumbens* (200 mg), EETP 400: Ethanolic extracts of *Tridax procumbens* (400 mg), EETP 200: Ethanolic extracts of *Tridax procumbens* (200 mg)

DISCUSSION

The results obtained from the present study could suggest the anti-diarrhoeal activity of *Tridax procumbens* by reducing the frequency of defecation as well as the total weight of wet faeces in castor oil induced diarrhoeal model, by decreasing the propulsion of charcoal meal in gastrointestinal motility test and reducing the amount of intestinal fluid in case of prostaglandin-E_2 induced diarrhoeal model.

The Ricinoleic acid is obtained by the hydrolysis of Castor oil is responsible to induce diarrhoea [15]. In a hyper-

secretory reaction ricinoleic acid plays a vital role in changing water and electrolytes transport [16].

Ricinoleic acid enhances peristaltic activity of a small intestine, and modify the electrolyte (Na+, K+) permeability by inhibiting the enteral Na+/K+ ATPase activity [17, 18]. The inhibition of enteral Na+/K+ ATPase activity reduces normal fluid absorption, by activating the adenylate cyclase or mucosal cAMP-mediated active secretion. Ricinoleic acid enhances prostaglandin formation and activates the platelet factor [19]. As a result increase in the volume of intestine content leads to the induction of diarrhoea [20].

Anti-diarrhoeal activity of the extracts in castor-oil induced diarrhoea may be due to the inhibition of electrolyte permeability. In case of prostaglandin-E_2 induced diarrhoeal model, synthesis of prostaglandin might be inhibited by the extracts and promoted its anti-diarrhoeal activity. The extract also enhances anti-diarrhoeal potential by decreasing gastro-intestinal motility in case of charcoal meal test.

The specific constituent accountable to show the anti-diarrheal activity of the whole plant of *Tridax procumbens* is yet to be identified. However, some of the phytoconstituents like tannins, saponins and flavonoids are likely to reduce the motility of the intestine and inhibit water and electrolyte secretion [21 - 25].

CONCLUSION

In conclusion, the results of the investigations revealed that, all the extracts of *Tridax procumbens* have a significant antidiarrheal activity and this might prove the utilization of the plant in traditional medicine. This investigation explored that the plant *Tridax procumbens* could be used as an alternative to the currently used anti-diarrhoeal drugs, which are not completely free from adverse effect. Extended pharmacological investigations are needed to explore the exact mechanism to exhibit antidiarrhoeal activity.

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Conflict of Interest

Declared None

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