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## • Research Article

# Medicinal potential of *Passiflora foetida* L. plant extracts: biological and pharmacological activities

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**OBJECTIVE:** To investigate analgesic, antidiarrhoeal and cytotoxic activities of the ethanol extract of *Passiflora foetida* L. (Passifloraceae) by three experimental methods.

**METHODS:** Analgesic activity of the ethanol extract of *Passiflora foetida* L. (EPPF) was carried out using acetic acid-induced writhing inhibition in mice. The method of castor oil-induced diarrhoea in mice was utilized to evaluate antidiarrhoeal activity. The cytotoxic activity of EPPF was explored with a brine shrimp lethality bioassay.

**RESULTS:** The extract showed 68.75% and 30.00% inhibition of writhing at the doses of 500 and 250 mg/kg body weight, respectively. The extract increased the mean latent period prior to diarrhoeal onset to about 1.55 h and 1.17 h, and decreased the mean number of stools to 4.4 and 5.6 at the doses of 500 and 250 mg/kg body weight. The extract also demonstrated cytotoxic activity in the brine shrimp lethality assay, and the median lethal concentration for brine shrimp nauplii was 80 µg/mL.

**CONCLUSION:** The results suggest that the plant extract has analgesic and antidiarrhoeal activities, supporting its uses in traditional medicine. The results also demonstrate that the plant extract possesses cytotoxic activities.

**KEYWORDS:** *Passiflora*; plant extracts; analgesics; antidiarrheals; cytotoxins; mice

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## 1 Introduction

For centuries plants have been the source of many traditional medicines throughout the world. They are one of the richest sources of bioactive compounds. Generally, people from the Indian subcontinent believe that ingestion of plant products will give them beneficial therapeutic effects.

The plant *Passiflora foetida* L. (Passifloraceae), commonly known as “foetid passion flower”, is a passion flower species. It is locally known as *Jhumka lata* and is indigenous to Mexico, southwestern United States (Arizona and southern Texas), the Caribbean and Central America. It is a fast

growing and spreading vine originated in dry forest floors, way-side thickets and riverbeds, and is also found near human settlements.

The plant has ovate to obovate leaves, 6-9 cm long, having 3 shallow lobes, often sinuate and ciliate, with heart-shaped base and pointed tip. The woody and wiry stems are thin, covered by yellow sticky hairs and the 2-3 cm diameter fruit is yellowish-orange to red (when ripe). Seeds are black and embedded in the pulp. Extracts made from the fruit and leaves of *P. foetida* have been used to treat asthma and biliousness. Root and leaf extracts have been used to treat hysteria<sup>[1]</sup>, and in case of headache, the paste of leaf is rubbed on head<sup>[2]</sup>. The herb is used in poultices or



lotions for skin diseases with inflammation and erysipelas in Brazil<sup>[3]</sup>. The present research was focused on the analgesic, antidiarrhoeal and cytotoxic activities of *P. foetida*.

## 2 Materials and methods

### 2.1 Collection of plant materials

The plant *P. foetida* was identified and collected by Md. Asadujjaman from Khulna University campus, Khulna, Bangladesh and was verified by Bangladesh National Herbarium, Dhaka, Bangladesh (Accession Number-34,404).

### 2.2 Preparation of the plant extract

Fresh collected *P. foetida* was separated from adhering materials, and rinsed with water. Plants were shade-dried for four weeks before they were ground into a coarse powder with a motorized plant grinder (capacitor start motor, Wuhu Motor Factory, China). The powder was kept in a dry, cool and dark place in a suitable airtight container until analysis commenced.

About 150 g of powered *P. foetida* was mixed with 900 mL of 95% ethanol in a clean, flat-bottomed glass container. This sealed container was kept for a week with occasional stirring and shaking. Coarse plant material was separated from the mixture by pouring through a clean cloth filter. This extract was passed through filter paper, and the filtrate was evaporated, yielding the ethanol extract.

### 2.3 Experimental animals

Mice of random sex (Swiss-Webstar strain, 20-28 g body weight) provided by Animal Resources Branch of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) were used for the experiments. For adaptation under ambient laboratory conditions (room temperature  $(25 \pm 2)^\circ\text{C}$ , relative humidity 55%-65%, and light:dark cycle for 12 h each), the mice were reserved at Pharmacy Discipline's animal house of Khulna University, Khulna, Bangladesh. The animals, having free access to tap water, were fed with ICDDR, B-formulated standard diets.

### 2.4 Chemicals and reagents

The described experiments used reagents such as acetic acid (Merck, Germany), dimethyl sulfoxide (DMSO; Gaylord Chemical Company, USA), Tween-80 (Loba Chemie Pvt Ltd., India), and castor oil (Loba Chemie Pvt Ltd., India).

### 2.5 Reference drugs

Diclofenac sodium, loperamide and chloramphenicol (Beximco Pharmaceutical Ltd., Bangladesh) were obtained from a local medicine shop.

### 2.6 Preliminary phytochemical analysis

Upon conducting standard procedures, preliminary phytochemical screening of ethanol extract of *Passiflora foetida* (EPPF) was carried out.

### 2.7 Determination of analgesic activity

The method of Ahmed *et al.*<sup>[4]</sup> was adopted with minor

modifications. Mice were randomly separated into four groups of five. Group I, the control group, received 1% (v/v) Tween-80 in water at 10 mL/kg body weight; group II, the positive control group, was treated with standard drug diclofenac sodium at 25 mg/kg body weight; group III and group IV were test groups which were given the extract at 250 and 500 mg/kg body weight, respectively. Treatments (control vehicle, extracts and standard drug) were administered orally 30 min prior to intraperitoneal injection of 0.6% acetic acid. Fifteen minutes after acid injection, the writhing (squirm) numbers were tallied for an interval of 5 min. The percentage of abdominal constriction protection was considered as an indicator of analgesia and calculated as<sup>[5]</sup>:

$$\left( \frac{\text{Number of writhing in control group} - \text{number of writhing in treated group}}{\text{number of writhing in control group}} \right) \times 100\%$$

### 2.8 Antidiarrhoeal activity

The method of Chatterjee<sup>[6]</sup> with minor modification was adapted to study the antidiarrhoeal activity of EPPF using castor oil-induced diarrhoea in mice. Initially all the mice were treated by 0.5 mL castor oil, and mice showing diarrhoea were used in the subsequent experiment. Mice were randomly divided into four groups consisting five mice in each group. Group I was defined as control group, and treated with 1% Tween-80 at 10 mL/kg of body weight. Group II was defined as positive control and treated with standard loperamide (antimotility drug) at 50 mg/kg of body weight. Group III and group IV were defined as test groups and were treated with 250 and 500 mg/kg body weight of EPPF, respectively. The control vehicle and EPPF were administered orally, 40 min prior to the oral administration of castor oil. After treatment with castor oil, mice from each experimental group were placed in individual cages, which had adsorbent paper underneath; diarrhoea episodes were counted every hour for the duration of the 5-hour study. Stool numbers and adsorbent paper staining fluid material were counted on each following hour for the whole experiment time (5 h). The latent period of each mouse was also documented.

### 2.9 Cytotoxicity screening

The brine shrimp lethality bioassay was employed to explore the cytotoxic activity of the plant extract. In the assessment of bioactive compounds this is a common method<sup>[7,8]</sup>.

This investigation was done on *Artemia salina* (brine shrimp). Saline water was prepared by dissolving pure NaCl amounting 20 g and edible normal NaCl amounting 18 g into 1 L of water. In the prepared water, brine shrimp cysts (one spoon) were allowed to hatch and develop into nauplii stage larvae (48 h). In the test, DMSO was used as solvent to prepare the extract at different concentrations. For test purposes, extracts were prepared in various concentra-

tions and were poured into test tubes. Each tube contained 10 shrimps, as well as saline water to adjust volume. The control (blank) group was incubated in only saline water. A known standard drug chloramphenicol (200 µg/mL) was used to treat positive control group. After 24 h the number of surviving nauplii in each tube was counted and the results were noted. Lastly, the median lethal concentration (LC<sub>50</sub>) of the plant extract was determined by the mortality percentage, which was calculated for each treatment concentration.

### 2.10 Statistical analysis

All experimental data were expressed as mean ± standard error of mean. Dunnett's *t* test was used to assess statistical significance by one-way analysis of variance. Statistical analysis was executed in Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). Results of the present study were considered as statistically significant when *P*<0.05.

## 3 Results

### 3.1 Phytochemical screening

The presence of reducing sugars, alkaloids, flavonoids, tannins, steroids, gums and glycosides was shown in the preliminary phytochemical screening of EEPF.

### 3.2 Analgesic activity

In the acetic acid-induced writhing test, EEPF significantly and dose-dependently suppressed the frequency of acetic acid-induced writhing in mice (*P*<0.01). The extract showed 68.75% and 30.00% inhibition of writhing at the doses of 500 and 250 mg/kg, respectively. Standard diclofenac sodium showed 72.5% (*P*<0.01) inhibition of writhing at the dose of 25 mg/kg (Table 1).

### 3.3 Antidiarrhoeal activity

In the castor oil-induced diarrhoea model, EEPF produced a considerable increase in the latent period. The extract showed 1.55 h and 1.17 h of latent period at the doses of 500 and 250 mg/kg body weight, respectively (*P*<0.01). The extract also substantially decreased the number of stool to 4.4 and 5.6 at the doses of 500 and 250 mg/kg body weight respectively (*P*<0.01, Table 2).

### 3.4 Cytotoxicity screening

In the brine shrimp lethality bioassay, EEPF produced

100% of mortality at a concentration of 320 µg/mL, and the LC<sub>50</sub> of the extract was 80 µg/mL (Table 3).

## 4 Discussion

The preliminary screening of EEPF for phytochemical analysis showed the presence of reducing sugars, alkaloids, flavonoids, tannins, steroids, gums and glycosides. It has been reported that alkaloids and flavonoids are responsible for the anti-inflammatory and antinociceptive activity of prostaglandin synthetase inhibition<sup>[9-12]</sup>. Therefore anti-inflammatory and antinociceptive activity of the plant extract may be attributable to the existence of alkaloids and flavonoids either in single form or in combination. Furthermore, presence of alkaloids, reducing sugars, flavonoids, and sterols in medicinal plants has been associated with antidiarrhoeal action<sup>[13,14]</sup>. Therefore the presence of individual or combinations of flavonoids and alkaloids may give the plant extract its possible antinociceptive and anti-inflammatory effects. Alkaloids, as well as flavonoids may also be accountable for the antidiarrhoeal action of EEPF.

The acetic acid-induced writhing model simulates a localized inflammatory response which triggered pain sensation. Acetic acid is used to induce writhing; it causes algia by releasing endogenous substances, which in turn excite nerve endings associated with pain sensation<sup>[15]</sup>. In the acetic acid-induced writhing test, local peritoneal receptors are assumed to be partly involved in the abdominal writhing response and the mechanism of the reaction to this nociceptive stimulus seems to be related to the prostanoid system<sup>[16]</sup>. Acetic acid produces the abdomen's constriction response. This is a procedure used to test effectiveness of peripheral analgesic agents. It has also been linked with prostanoids in general, for example, increased levels of prostaglandin E<sub>2</sub> and prostaglandin F<sub>2α</sub> in peritoneal fluids<sup>[17,18]</sup> as well as lipoxigenase products<sup>[19,20]</sup>.

Change in the regular bowel movement, associated with increased bowel sound, frequency and movement, wet stool, and abdominal pain, is called diarrhoea<sup>[21]</sup>. In developing countries, diarrhoeal diseases are one of the prominent causes of mortality and morbidity<sup>[22]</sup>. The World Health Organization has given special focus to studies of tradi-

**Table 1** Effects of EEPF on acetic acid-induced writhing in mice

Group	<i>n</i>	Number of writhes	Inhibition (%)
Control (1% Tween-80 solution in water, 10 mL/kg, p.o.)	5	16.00±1.41	—
Positive control (diclofenac sodium 25 mg/kg, p.o.)	5	4.40±0.51**	72.50
Test group I (EEPF 250 mg/kg, p.o.)	5	11.20±0.92**	30.00
Test group II (EEPF 500 mg/kg, p.o.)	5	5.00±1.88**	68.75

Numbers of writhes are expressed as mean ± standard error of mean. \*\**P* < 0.01, vs control group. EEPF: ethanol extract of *Passiflora foetida*; p.o.: per oral.

**Table 2** Effects of EEPF on castor oil-induced diarrhoea in mice

(Mean ± standard error of mean)

Group	n	Latent period (h)	Number of stools
Control (1% Tween-80 solution in water, 10 mL/kg, p.o.)	5	0.750±0.063	7.60±1.19
Positive control (loperamide, 50 mg/kg, p.o.)	5	2.280±0.198**	2.40±0.89**
Test group I (EEPF 250 mg/kg, p.o.)	5	1.170±0.149**	5.60±1.08**
Test group II (EEPF 500 mg/kg, p.o.)	5	1.550±0.383**	4.40±1.22**

\*\**P* < 0.01, vs control group.EEPF: ethanol extract of *Passiflora foetida*; p.o.: per oral.**Table 3** Result of brine shrimp lethality bioassay of EEPF

Group	Concentration (µg/mL)	Average number of alive shrimp (sample)	Average number of alive shrimp (control)	Mortality (%)
EEPF	5	10.0	10	0
EEPF	10	7.5	10	25
EEPF	20	6.0	10	40
EEPF	40	5.5	10	45
EEPF	80	5.0	10	50
EEPF	160	3.5	10	65
EEPF	320	0	10	100
Chloramphenicol	100	0	10	100

EEPF: ethanol extract of *Passiflora foetida*.

tional medical practices concerning the prevention and treatment of diarrhoeal diseases<sup>[23]</sup>. In the present study, castor oil used to induce diarrhoea is hydrolyzed to form ricinoleic acid in the upper small intestine<sup>[24]</sup>. Castor oil is believed to link with the liberation of ricinoleate salts, which stimulates adenyl cyclase in the intestinal epithelial cells<sup>[25]</sup> or the release of prostaglandin<sup>[26]</sup>. It is believed ricinoleic acid acts by irritating the gastrointestinal tract mucosa and reducing sodium ion and chloride ion permeability, which in turn results in increased intestinal motility followed by diarrhoea<sup>[27,28]</sup>. However, pre-treatment with EEPF delayed the onset of diarrhoea and reduced the number of diarrhoeal episodes (number of stools), indicating the potential treatment value of the extract. Since, by preventing fluid and electrolyte absorption, castor oil does induce diarrhoea<sup>[29]</sup>, one of the possible mechanisms through which the extract might act is the enhancement of fluid and electrolyte absorption through the gastrointestinal tract. The extract might contain certain components that act as agonist to opioid receptors present in the gastrointestinal mucosa and thus relieving diarrhoea<sup>[29]</sup>. The findings of our study support the traditional medicinal use of this plant, especially in treatment of diarrhoea<sup>[30,31]</sup>. Considering the impact of diarrhoeal disease in the developing countries<sup>[32]</sup>, this plant could play a potential role in developing a new drug molecule.

Since the response variable is binary (either dead or alive), and this lethality (*in vivo*) test is based on a simple model organism (i.e., brine shrimp), it provides a simple and rapid assessment method for screening the extracts plant materials<sup>[33,34]</sup>. In this study, EEPF displayed substantial lethality against nauplii of brine shrimp with LC<sub>50</sub> of 80 µg/mL. There is a general agreement in toxicity tests that LC<sub>50</sub>s greater than 100 µg/mL characterize non-toxic compounds, whilst LC<sub>50</sub>s lower than 100 µg/mL characterize compounds that can be said to have varying degrees of toxicity. The substantial lethality of the EEPF indicates the presence of potent cytotoxic compounds. However, to isolate the active component(s) accountable for this toxicity, advanced explorations using carcinoma cell line are essential.

## 5 Conclusions

EEPF contains reducing sugars, alkaloids, flavonoids, tannins, steroids, gums and glycosides and the extract possesses analgesic, antidiarrhoeal and cytotoxic activities. Results of the experiment tend to suggest that the plant could be a good source of alternative medicine for rheumatism, inflammation, abdominal pain and diarrhoea. Further research is essential to identify active principle(s) and explore the mechanisms involved for their bio-activities.

Overall, the findings of this research support some of

the traditional uses of *P. foetida* in different ailments. This work indicates a need to pursue the isolation of bioactive compounds from EEPF, as well as investigate the pharmacological utility of these isolates.

## 6 Competing interests

The authors declare that they have no competing interests.

## REFERENCES

- Ambasta SP. The useful plants of India. New Delhi: Publication and Information Directorate, Council of Scientific and Industrial Research, India. 1986: 433-437.
- Chopra RL, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research, India. 1956: 186-187.
- Chopra RN, Badhwar RL, Ghosh S. Poisonous plants of India. Calcutta: Public Service Commission, Government of West Bengal, India. 1944: 469-472.
- Ahmed F, Selim MS, Das AK, Choudhuri MS. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie. 2004; 59(4): 329-330.
- Ali K, Ashraf A, Nath Biswas N. Analgesic, anti-inflammatory and anti-diarrheal activities of ethanolic leaf extract of *Typhonium trilobatum* L. Schott. Asian Pac J Trop Biomed. 2012; 2(9): 722-726.
- Chatterjee TK. Handbook of laboratory mice and rats. Calcutta: Jadavpur University, India. 1993: 133-139.
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982; 45(5): 31-34.
- Zhao G, Hui Y, Rupprecht JK, McLaughlin JL, Wood KV. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. J Nat Prod. 1992; 55(3): 347-356.
- Deb D, Dev S, Das AK, Khanam D, Banu H, Shahriar M, Ashraf A, Choudhuri MSK, Basher SAMK. Antinociceptive, anti-inflammatory and anti-diarrheal activities of the hydroalcoholic extract of *Lasia spinosa* Linn. (Araceae) roots. Lat Am J Pharm. 2010; 29(8): 1269-1276.
- Sabina EP, Chandel S, Rasool MK. Evaluation of analgesic, antipyretic and ulcerogenic effect of withaferin A. Int J Integr Biol. 2009; 6(2): 52-56.
- Çitoğlu GS, Özbek H, Sever B. Antinociceptive activity of *Ballota glandulosissima* Hub. -Mor & Patzak. East J Med. 2005; 10(1-2): 24-28.
- Owoyele BV, Nafiu AB, Oyewole IA, Oyewole LA, Soladoye AO. Studies on the analgesic, anti-inflammatory and antipyretic effects of *Parquetina nigrescens* leaf extract. J Ethnopharmacol. 2009; 122(1): 86-90.
- Ezenwali MO, Njoku OU, Okoli CO. Studies on the anti-diarrheal properties of seed extract of *Monodora tenuifolia*. Int J App Res Nat Prod. 2009; 2(4): 20-26.
- Saralaya MG, Patel P, Patel M, Roy SP, Patel AN. Antidiarrheal activity of methanolic extract of *Moringa oleifera* Lam roots in experimental animal models. Int J Pharm Res. 2010; 2(2): 35-39.
- Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJ. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J Ethnopharmacol. 2003; 84(1): 31-35.
- Nguemfo EL, Dimo T, Azebaze AG, Asongalem EA, Alaoui K, Dongmo AB, Cherrah Y, Kamtchouing P. Anti-inflammatory and anti-nociceptive activities of the stem bark extracts from *Allanblackia monticola* STANER L.C. (Guttiferae). J Ethnopharmacol. 2007; 114(3): 417-424.
- Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH, Cunha FQ. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol. 2000; 387(1): 111-118.
- Deraedt R, Jouquey S, Delevallée F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol. 1980; 61(1): 17-24.
- Levine JD, Lau W, Kwiat G, Goetzl EJ. Leukotriene B4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes. Science. 1984; 225(4663): 743-745.
- Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AK. Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of *Tragia involucreta* Linn. J Ethnopharmacol. 2000; 72(1-2): 265-268.
- Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, Hennessy T, Griffin PM, DuPont H, Sack RB, Tarr P, Neill M, Nachamkin I, Reller LB, Osterholm MT, Bennis ML, Pickering LK; Infectious Diseases Society of America. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis. 2001; 32(3): 331-351.
- Carlos CC, Saniel MC. Etiology and epidemiology of diarrhea. Phil J Microbiol Infect Dis. 1990; 19(2): 51-53.
- Atta AH, Mouneir SM. Antidiarrheal activity of some Egyptian medicinal plant extracts. J Ethnopharmacol. 2004; 92(2-3): 303-309.
- Altman DF. Drugs used in gastrointestinal diseases. In: Katzung BG. Basic and clinical pharmacology. 8th ed. San Francisco: McGraw Hill. 2001: 1070-1071.
- Racusen LC, Binder HJ. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. J Clin Invest. 1979; 63(4): 743-749.
- Beubler E, Juan H. Effect of ricinoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon. J Pharm Pharmacol. 1979; 31(10): 681-685.
- Zavala MA, Pérez S, Pérez C, Vargas R, Pérez RM. Antidiarrhoeal activity of *Waltheria americana*, *Commelina coelestis* and *Alternanthera repens*. J Ethnopharmacol. 1998; 61(1): 41-47.
- Gaginella TS, Phillips SF. Ricinoleic acid: current view of an ancient oil. Am J Dig Dis. 1975; 20(12): 1171-1177.
- Goodman A, Gillman A. The pharmacological basis of therapeutics. 9th ed. New York: Macmillan Publishers. 1996: 924-926.
- Gani A. Medicinal plants of Bangladesh. Chemical constituents and uses. Dhaka: Asiatic Society of Bangladesh. 2003: 434.
- Yusuf M, Chowdhury JU, Wahab MA, Begum J. Medicinal



- plants of Bangladesh. Dhaka: BCSIR. 1994: 17-266.
- 32 Das AK, Mandal SC, Banerjee SK, Sinha S, Das J, Saha BP, Pal M. Studies on antidiarrhoeal activity of *Punica granatum* seed extract in rats. *J Ethnopharmacol.* 1999; 68(1-3): 205-208.
- 33 Pimentel-Montanher AB, Pizzolatti MG, Costa-Brighente IM. An application of the brine shrimp bioassay for general screening of Brazilian medicinal plants. *Acta Farm Bonaerense.* 2002; 21(3): 175-178.
- 34 Olorunnisola OS, Bradley G, Afolayan AJ. Antioxidant properties and cytotoxicity evaluation of methanolic extract of dried and fresh rhizomes of *Tulbaghia violacea*. *Afr J Pharm Pharmacol.* 2011; 5(22): 2490-2497.



## Submission Guide

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