ORIGINAL ARTICLE

Discovery Phytomedicine 2019, Volume 7, Number 3: 128-137

Chemical group characterization and determination of pharmacological activities of *Pandanus fascicularis L.* fruit

Md. Shahadat Hossain,[1] Shammi Akhter,[2] Biddut Deb Nath,[3] Mst. Monira Khanom,[1] Md. Eleas Kobir,[1] Amina Khanom,[4] Md. Tofikul Islam,[1] Mst. Nilufa Yasmin,[1] Md. Rakibul Islam,[1] Sulata Bayen,[1] Sonia Ferdousy[1*]

ABSTRACT

The present study was designed to investigate analgesic, anti-pyretic, anti-inflammatory, GI motility and cytotoxic activities of fruit extract of *P. fascicularis* L. The analgesic effect was evaluated by using the acetic acid-induced writhing test. Antipyretic study was done by yeast induced pyrexia. The cytotoxic activity was evaluated by using brine shrimp lethality bioassay. The extracts are used for anti-inflammatory studies by albumin induced edema over a period of time. GI motility test was performed using charcoal. The methanolic, ethanolic and chloroform extracts show presence of maximum phytochemicals such as alkaloid, tannin, saponin, steroid, terpenoid, protein, flavonoid etc. The dose of 400mg/kg is capable of inhibiting 62.96% writhing in comparison to standard Diclofenac sodium. The initial and final rectal temperatures treated with chloroform extract (400 mg/kg) were 38.7±0.45°C and 37.83±0.37°C; where the values for Acetyl salicylic acid (100mg/kg) were 38.4±0.11°C and 37.5±0.010°C respectively. During cytotoxic bioassay LC50 value was found 1.0636 mg/ml. Oedema reduced from 0.62 ± 0.06 to 0.45 ± 0.02 after 5hr administering of chloroform extract (400 mg/kg), where aspirin did this from 0.60±.002 to 0.42±0.01 at 200mg/kg. The motility rates of castor oil and chloroform extract were 53.24%& 31.78% at 10 ml/kg and 400 mg/kg respectively, where the control showed 63.68% motility rate. These results show that, chloroform extract of fruit of *P. fascicularis L*. has significant analgesic, anti-pyretic, cytotoxic, anti-inflammatory and GI motility effects that increase with the increase of concentration. It can be assumed that, phytochemicals present, are the source of potency of the chloroform extract.

Keyword: *P. fascicularis L*., pyrexia, Oedema, GI motility, LC50.

*Correspondence to:
Sonia Ferdousy, Lecturer, Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka-1230, Bangladesh.
sonia.ferdousy@gmail.com

Cite This Article: Hossain, M.S., Akhter, S., Nath, B.D., Khanom, M.M., Kobir, M.E., Khanom, A., Islam, M.T., Yasmin, M.N., Islam, M.R., Bayen, S., Ferdousy, S. 2020. Chemical group characterization and determination of pharmacological activities of Pandanus fascicularis L. fruit. *Discovery Phytomedicine* 7(3): 128-137. DOI: 10.15562/phytomedicine.2020.137

INTRODUCTION

Medicinal plants have always been considered a healthy source of life for all people. These plants are very useful in healing various diseases because of their therapeutic properties and the advantage of these medicinal plants is being 100% natural. Since ancient times, herbal preparations have been used for the treatment of several diseases. Herbal products are often perceived as safe because they are "natural".[1] World Health Organization (WHO) directed medicinal plants as an accessible, affordable and culturally appropriate source of primary health care for more than 80% of Asia's population. Not only Asia, but also the whole world is used to depend on natural sources for health related problems. Because of their possible medicinal value a large number of plants are continuatively being screened.[2] Botanical medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes.[3] Even among prescription drugs, minimum 25% contain at least one compound derived from plants. The percentage might be higher if we include over-the-counter (OTC) drugs.[4] In developing countries, about 75% of the world populations rely on traditional medicine for their primary health care.[5] Herbal medicines are gaining interest because of their cost effective and eco-friendly attributes.[6]

*P. fascicularis L.*is a coastal plant which belongs to the family of Pandanaceae (screw pine family). In Bangladesh it is known as Keora, Keya and Ketaki. Although pandanus trees grow almost everywhere in tropical Asia, kewra water is still mainly a Northern Indian flavouring that is not used anywhere else. Indian emigrants, however, have taken their likening for this flavour with them, and have transported pandanus trees to other tropical areas. In Western cooking, kewra water makes a fine alternative to the flower essences already in use, like rose or orange.The ripe fruits of *P. fascicularis* have concern with their scent to an essential oil

[1]Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka-1230, Bangladesh.
[2]Department of Pharmacy, Varendra University, Rajshahi-6204, Bangladesh..
[3]Department of Physiotherapy, Centre for the Rehabilitation of the Paralysed (CRP), Dhaka-1206, Bangladesh
[4]Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh.

dominated by esters: Besides geranyl acetate, a couple of hemiterpenoid esters were found: isopentenyl (3-methylbut-3-enyl) and, to a lesser degree, dimethyl allyl (3-methylbut-2-enyl) acetates and cinnamates.[7]

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[8] By acting in the CNS or on the peripheral pain mechanism, analgesic compounds selectively relieves pain without significant alteration of consciousness. Actually analgesics are applied when the noxious stimulus cannot be removed or as adjuvant to more etiological approach to pain.[9]

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature.[10] Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness, and inability to concentrate. This increase in set point triggers increased muscle tone and shivering. However antipyretic medication can be effective at lowering the temperature which may include the affected person's comfort.[11] Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature. Drugs like paracetamol do not influence body temperature when elevated by factors such as exercise or increase in ambient temperature.[12]

One of the most important pathological disorder is inflammation. It is a part of non-specific immune response that occurs in reaction to any type of bodily injury, is a complex biological response of vascular tissues to harmful stimuli.[13] It involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair. Non-steroidal anti-inflammatory drugs (NSAID) are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders. However, their use is associated with adverse effects at the level of digestive tract, ranging from dyspeptic symptoms, gastrointestinal erosions and peptic ulcers to more serious complications, such as over bleeding or perforation.[14] Development of new anti-inflammatory drugs is still necessary and the natural product such as medicinal plants could lead in discovering new anti-inflammatory drugs with less undesirable effects.[15]

Diarrhoeal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year.[16] It can be defined as the increased frequency of bowel movements accompanied by a loose consistency of stools.[17] Modern estimate suggests that approximately 2.5 billion cases of diarrhoea occur every year which results in 1.5 million deaths among children under the age five. Diarrhoea still remains the second leading cause of infant mortality.[18] Diarrhoea results from hyper peristalsis of the small intestine or colon. Large amounts of Na+ and K+ and water are washed out of the colon and small intestine in diarrhoeal stools, causing dehydration, hypovolemia and eventually shock and cardiovascular collapse. The conditions of diarrhoea are particularly dangerous in infants and young children because of the rapidity with which serious dehydration occur.[18,19]

Cancer or tumor is the most common cause of death in both developed and developing countries. There are many methods are available to describe how cancer spread throughout the body. One method showed cancer is preliminary effect on specific part of our body and then invade to the other parts of our body very quickly and ultimatelycauses death of the patient.[19] So it is very necessary to identify or diagnosis of cancer at early stage otherwise if it is spread other part of the body then difficult to treat. However, there are several approaches of cancer treatments are available including surgery, radiation therapy and chemotherapy. All of these approaches are aimed to destroy cancerous cell from the body. Each approach possesses several side effect.[20] That is why it is now demand of the present era to discover drug with fewer side effect. We know that plant is always the safer source for treating any kind of disease. By considering this universal truth our present study was undertaken to discover drug from natural source with fewer side effect for treating different types of cancer. *Pandanus fascicularis L.* was selected due to its availability in Bangladesh. The present study was cherished to evaluate possible in-vivo analgesic, anti-pyretic, anti-inflammatory, gastro-intestinal motility and cytotoxic activities of chloroform extract of *P. fascicularis L.* (fruits).

MATERIALS AND METHODS

Chemicals and reference drugs

All the chemicals and used in this study were of analytical grade, and purchased from Sigma Chemical Co. (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

Collection of plant materials

The fresh fruits of *P. fascicularis L.* were collected from market.

Plant extracts preparation and isolation

The fruits of *P. fascicularis L.* were thoroughly washed with water after collection.Mechanical graded aluminum foil was used to air-dry the fruit-sand finally kept at room temperature for 14 days.[21] Then the collected plant materials were chopped and powdered. About 500g of the powdered materials of the plant were soaked in 1.5 liters of chloroform at room temperature for two weeks. The sample mixture were shacked and stirred at regular interval during this time. Then the solution was filtered using filter cloth and Whatman's filter paper and concentrated with a rotary evaporator. It rendered a brown granulars which was then designated as crude chloroform extracts for further study.

Test animals

For the evaluation of in-vivo analgesic, anti-pyretic, anti-inflammatory, gastro-intestinal motility and cytotoxic activities of chloroform extract of *P. fascicularis L.* (fruits), young Swiss-albino mice (aged 20–25 days) of either sex, average weight 20–25 g were used. They were collected from the Animal Resources Branch of ICDDR, B (International Centre for Diarrheal Disease and Research, Bangladesh). After collection, they were kept in favorable condition (temperature: 25.0±1.0°C, relative humidity: 55-65% and 12 h light/ dark cycle) for one week for adaptation and fed rodent food and water *ad libitum* formulated by ICDDR, B. The experiment was carried out according to the protocol approved by the Animal Ethics Committee of NSTU Research Cell, Noakhali Science and Technology University, and the internationally recognized principles was followed for laboratory animal use and care.

Phytochemical screening

Small quantity of freshly prepared chloroform extracts of *P. fascicularis L.* (fruits) were subjected to investigatequantitativepreliminary phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, gums and mucilages, fats & fixed oils using the following standard methods given by Roopashree et al., 2008.[22]

Analgesic activity

The crude chloroform extract of *P. fascicularis L.* (fruits) was used to evaluate analgesic potential by using the model of acetic acid induced writhing in mice.[23] Experimental animals (n=16) were randomly selected and divided into four groups denoted as group I, group II, group III, group IV. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Each group was designed to receive the followingtreatment such as; control, positive control (standard Diclofenac Na) and two doses (200 and 400 mg/kg-body weight) of the extract solution respectively. Positive control group was treated with the dose of 25 mg/kg-body weight and control group was administered with 1% Tween 80 in water at the dose of 15 ml/kg-body weight. Control, standard drug and test samples were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, writhing of the mice were observed for 5 min.

Anti-pyretic activity

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in experimental animal.[24] Hyperpyrexia was induced by subcutaneous administration of 20% aqueous suspension of brewer's yeast at the dose of 10 ml/kg body weight. The test animals were fasted overnight with water *ad libitum* before the experiments. By using an Ellab thermometer (33.19 ± 0.40°C) the initial rectal temperature of test animals were recorded. After 18 h of subcutaneous injection, the animals that showed an increase of 0.3–0.5°C in rectal temperature were selected for the antipyretic activity. Crude chloroform extracts of plant were given orally at the dose of 200 mg/kg and 400mg/kg respectively. Paracetamol (150 mg/kg orally) was used as reference drug whereas; distilled water (10 ml/kg) was treated as control. The rectal temperature was recorded for 4 times at 1 h intervals after treatment.

Anti-inflammatory activity

The rat paw oedema method of Winter et al was used. Garden egg extract (100, 200 and 400 mg/kg, i.p.) or indomethacin (10 mg/kg, i.p.) suspended in normal saline were administered to the rats. Control group received normal saline (1 mL/kg, i.p.). One hour post treatment, inflammation of the hind paw was induced by injecting 0.1 mL of undiluted fresh egg albumin into the subplantar surface ofthe right hind paw of rats. The right hind paw volumes ofthe rats were taken on the principle of volume displacement using LETICA Digital Plethysmometer (LE 7500) immediately before the experiment (zero time) and at 1hr intervals after the injection of egg albumin for a period of 5 h. The average oedema at every interval was assessed in terms of difference in volume displacement after injecting the egg albumin and zero time volume displacement of the injected paw (Vt -V0). Percent

inhibition of oedema was also calculated for each treated group.

GI motility activity

Castor Oil-Induced Diarrhea in Rats

Rats of both sexes (95–100 g) were fasted for 18 hours.The selected rats for castor oil-induced diarrheal test were divided into four groups (n = 5). Group I was given normal saline (2 mL/kg) orally as control group and Group II received loperamide (5 mg/kg) as standard group. Groups III-IV received MEMA (200 and 400 mg/kg b. wt. i.p., resp.). After 1 h, all groups received castor oil 1 mL each orally. Then they were placed in cages lined with adsorbent papers and observed for 4 h for the presence of characteristic diarrheal droppings. 100% was considered as the total number of feces of control group.[25,26]

The activity was expressed as% inhibition of diarrhea. The percent (%) inhibition of defecation was measured using the following formula:

$$\text{Percent (\%) inhibition of defecation} = [(A-B)/A] \times 100$$

Where A is mean number of defecation time caused by castor oil and B is mean number of defecation time caused by drug or extract.

Castor Oil-Induced Enter pooling

Castor oil-induced enter pooling test helps to determine the prevention of fluid accumulation ability of extract. Here also rats of both sexes (95–100 g) were fasted for 18 hours. The selected rats for this test were divided into four groups (n=5). Group I (controlled group) was given normal saline (2 mL/kg) orally while Group II (standard group) received loperamide (5 mg/kg). The rest of the groups (Groups III-IV) received MEMA (200 and 400 mg/kg b. wt. i.p. resp.). After 1 h, all groups received castor oil, 1 mL orally per animal. Two hours later, all rats were sacrificed and the small intestine from the pylorus to the caecum was isolated. The intestinal contents were collected by milking into a graduated tube and their volume was measured.[26]

Gastrointestinal Motility Test

This test was done according to the method of Mascolo et al. and Rahman et al. For this test, selected rats were divided into four groups of five rats in each. At first, 1 mL castor oil was given orally in every rat of each group to produce diarrhea. After 1 h, Group I (control group) received saline (2 mL/kg) orally. Group II received standard drug (loperamide 5 mg/kg b. wt. i.p) and Groups III-IV (the rest of the two groups) received MEMA (200 and 400 mg/kg b. wt. i.p. resp.). After 1 h, all animals received 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour after following the charcoal meal administration, all animals were sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as percentage of distance moved.[27]

Brine shrimp lethality bioassay

The measurement of toxicity plays a vital role in drug discovery and is a useful tool in biological, especially ecological investigations. It also serves as a tool for screening plant extracts of possible medicinal value. In this study, we used simple brine shrimp bioassay test of Meyer with slight modification by using *Artimiasalina*as test organism, which was collected from a pet shop.[28]

Brine shrimp hatching

Sea water was prepared by dissolving 38 g sea salt (pure NaCl) in one liter of distilled water, which is then filtered to get clear solution of 3.8% concentration.[29] In a suitable plastic or glass vessel sea water was taken and shrimp eggs were added to one side of the vessel and allowed to hatch for 24 h till the mature nauplii were found. Continuous oxygen and light supply were provided to support the hatching process.

Sample preparation

All the test samples were taken in vials and dissolved in 100 µl of pure dimethyl sulfoxide (DMSO) to get stock solutions. Then 50 µlof solution was taken in the first test tube containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In every case, 50 µl samples were added to test tube and fresh 50 µl DMSO was added to vial.

Negative control group test

100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as negative control groups.

Positive control group test

Here we used vincristine sulphate (VINCRIRST®, Techno Drugs Ltd., Bangladesh) as a positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 40 µg/ml from which serial dilutions were made using DMSO to get 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 and 0.078125 µg/ml respectively. Then the positive control solutions were added to

the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.[30]

Counting of nouplii

After 24 h, the number of survived nauplii in each vial was counted by using magniflying glass. From this data the percent (%) of mortality of brine shrimp nauplii was calculated for each concentration.

Statistical analysis

All the above assays were conducted in triplicate and repeated threes for consistency of results and statistical purpose. The data were expressed as Mean ± SD and analyzed by using SPSS software of 10 version.

RESULTS

Phytochemical nature

Phytochemical analysis of chloroform extract fruits of *P. fascicularis* revealed the presence of alkaloids, steroids, terpenoids, flavonoids, saponis, proteins and tannins (Table 1).

Analgesic activity

The number of writings of all test animals and mean cumulative total of writings in the intervention groups are presented in Table 2. From Table 1 it may be seen that there was a reduction in the cumulative total of writings in the chloroform extract groups and the Diclofenac sodium group, in comparison with the negative controls receiving 1% tween 80. This indicates that Diclofenac and *P. fascicularis* chloroform extract at the two dosages were capable of reducing writhing in mice. The dose of 400mg/kg is significantly capable of inhibition (%) of writhes at 62.96% comparison with standard Diclofenac sodium (150mg/kg b.w) at 67.74%.That means Chloroform extract of P. *fascicularis* (fruit) at 400mg/kg dose shows significant analgesic activity.

Table 1 **Result of phytochemical screening of different fractions of fruits of *P.fascicularis***

Bioactive constituents	Chloroform extract
Alkaloids	+
Steroids	+
Terpenoids	+
Phenols	-
Glycosides	-
Carbohydrates	-
Proteins	+
Flavanoids	+
Saponins	+
Tannins	+

Table 2 **Effect of chloroform extract of *P. fascicularis* (fruit) on acetic acid induced writhing in mice**

Sample/Drugs	Dose (mg/kg)	Writhing count (Mean ± S.E.M.) (15 min)	% of Inhibition
Control	10ml/kg	54±4.848	0
Diclofenac sodium	150mg/kg	17.6±3.701	67.74
Chloroform extract	200mg/kg	33±2	37.03
Chloroform extract	400mg/kg	18.66±4.04	62.96

Antipyretic activity

Chloroform extract produced significant antipyretic activity ($p < 0.05$). In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus. Therefore, the antipyretic activity of Chloroform extract of *P. fascicularis is* probably by inhibition of prostaglandin synthesis in hypothalamus. Further, Chloroform extract was found to contain alkaloids, steroids, terpenoids, flavonoids, and tannins through preliminary photochemical screening. The antipyretic activity may be due to one/more group of above phyto-constituents. Extract reduced the hyperthermia at both 200 and 400 mg/kg doses 1 hr after administration. The initial and final rectal temperatures in the groups treated with Chloroform extract (400 mg/kg) and Acetyl salicylic acid (100mg/kg) were 38.7±0.45 and 37.83±0.37; and 38.4±0.11and 37.5±0.01 respectively which are represented in table 3.

Anti-inflammatory activity

Chloroform extract produced significant anti-inflammatory activity ($p < 0.05$). In general, non-steroidal anti-inflammatory drugs produce their anti-inflammatory action through the inhibition of prostaglandin synthetase within the hypothalamus. Therefore, the anti-inflammatory activity of Chloroform extract of *P. fascicularis* is probably by inhibition of prostaglandin synthesis in hypothalamus.

Further, Chloroform extract was found to contain alkaloids, terpenoids, proteins, flavonoids and tannins through preliminary photochemical screening. The anti-inflammatory activity may be due to one/more group of above Phytoconstituents. Extract reduced the paw oedema at both 200 and 400 mg/kg doses 1 hr after administration. The initial and final edema are 0.62±0.06 and 0.45±0.02 for Chloroform extract (400 mg/kg) and 0.60±.002 and 0.42±0 for Aspirin (100 mg/kg) treatment. Both Aspirin and Chloroform extract showed significant

Table 3 **Effect of the chloroform extract (fruit) of *P. fascicularis* on yeast induced pyrexia in rat**

Sample	Dose	Before yeast	After yeast (Time Interval (hr) after treatment)				
			0	1	2	3	4
Control	10ml/kg	37.6±0.38	38.3±0.34	38.5±0.87	38.6±0.73	38.7±0.75	38.7±0.81
ASA	100mg/kg	37.4±0.01	38.4±0.11	38.2±0.07	38.1±0.05	37.8±0.03	37.5±0.01
Chloroform extract	200mg/kg	37.9±0.36	38.8±0.44	38.9±0.53	37.9±0.4	37.8±0.36	38.13±0.1
	400mg/kg	37.53±0.057	38.7±0.45	38.36±0.85	38.03±0.7	37.83±0.40	37.83±0.4

Table 4 **Effect of chloroform extract of *P. fascicularis* (fruit) on egg-albumin induced edema in mice**

Sample/Drug	Dose (mg/kg)	Before egg Albumin (cm)	After egg- Albumin(cm) (Time intervals (hr) after treatment)					
			0	1	2	3	4	5
Control		0.39±0.15	0.62±0.07	0.64±0.11	0.67±0.02	0.68±0.05	0.65±0.03	0.63±0.001
Aspirin	200	0.40±.01	0.60±.002	0.52±0.04	0.50±0.003	0.45±0.001	0.44±0.01	0.42±0.01
Chloroform extract	200	0.38±0.026	0.6±0.05	0.52±0.01	0.50±0.005	0.50±0	0.49±0.01	0.48±0.01
	400	0.39±0.01	0.62±0.06	0.56±0.03	0.52±0	0.50±0.01	0.48±0.02	0.45±0.02

Table 5 **GIT motility activity of chloroform extract of fruits of *P. fascicularis***

Treatment	Dose	Total length of intestine	Distance covered by charcoal	% Motility
Control	10ml/kg	51.88±5.23	18.77±5.35	63.68
Castor oil	10ml/kg	50.25±7.27	23.5±3.31	53.24
Chloroform extract	200 mg/kg	50.75±2.98	32.5±4.50	35.03
	400 mg/kg	53.5±9.03	36.5±6.02	31.78

Table 6 **Effect of chloroform extract (fruit) of *P. fascicularis* on brine shrimp nauplii**

Conc. (C) (µg/ml)	Log C	No. of alive nauplii added	No. of dead nauplii	% Mortality	LD_{50} (mg/ml)
1	0	10	4	40	1.0636
5	0.6989	10	7.5	75	
10	1	10	8.5	85	
20	1.301	10	10	100	
50	1.6989	10	10	100	
100	2	10	10	100	

Table 7 **Effect of vincristine sulphate on brine shrimp nauplii**

Conc (C) (mg/ml)	Log C	No. of alive nauplii added	No. of dead nauplii	% Mortality	LD_{50} (mg/ml)
1	0	10	6	60	0.2
5	0.6989	10	8	80	
10	1	10	9	90	
20	1.301	10	9	90	
50	1.6989	10	10	100	
100	2	10	10	100	

anti-inflammatory activity throughout the test period of 5 hr which are imparted in Table 4.

GI motility

The activity of GI motility of chloroform extract of *P. fascicularis* are tabulated in table 5. The results were articulated as mean ± SEM of six animals. Effects were considered to be significant at the P< 0.05 level. From the table 5, it is seen that Chloroform extract exert GI motility activity.The both dose 200mg/kg & 400mg/kg decrease motility (%) rate of mice comparison with castor oil. The Total length of intestine and distance covered by charcoal are 50.25±7.27 & 23.5±3.31 for castor oil at a dose of 10ml/kg and 53.5±9.03 & 36.5±6.02 for chloroform extract at a dose of 400mg/kg.The motility rate of castor oil and chloroform extract are 53.24 & 31.78% respectively.

Cytotoxic activity

By using brine shrimp bioassay, developed by Meyer we could understand the cytotoxic potential and anti-tumor properties. In our current study we used chloroform soluble extracts of *P. fascicularis*. Plotting of log of concentration versus percent mortality for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC50, the concentration at which 50% mortality of brine shrimp nauplii occurred) was determined for the samples. The positive control groups showed nonlinear mortality rates at lower concentrations and linear rates at higher concentrations. There was no mortality in the negative control groups indicating the test as a valid one and the results obtained are only due

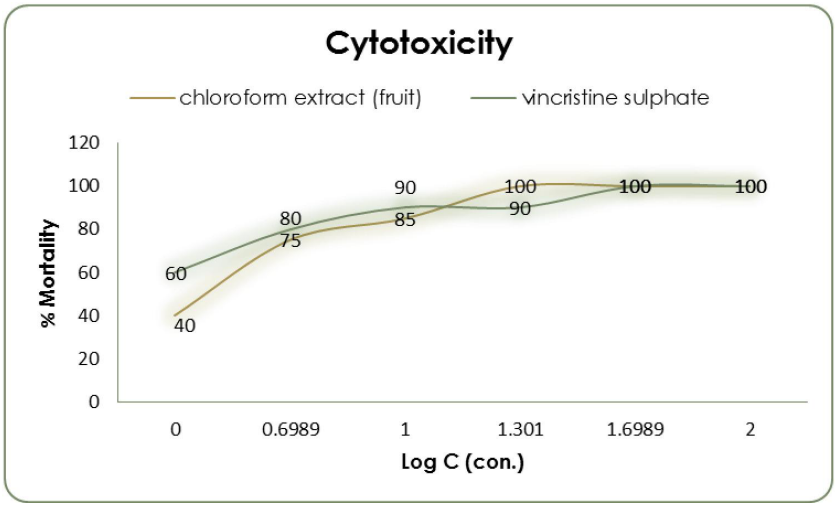

Figure 1 Comparative presentation of cytotoxic activity of chloroform soluble extracts of *P. fascicularis* with standard vincristine sulphate

to the activity of the test agents. The Brine Shrimp Lethality test represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties.[29] Following the procedure of Meyer (Meyer et al., 1982) the lethality of the Dimethyl-Sulphoxide (DMSO) soluble Chloroform extract was determined and the summary of the results is expressed in Table 6.

The LC50 values of Chloroform extract for fruit of the plant was found 1.0636 µg/ml. The positive control vincristine sulphate showed LC50 at a concentration of 0.200µg/ml (Table 7). When this result is compared with data obtained for Chloroform extract of *P. fascicularis* was found almost identical and the value is represented at Figure 1.

DISCUSSION

Results of the present study showed that the chloroform extract of *P. fascicularis* L. has considered the level of antipyretic, analgesic, anti-inflammatory, GIT motility and cytotoxic effects with a marked safety profile. When Brewer's yeast injected subcutaneously induced pyrexia by ameliorating the synthesis of prostaglandin thus anuseful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect.[31] This Pyrexia induced through Yeast is called pathogenic fever.[32] Thus, depletion of prostaglandin synthesis could be the possible mechanism of antipyretic action as that paracetamol do by blocking the cyclooxygenase enzyme activity.[33] The intraperitoneal administration of chloroform extract of *P. fascicularis* L. significantly reduced rectal temperature of yeast induced febrile mice. Thus it can be assumed that chloroform extract of *P. fascicularis* L. contained pharmacologically active compounds that interfere with the release of prostaglandins[34] and the antipyretic action of the n-hexane fraction of *Viola odorata*[35] supplement the antipyretic activity of our tested extract. Acetic acid-induced writhing is a well recommendedprotocol in evaluating medicinal agents for their analgesicproperty. Some endogenous pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis causes induction of pain.[36] This pain paradigm is widely used forthe assessment of peripheral analgesic activity due to itssensitivity and response to the compounds at a dose whichis not effective in other methods. The probable cause of abdominal writhing is local peritoneal receptor.[37] In acetic acid induced writhing paradigm painsensation is determinedby producing localized inflammatory response due toproducing prostaglandinspecifically PGE2 and PGF2α with increasing the level of lipoxygenase products may also in peritoneal fluids.[37] These lipoxygenase and prostaglandin products cause inflammationand pain by increasing capillary permeability. Thus substance with analgesic effect causesinhibitionof writhing's preferably by inhibition of prostaglandin synthesis.[36] Regarding the effects of our extract in acetic acid-induced abdominal constrictionassay, a prominent inhibition of writhing refluxwas observed. These findings strongly recommend that chloroform extract of *P. fascicularis L.* has peripheral analgesic activity and their mechanismsof action may be mediated through inhibition of localperitoneal receptors. The profound analgesicactivity of chloroform extract of *P. fascicularis* L. may be due to the interference of their activeprinciple(s) with the release of pain mediators.The present study also examined the anti-inflammatory activity of the chloroform extract of *P. fascicularis* L. The plant extract showed good anti-inflammatory activity through suppressing the egg albumin induced rat paw oedema both at the early and later phases of oedema. The results obtained was comparable to thatobtained for standard aspirin. Several inflammatory mediators such as histamine, serotonin and bradykinin at the site of a local inflammatory insult leading to oedema through increasing vascular permeability and blood flow.[37] Egg albumin induced oedema formation in the rat paw is a biphasic event. In the primary phase probable release of histamine and serotonin induced immediate oedema after the administration of the irritant and lasting up to 2 h is, while at later phase, oedema formed after 3 to 5 h of administration of the irritant may be due to the release of bradykinin, protease, prostaglandins and lysosome.[38] Oedema produced into the rat paw after subcutaneousinjection of egg albumin which is resulting from plasma extravasation, while

increasing tissuewater and plasma protein exudation along with neutrophilextravasation.[39] In the first phase reduction of oedemogenesis evinced by both the garden egg extract and the gardenegg incorporated diet in this study replicated that the plantis very effective in reducing the release or action of the earlyphase mediators. Suppression of oedema in the second phase of inflammation suggests that the anti-inflammatoryactivity of chloroform extract of *P. fascicularis* L. may also be due to the suppressionof egg albumin induced prostaglandins and kinnin formation within this period. Thus continuous intakeof the plant could confer protection against inflammatory oedema and thus could be useful in the treatment andmanagement of inflammatory conditions. Report by Mutalik et al support our present finding very amorously.[40]

For evaluating cytotoxic activity of our present plant extract this method was chosen because this is easiest to conduct than any other methods and also said that the cytotoxic compounds generally exhibit significant activity in the BSLB; for all of this aspect this method become one of the recommended guideline for the detection of antitumour compounds and pesticides due to its low cost.[41] This bioassay also found to exhibit positive relationship with the human solid tumour cell lines. It is now become a proverb that if correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts have been established than cytotoxic effects of the plant extracts will enunciate for further cell line assay.[42] Not only Bangladesh but also the country of the third world this methods got the main consideration for conducting cytotoxic activity of plant extract. BSLB also hold the basement while conducting our present study to enunciate the cytotoxic activity of chloroform extract of *P. fascicularis* L. Present study data showed various rate of mortality at different concentration as the report of Anderson et al.[42,43] though there was no rate of mortality obtained. We want to conclude here with a massage that significant lethality (as LC50 value less than 100 ppm or µg/ml) of the plant extract to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigations.

Several studies have established the medicinal use of plants extract by investigating the biological activity of extracts of plants extract with antispasmodic effects which delay intestinal transit, stimulate water adsorption, suppress gut motility, and reduce the intraluminal fluid accumulation.[43] The present study was also accused to validate antidiarrheal efficacy chloroform extract of *P. fascicularis L.* in the experimental models of mice with diarrhea. In the current study the chloroform extract of *P. fascicularis* L. have ressembled antidiarrheal activity in experimental animal models. Despite the heterogenicity of etiologies of diarrhea, previous study data state four major pathophysiologies may responsible for clinical development of diarrhea. These include increased electrolytes secretion (secretory diarrhea), increased luminal osmolarity (osmotic diarrhea), decreased electrolytes absorption, and a decreased transit time via deranged intestinal motility.[44] Most of the antidiarrheal agents revealedantidiarrheal effects by reducing the gastrointestinal motility and/or the secretions,[45] while diarrhea causing agents like laxatives promote this two distinct of pathological development of diarrhea. During laboratory study castor oilused as an inducer of diarrhea; because of its well established laxative effects. Castor oil is induce diarrhea through changing electrolyte permeability of the intestinal membrane and through elevated prostaglandin biosynthesis and release.[46,47] Different researches have shown that castor oil induce diarrhea within 1–2 h just after administration of 0.1-0.3 ml on experimental animal model.[48] In our present study diarrhea was occurred within 1 h because of the high dose ofcastor oil (0.5 ml/mice). Mice's that showed the diarrheal response were only subjected for the evaluation of anti-diarrheal activity of chloroform extract of *P. fascicularis* L. The plant extract demonstrated significant reductions of frequency of defecation, water contents, and intestinal fluid accumulation. On the other hand, findings from gastrointestinal motility test indicated its poor antimotility effect. The overall findings show that experimental plant extract demonstrated a considerable antidiarrheal activity.

CONCLUSION

In conclusion, the chloroform extract of *P. fascicularis* L was proved a natural safe remedy for the treatment of pyrexia, analgesia, diarrhea and inflammation. Our recent findings supported scientific rationale for the folk use of the plant as antidiarrheal, antipyretic, analgesic and anti-inflammatory and anticancer agent. Nevertheless, present study also demands the isolation of pure secondary metabolites from the plant will help us further in narrating the biological pathways of these activities and identification of major responsible compounds of clinical utility.

COMPETING INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Pharmacy, Atish Dipankar University of Science and Technology Bangladesh for their support throughout the project work.

REFERENCES

1. Raju GS, Moghal MMR, Dewan SMR, Amin MN, Billah MM. Characterization of phytoconstituents and evaluation of total phenolic content, anthelmintic, and antimicrobial activities of Solanum violaceum Ortega. Avicenna Journal of Phytomedicine. 2013, 3(4): 313-320.
2. Baul S, Amin MN, Hussain MS, Mukul MEH, Millat MS, Rashed MSU et al. Phytochemical Nature and Pharmacological Evaluation of Chloroform Extract of Pandanus fascicularis L. (Fruits) An in vivo Study. Journal of Bioanalysis & Biomedicine 2017, 9(4): 223-228.
3. Dewan SMR, Amin MN, Adnan T, Uddin SMN, ShahidUd-Daula AFM, Sarwar G et al. Investigation of analgesic potential and in vitro antioxidant activity of two plants of Asteraceae family growing in Bangladesh. Journal of Pharmacy Research. 2013, 6(6): 599-603.
4. Uddin SMN, Amin MN, Shahid-Ud-Daula AFM, Hossain H, Haque MM, Rahman MS. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of Stephania japonica Linn. Journal of Medicinal Plant Resrearch, 2014; 8(37): 1127-1133.
5. Amin MN, Dewan SMR, Noor W, Shahid-Ud-Daula AFM. Characterization of chemical groups and determination of total phenolic content and in-vitro antioxidant Activities of ethanolic extract of Ocimum sanctum leaves growing in Bangladesh. European Journal of Experimental Biology, 2013; 3(1): 449-454.
6. Amin MN, Banik S, Ibrahim M, Moghal MMR, Majumder MS, Siddika R. A Study on Ardisia solanacea for Evaluation of Phytochemical and Pharmacological Properties. International Journal of Pharmacognosy and Phytochemical Research 2015; 7(1); 8-15.
7. Tanna MTH, Amin MN, Ibrahim M, Mukul MEH, Kabir A. Evaluation of antioxidants, membrane stabilizing, cytotoxic and anthelmintic activity with phytochemical screening of Chromolaena odorata: A medicinal shrub. International Journal of Pharmacy, 2016, 6(1): 53-61.
8. Rahaman M.Z., Akhter S., Islam MR., Begum S., Mondal KK., Mottakin M., et al. Assessment of thrombolytic, antioxidant and analgesic properties of a medicinal plant of Asteraceae family growing in Bangladesh. Discovery Phytomedicine, 2020, 7(1): 47-52.
9. Meyer BN., Ferrigni NR., Putnam JE., Jacobsen LB., Nichols DJ, & McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta medica, 1982; 45(05): 31-34.
10. Sikder MAA, Kaisar MA, Rashid MA., Millat MS, & Sultana A. In vitro membrane stabilizing activity, total phenolic content, cytotoxic, thrombolytic and antimicrobial activities of Calliandrasurinamensis (Wall.). J. Pharmacog. Phytochem, 2012, *1*(3): 45-50.
11. Duraisankar M. and Ravichandran V. Antipyretic Potential of Polyherbal Ayurvedic Products. Asian Journal Pharmaceutical and Clinical Research, 2012, 5 (2):146 – 150.
12. Gomathi P, Mazumder UK, Gupta M. Antipyretic potential of *Galega pupurea* root,.Intl. Research Journal of Pharmacy, 2011; 2(11): 151-152.
13. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanumaethiopicum*). DARU J Pharm Sci. 2012; 20:76.
14. Hossain H, Shahid-Ud-Daula AFM, Hasan K, Mansur AA, Haq MM. Anti-inflammatory activity, total flavonoids and tannins content from theethanolic extract of *Spilanthes paniculata* leaf growing in Bangladesh. Int J Pharm 2012, 2:271–277.
15. Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease: where are we now? J Lab Clin Med 1992, 119:598–620.
16. Baul S, Amin MN, Hussain MS, Mukul MEH, Millat MS, Rashed MSU et al. Phytochemical Nature and Pharmacological Evaluation of Chloroform Extract of Pandanus fascicularis L. (Fruits) An in vivo Study. Journal of Bioanalysis & Biomedicine 2017, 9(4): 223-228.
17. Dewan SMR, Amin MN, Adnan T, Uddin SMN, ShahidUd-Daula AFM, Sarwar G et al. Investigation of analgesic potential and in vitro antioxidant activity of two plants of Asteraceae family growing in Bangladesh. Journal of Pharmacy Research. 2013, 6(6): 599-603.
18. Uddin SMN, Amin MN, Shahid-Ud-Daula AFM, Hossain H, Haque MM, Rahman MS. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of Stephania japonica Linn. Journal of Medicinal Plant Resrearch, 2014; 8(37): 1127-1133.
19. Amin MN, Dewan SMR, Noor W, Shahid-Ud-Daula AFM. Characterization of chemical groups and determination of total phenolic content and in-vitro antioxidant Activities of ethanolic extract of Ocimum sanctum leaves growing in Bangladesh. European Journal of Experimental Biology, 2013; 3(1): 449-454.
20. Amin MN, Banik S, Ibrahim M, Moghal MMR, Majumder MS, Siddika R. A Study on Ardisia solanacea for Evaluation of Phytochemical and Pharmacological Properties. International Journal of Pharmacognosy and Phytochemical Research 2015; 7(1); 8-15.
21. Atata RF, Sani A, Ajewole SM. Effect of Stem Bark Extracts of *Enantiachloranta* on some clinical isolates. Niger. Soc. Exp. Biol. 2003; 15(2):84-92.
22. Tanna MTH, Amin MN, Ibrahim M, Mukul MEH, Kabir A. Evaluation of antioxidants, membrane stabilizing, cytotoxic and anthelmintic activity with phytochemical screening of Chromolaena odorata: A medicinal shrub. International Journal of Pharmacy, 2016, 6(1): 53-61.
23. Ahmed F, Selim MST, Das AK, Choudhuri MSK. Antiinflammatory and antinociceptive activities of *Lippianodiflora* Linn. Pharmazie. 2004; 59:329e333.
24. Raju GS, Moghal MMR, Dewan SMR, Amin MN, Billah MM. Characterization of phytoconstituents and evaluation of total phenolic content, anthelmintic, and antimicrobial activities of Solanum violaceum Ortega. Avicenna Journal of Phytomedicine. 2013, 3(4): 313-320.
25. Lanhers MC, Fleurentin J, Dorfman P, Motrier F, Pelt JM. Analgesic, antipyretic and anti- inflammatory properteis of *Euphorbia hirta*. Planta Medica 1991, 57:225–231.
26. Baul S, Amin MN, Hussain MS, Mukul MEH, Millat MS, Rashed MSU et al. Phytochemical Nature and Pharmacological Evaluation of Chloroform Extract of Pandanus fascicularis L. (Fruits) An in vivo Study. Journal of Bioanalysis & Biomedicine 2017, 9(4): 223-228.
27. Dewan SMR, Amin MN, Adnan T, Uddin SMN, ShahidUd-Daula AFM, Sarwar G et al. Investigation of analgesic potential and in vitro antioxidant activity of two plants of Asteraceae family growing in Bangladesh. Journal of Pharmacy Research. 2013, 6(6): 599-603.
28. Lanhers MC, Fleurentin J, Dorfman P, Motrier F, Pelt JM. Analgesic, antipyretic and anti- inflammatory properteis of *Euphorbia hirta*. Planta Medica 1991, 57:225–231.
29. 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; 45:31-34.
30. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). Int. J. Appl. Sci. Eng. 2006; 4(2):115-125.

31. Devi BP, Boominathan R, Mandal SC. Evaluation of antipyretic potential of *Cleome viscosa* Linn. (Capparidaceae) extract in rats. J Ethnopharmacol, 2003, 87(1):11–13.
32. Dewan SMR, Amin MN, Adnan T, Uddin SMN, ShahidUd-Daula AFM, Sarwar G et al. Investigation of analgesic potential and in vitro antioxidant activity of two plants of Asteraceae family growing in Bangladesh. Journal of Pharmacy Research. 2013, 6(6): 599-603.
33. Uddin SMN, Amin MN, Shahid-Ud-Daula AFM, Hossain H, Haque MM, Rahman MS. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of Stephania japonica Linn. Journal of Medicinal Plant Resrearch, 2014; 8(37): 1127-1133.
34. Toiu A, Vlase L, Oniga I, Tamas M. Quantitative analysis of some phenolic compounds from viola species tinctures. Farma J 2008, 56(4):440–445.
35. Khattak SG, Gilani SN, Ikram M. Antipyretic studies on some indigenous Pakistani medicinal plants. J Ethnopharmacol 1985, 14(1):45–51.
36. Duarte I, Nakamura M, Ferreira S. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med and Bio Res 1988, 21(2):341.
37. Kang HS, Lee JY, Kim CJ. Anti-inflammatory activity ofarctigenin from *Forsythiae fructus*. J Ethnopharmacol 2008; 116:305-310.
38. Wallace JM. Nutritional and botanical modulation of the inflammatory cascade: eicosanoids, cyclooxygenase and lipoxygenase-as an adjunct in cancer therapy. Integr Cancer Ther2002; 1: 7-37.
39. Yankanchi SR, Koli SA. Anti-inflammatory and analgesic activityof mature leaves of methanol extract of *Cleroden druminerme* L.(Gaertn). J Pharm Sci Res 2010; 11(2): 782-785.
40. Mutalik S, Paridhavi K, Mallikarjuna C. Udupa N. Antipyreticand analgesic effect of leaves of *Solanumme longena* in rodents. Indian J Pharmacol, 2003; 35: 312-315.
41. 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: 31–34.
42. Manilal A, Sujith S, Seghal KG, Selvin J, Shakir C. Cytotoxic potentials of red alga, *laurencia brandenii* collected from the Indian coast. Global J Pharmacol 2009; 3: 90–94.
43. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor pre-screens. Phytochem Ana 1991; 2: 107–111.
44. Gutiérrez SP, Mendoz DZ, Munive AH, Martínez AM, González CP, Mendoza ES. Antidiarrheal activity of 19-deoxyicetexone isolated from Salvia ballotiflora Benth in mice and rats. Molecules 2013, 18:8895–8905.
45. Uddin SMN, Amin MN, Shahid-Ud-Daula AFM, Hossain H, Haque MM, Rahman MS. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of Stephania japonica Linn. Journal of Medicinal Plant Resrearch, 2014; 8(37): 1127-1133.
46. Agbor GA, Léopold T, Jeanne NY. The antidiarrhoeal activity of *Alchorneacordifolia* leaf extract. Phytother Res 2004, 18:873–876.
47. Besra SE, Gomes A, Chaudhury L, Vedasiromoni JR, Ganguly DK. Antidiarrheal activity of seed extract of *Albizzialebbek*Benth. Phytother Res, 2002, 16:529–533.
48. Brijesh S, Daswani P, Tetali P, Antia N, Birdi T. Studies on the antidiarrhoealactivity of *Aegle marmelos* unripe fruit: Validating its traditional usage. BMC Complement Altern Med 2009, 9(Suppl 47):1–12.