

Phytochemical group test and pharmacological investigations of *Persicaria barbata* (L.) H. Hara**Mohammed Shamiul Shahid¹, Mohammed Ibrahim¹, Md. Mizanur Rahaman², Tawhida Islam², Md. Shimul Bhuia², Shamia Zaman², Muhammad Torequl Islam^{2*}**¹Department of Pharmacy, Southern University Bangladesh, Chattogram 4210, Bangladesh²Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj 8100, Bangladesh**ABSTRACT**

Traditionally, various parts of *Persicaria barbata* (family: Polygonaceae) are used in many countries in eastern South Asia. This study evaluates the phytochemical groups and pharmacological activities of the aerial parts of *P. barbata*. For this, extraction was done with ethanol and fractioned with chloroform and n-hexane. Preliminary chemical groups, DPPH scavenging, egg albumin denaturation, and disc diffusion assays were performed for phytochemical, antioxidant, anti-inflammatory, and anti-bacterial activity screening, respectively. *In vivo* anti-diarrheal, analgesic, and anti-pyretic activity screening was done by applying the castor oil-induced diarrheal model, the hot plate test, and the yeast-induced pyrexia model, respectively. Additionally, open-field and swing test models were used to assess *in vivo* anxiolytic activity. The phytochemical study suggests that *P. barbata* possesses alkaloids, steroids, glycosides, flavonoids, tannins, gums, and saponins. The hydro-ethanol extract showed concentration-dependent DPPH radical scavenging and inhibition of protein denaturation capacity. It also showed moderate (zone of inhibition ranging from 9 to 16 mm) but broad-spectrum anti-bacterial effects against *L. casei*, *L. coryniformis*, *B. cereus*, *S. aureus*, *P. azotoformis*, *E. coli*, *P. aeruginosa*, and *K. pneumonia*. Moreover, all the extracts also showed significant anti-diarrheal, analgesic, anxiolytic, and antipyretic effects in Swiss albino mice. In conclusion, *P. barbata* might be a good source of phytotherapeutic agents. Further extensive investigations should be conducted to isolate and distinguish the active principles of this plant and to interpret the exact mechanism of each action.

Keywords: *Persicaria barbata*; organic extracts; phytochemicals; pharmacological effects*Corresponding author: dmt.islam@bsmrstu.edu.bd; mti031124@gmail.com (M. T. Islam)

1. INTRODUCTION

Researchers are trying to find new medications with enhanced or developed therapeutic potential [1]. Medicinal plants are a great source of lead compounds for producing advanced, newer medications with fewer side effects [2]. Since early antiquity, traditional medicines have been used consistently to treat various diseases and human suffering. Medicinal plants yield a variety of bioactive natural compounds, which are used as starting components for the development of novel drugs [3]. Many phytochemicals that are synthesized from plants have many therapeutic and pharmacological activities and are responsible for fighting various diseases, such as alkaloids, flavonoids, saponins, glycosides, steroids, carbohydrates, gums, phenolic compounds, tannins, essential oils, volatile oils, etc. [4-6]. The potential health advantages of natural compounds with antioxidant, antibacterial, analgesic, hypoglycemic, anti-cancer, and other properties have received a lot of attention in recent years. As a result, research on various medicinal plants has significantly increased in an effort to identify the active compounds behind such pharmacological effects.

Persicaria barbata (L.) H. Hara (Family: Polygonaceae) terrestrial or aquatic, perennial, erect herb, up to 200 cm long. The seeds are used to relieve colic discomfort, and the aerial portions have anti-nociceptive, anti-inflammatory, and diuretic functions. Roots are employed as a cooling and astringent treatment. The decoction of leaves and shoots is applied topically to ulcers as a stimulating wash that promotes the healing of scar tissue. Scabies is treated with an external application of root paste [7]. In an investigation, it was demonstrated that the ethanol extract of *P. barbata* showed radical scavenging activity with a maximum percentage inhibition of 97.62 at 100 µg/ml. The median half-inhibitory concentrations (IC₅₀s) calculated for the extract are 58.69 µg/ml. In a study, it was seen that alcoholic extracts of *P. barbata* at 100 mg/kg p.o. doses remarkably ($P < 0.01$) diminished the ulcer number, ulcer score, ulcer index, free acidic contents, and total acidic contents in pylorus ligation and ethanol-mediated gastric mucosal destruction ulcer models in rats [8]. *In vitro* phytochemical analysis of the leaf extract of *P. barbata* yields two flavanones and a saponin [9]. The ethanol extract of *P. barbata* contains alkaloids, steroids, glycosides, tannins, flavonoids, saponins, reducing sugars, and gums. The n-hexane extract of *P. barbata* contains glycosides, tannins, flavonoids, saponins, and gums. The chloroform extract of *P. barbata* contains alkaloids, glycosides, flavonoids, tannins, saponins, and gums. The hydro-ethanol fraction of *P. barbata* showed maximum inhibition of protein denaturation at a concentration of 500 µg/ml. Zones of inhibition were used to quantify how sensitive various bacterial strains were to plant extracts [10]. Sensitive bacteria were defined as those with an evident zone of inhibition larger than 12 mm. The viable cell count method was utilized to estimate the bactericidal effect of the chosen plant parts, and the output was presented as the number of organisms' cells as the portion of the control [11]. We assume that this plant's extracts may include chemicals with antibacterial properties.

Diarrhea, among other diseases, is a leading cause of illness and death, especially in undeveloped countries [12]. WHO has devised a diarrhea infection prevention strategy for the management and treatment of the disease that combines conventional medical procedures with health promotion and disease prevention methods (Syder medicine), which are primarily designed on the basis of medicinal herbs [13]. However, studies have been conducted to assess the *in vivo* antidiarrheal

efficacy of the *P. barbata* extract at a lower concentration than the standard. On account of this, our concentration has also been focused particularly on the analgesic, anxiolytic, and antipyretic effects of *P. barbata*. The results of the studies mentioned above provided the framework for our theory that the plant's extract might have bioactivity that is advantageous to human hygiene. Therefore, we were prompted to conduct an *in vivo* assessment of the plant extract using animal models.

Thus, this study aims to investigate the analgesic, anxiolytic, radical scavenging, protein-protection, anti-bacterial, anti-diarrheal, and anti-pyretic effects of the organic extracts of *P. barbata* in various test systems.

2. MATERIALS AND METHODS

2.1. Collection and identification

The plant *P. barbata* was gathered from the hill tracts of Bandarban, Chittagong, and Bangladesh in the middle of July, 2016. It was identified by an expert of the Bangladesh Forest Research Institute (BFRI), Chittagong. The accession number for *P. barbata* is SA (291).

2.2. Extraction

The aerial parts of *P. barbata* were separated from undesirable materials like other plants, dust, etc. and thoroughly washed by using running water. The plant sample was subsequently air-dried at room temperature for 7–15 days. Then the dried materials were ground into coarse powder with the aid of a mechanical grinder, and the powdered materials were kept in an airtight vessel prior to use. In warm extraction, about 200 g of coarse powder were subjected to 1000 ml of absolute ethanol (99%) in a Soxhlet apparatus. After 8 hours, the extract was collected and filtered through Whatman filter paper No. 1, which uses a rota evaporator to evaporate the solvent below 50 °C. After solvent evaporation, a gelatinous concentration referred to as "hot ethanol crude extract" of the plant was produced. The extract yield was 5%.

2.3. Fractionation (solvent-solvent portioning)

Using the procedure developed by Ghribia et al. [14], the ethanolic extract of *P. barbata* was subjected to solvent-solvent partitioning. In brief, 5 g of extract was triturated by dissolving it in 10% aqueous ethanol to create the master solution, which was then partitioned using three solvents, namely chloroform, n-hexane, and hydro-ethanol, in order to enhance polarity, utilizing a separating funnel. The resulting fractions of every plant extract were air dried. All extracts were stored in an airtight vessel at 4 °C. The weights of hydro-ethanol, n-hexane, and chloroform extracts were 1.8, 1.4, and 1.7 gm, respectively.

2.4. Phytochemical investigation

The phytochemical screening of different fractional parts of *P. barbata* was done by the method described by Islam [15].

2.5. *In vitro* pharmacological activity analysis

2.5.1. Free radical scavenging capacity

This experiment was conducted using a slightly modified version of Manzocco et al. [16]. A different concentration of extracts or positive control at a volume of 0.1 ml was added to 2.9 ml of DPPH (0.1 mM). After rapidly stirring the reaction mixture, we let it sit alone for 30 minutes at room temperature and without light. After 30 minutes, the absorbance of the mixture was calculated at 517 nm (after absorbance). 3 ml of DPPH was taken as a control (before absorbance). The following equation was utilized to measure the percentage of DPPH radical scavenging:

$$\% \text{ Radical scavenging} = \frac{C - T}{C} \times 100$$

Where, C and T stand for the absorbance of control and test, respectively.

2.5.2. Anti-inflammatory activity screening

The protein denaturation assay method of Mizushima and Kobayashi [17] was followed to perform this study with slight modifications. Briefly, 3 ml of different concentrations of extract or standard drug were placed in variously marked test tubes. After that, 3 ml of egg albumin solution was added to each tube. All reaction mixture's pH (5.6 ± 0.2) was adjusted with 0.1 N of HCl. The solutions were then incubated for 15 minutes at 37 ± 1 °C in a BOD incubator (Labline Technologies) before being heated for 10 minutes at 70 ± 1 °C in a water bath. After cooling, their absorbance was calculated at 660 nm. The percentage of inhibition of denaturation was determined relative to a control in which no medications or samples were employed. Every experiment was triplicated. The following calculation was used to determine the proportion of protein denaturation that was prevented:

$$\% \text{ Protein protection} = \frac{C - T}{C} \times 100$$

Where, C and T stand for the absorbance of control and test, respectively.

2.5.3. Anti-bacterial activity screening

The disk-diffusion method was adopted to investigate the anti-bacterial activity of the different fractions of *P. barbata*. This method was established by Bauer et al. (1966)[10]. In this study, using azithromycin, a broad-spectrum anti-bacterial, we tested it against four gram-positive and five gram-negative pathogens.

2.6. In vivo pharmacological studies

2.6.1. Experimental animal

Swiss albino mice (18–22 g, b.w.) of both sexes were purchased from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). The mice had unrestricted access to the normal laboratory diet and water. They were acclimated to laboratory conditions for a week before the studies began, during which time they were housed at a temperature of 26 ± 2 °C with a 12-

hour light/dark cycle. Except for water, the food supply was stopped 24 hours before each experiment started. This research was approved by the Pharmacy Department at the Southern University of Bangladesh in Chattagram (#Sub/999-16-08).

2.6.2. Anti-diarrheal activity (*Castor oil model*)

The study was performed by following the model of Shoba and Thomas [18]. Five mice were included in each of the three control, standard, and six experimental groups. The standard group received 3 mg/kg of loperamide orally, whereas the control group took 1% v/v tween-80 in distilled water. The rest groups were given hydro-ethanol, n-hexane, and chloroform extracts of *P. barbata* at a dose of 500 mg/kg orally. All the groups were treated at 10 ml/kg. After 30 minutes, the animals were treated orally with 0.4 ml of castor oil. Each animal was given its own cage with blotting paper laid out on the bottom that was replaced every hour. Total latency durations (time until first diarrheal stool following delivery of samples or controls) and the number of diarrheal stools were recorded over the course of the 4-hour observation period. The following is a numerical evaluation of stool consistency: Stools that are regular in consistency equal 1, while loose, watery stools equal 2.

2.6.3. Analgesic activity screening (*Hot plate model*)

The investigation was carried out using the model of Eddy and Leimbach [19]. To accomplish this, each overnight fasting mouse was placed atop a thermostatically controlled heated beaker. Each mouse's reaction time was noted. The hot plate was placed at a temperature of 45 ± 1 °C. The mouse was kept on the hot plate, and the reaction time was estimated as the amount of time that passed between that point and the mouse exhibiting symptoms of acute discomfort, such as flicking or licking of the forepaw or hindpaw or jumping to try to get away from the pain. For this investigation, mice with an initial reaction time of 11 seconds or less were chosen, and they were separated into 8 groups (3 in each group). After regrouping, each group provided a distinct treatment. In this case, the reference drug, diclofenac sodium, was administered intraperitoneally at a dose of 9 mg/kg. As mentioned in the previous study, control and test samples were given orally. Following treatment with the appropriate chemicals, each mouse's initial latent reaction time in a heated beaker was recorded at "0" minute, and lastly, 30 minutes later, the latent reaction time of each mouse was recorded.

2.6.4. Anxiolytic activity

2.6.4.1. *Open field test*

The study was carried out using the model of Gupta et al. [20] with slight modifications. Diazepam was administered at a dose of 4 mg/kg (i.p.) as a reference drug. The control and extract groups were similar, as mentioned in the previous studies. After giving out samples and controls, we waited 15 minutes. Then, for the next five minutes, we recorded how many squares each mouse had traversed.

2.6.4.2. *Swing test*

The study was carried out using the apparatus and method provided by Islam et al. [21]. One of the goals of this research was to use the swing test to better understand the mice's emotional

behavior. Treatments were given similarly, as mentioned in the open-file study. After administering the samples and controls, a 15-minute wait period was conducted. The animals were then kept in the swing box, and their swinging abilities were evaluated over the course of 5 minutes.

2.6.5. Anti-pyretic activity (*Brewer's yeast model*)

This investigation was carried out using the model provided by Abena et al. [22]. By injecting a 10% Brewer's yeast aqueous suspension (10 ml/kg b.w.), pyrexia was elicited. All the groups went without eating and drinking for 24 hours (with free access to drinking water). 18 hours later, their rectal temperature was checked and recorded using a digital thermometer. A rectal temperature increase of at least 2 °F was used to select the test animals. In this case, paracetamol, the standard antipyretic medication, was administered at a dose of 150 mg/kg orally as a positive control. Control and extract groups were administered similarly. Animals' rectal temperatures were taken every hour for four hours after receiving samples or controls.

2.7. Statistical analysis

Using GraphPad Prism (version 6.0, San Diego, California, USA, copyright 1994–1999), we ran a one-way analysis of variance (ANOVA) with time as the independent variable and then performed a Dunnett's post hoc multiple comparison test, adjusting for significance at the $P < 0.05$ level and a 95% confidence interval.

3. RESULTS

The phytochemical report proposes that the hydro-ethanol fraction of *P. barbata* contains alkaloids, steroids, tannins, glycosides, flavonoids, reducing sugars, gums, and saponins. The n-hexane fraction contains glycosides, tannins, flavonoids, saponins, and gums, while the chloroform fraction possesses alkaloids, glycosides, tannins, saponins, gums, and flavonoids.

In the DPPH scavenging assay, the ethanol extract of *P. barbata* demonstrated a maximum percentage inhibition of 97.62 at a concentration of 100 µg/ml, whereas the reference drug ascorbic acid demonstrated 94.65% at the same concentration. Both the extract and standard concentration-dependently reduced DPPH radicals in the reaction mixtures. The median half-inhibitory concentrations (IC₅₀s) calculated for extract and ascorbic acid were 58.69 and 60.79 µg/ml, respectively (Table 1).

Table 1. Radical scavenging effect of ethanol extract of *P. barbata* and controls

Test groups	Concentration (µg/mL)	Percentage DPPH radical scavenging	IC ₅₀ (µg/mL)
Vehicle (Control)	-	0	-
Ascorbic Acid (Standard)	20	17.24	60.79
	40	33.43	
	60	51.71	
	80	73.10	
	100	94.65	
Ethanol extract of <i>P. barbata</i>	20	18.57	58.69
	40	36.40	
	60	54.68	
	80	76.08	
	100	97.62	

The hydro-ethanol, n-hexane, and chloroform extracts of *P. barbata* exhibited mean inhibition of protein denaturation of 91.73, 82.56, and 82.56% by 500 µg/ml; 82.79, 64.68, and 58.26% by 250 µg/ml and 72.93, 45.87, and 37.61% by 125 µg/ml, respectively. The standard drug acetyl salicylic acid exhibited mean inhibition of protein denaturation by 90.83, 81.65, and 70.18% at 500, 250, and 125 µg/ml, respectively (Table 2).

Table 2. Inhibition of protein denaturation by different fractional parts of *P. barbata* and controls

Treatments	Concentration (µg/mL)	%inhibition of protein denaturation
Control (Vehicle)	-	-
Standard (Acetyl salicylic acid)	125	70.18
	250	81.65
	500	90.83
Hydro-ethanol fraction of <i>P. barbata</i>	125	72.93
	250	82.79
	500	91.73
n-Hexane fraction of <i>P. barbata</i>	125	45.87
	250	64.68
	500	82.56
Chloroform fraction of <i>P. barbata</i>	125	37.61
	250	58.26
	500	82.56

The hydro-ethanol fraction of *P. barbata* inhibited the growth of eight bacterial species, namely *L. casei*, *L. coryniformis*, *B. cereus*, *S. aureus*, *P. azotoformis*, *E. coli*, *P. aeruginosa*, and *K. pneumonia*, while the n-hexane fraction acted only against *K. pneumonia* and the chloroform fraction exerted an inhibitory effect on *P. azotoformis*, *E. coli*, and *P. aeruginosa* (Table 3).

Table 3. Anti-bacterial effect of various fractions *P. barbata* and standard drug

Test microorganisms	Zone of inhibition (diameter in mm)			
	Hydro-ethanol fraction of <i>P. barbata</i> (500 µg/disc)	n-Hexane fraction of <i>P. barbata</i> (500 µg/disc)	Chloroform fraction of <i>P. barbata</i> (500 µg/disc)	Azithromycin (30 µg/disc)
Gm(+) species				
<i>Lacticaseibacillus casei</i>	14	NR	NR	30
<i>Lactobacillus coryniformis</i>	10	NR	NR	30
<i>Bacillus cereus</i>	14	NR	NR	29
<i>Staphylococcus aureus</i>	14	NR	11	24
Gm(-) species				
<i>Pseudomonas azotoformis</i>	11	NR	9	24
<i>Escherichia coli</i>	16	10	10	31
<i>Pseudomonas aeruginosa</i>	14	NR	11	26
<i>Klebsiella pneumonia</i>	11	14	NR	24
<i>Vibrio cholera</i>	NR	NR	NR	25

NR = No Response

Hydro-ethanol, n-hexane, and chloroform extracts of *P. barbata* at 500 mg/kg showed mean defecation of 7.33 ± 0.41 , 7.33 ± 1.08 , and 6.33 ± 0.41 , respectively. Standard drug loperamide (3 mg/kg) resulted a mean defecation rate of 6.00 ± 0.71 . All the extracts and the standard increased in mean latent period ($P < 0.05$) in animals. However, chloroform fraction showed better anti-diarrheal effect than the hydro-ethanol and n-hexane fractions (Table 4).

Table 4. Results of the treatment groups compared to the control group during the 4 hour defecation interval

Test groups	Latent period (h)	Defecation (240 min)
Control (1% Tween-80 dissolved in normal saline)	5.66 ± 0.41	14.00 ± 1.87
Standard (Loperamide) (3 mg/kg)	14.33 ± 0.41	6.00 ± 0.71
Hydro-ethanol fraction of <i>P. barbata</i> (500 mg/kg)	10.33 ± 0.41	7.33 ± 0.41
n-Hexane fraction of <i>P. barbata</i> (500 mg/kg)	9.33 ± 0.41	7.33 ± 1.08
Chloroform fraction of <i>P. barbata</i> (500 mg/kg)	11.66 ± 0.41	6.33 ± 0.41

Values are mean ± SEM (n = 5)

Table 5 suggests that all the fractions of *P. barbata* at 500 mg/kg increased the latency compared to the control group after 30 minutes. However, the standard showed better activity than the crude fractions.

Table 5. Results of the treatment groups relative to the latency period of the control group

Test groups	Latency period (Sec)	
	Initial	After 30 minutes
Control (1% Tween-80 dissolved in normal saline)	2.33 ± 0.408	2.67 ± 0.816
Standard (Diclofenac-Na) (9 mg/kg)	2.45 ± 0.408	10.67 ± 1.08
Hydro-ethanol fraction of <i>P. barbata</i> (500 mg/kg)	2.45 ± 0.3169	6.67 ± 0.408
n-Hexane fraction of <i>P. barbata</i> (500 mg/kg)	2.50 ± 0.204	7.67 ± 0.408
Chloroform fraction of <i>P. barbata</i> (500 mg/kg)	2.52 ± 0.500	8.67 ± 0.816

At the time of the open field test, squares crossed by the group receiving the reference drug diazepam were 63.00 ± 1.00, while for the control group they were 101.34 ± 1.53. The hydro-ethanol, n-hexane, and chloroform fractions of *P. barbata* at 500 mg/kg exhibited square crossings of 75.30 ± 0.58, 76.00 ± 0.00, and 77.60 ± 1.15, respectively. At the time of the swing test, the

number of swings recorded after diazepam administration were 3.67 ± 0.58 , and for control, it was 13.33 ± 1.15 . The hydro-ethanol, n-hexane, and chloroform fractions of *P. barbata* at 500 mg/kg exhibited swings of 5.67 ± 0.58 , 6.67 ± 0.58 , and 5.33 ± 0.58 , respectively (Table 6).

Table 6. Number of square crossing and swings observed in *P. barbata* and control groups

Test groups	No. of field cross	No. of swings
Control (1% Tween-80 dissolved in normal saline)	101.34 ± 1.53	13.33 ± 1.15
Standard (Diazepam) (4 mg/kg)	$63.00 \pm 1.00^*$	$3.67 \pm 0.58^*$
Hydro-ethanol fraction of <i>P. barbata</i> (500 mg/kg)	$75.30 \pm 0.58^*$	$5.67 \pm 0.58^*$
n-Hexane fraction of <i>P. barbata</i> (500 mg/kg)	$76.00 \pm 0.00^*$	$6.67 \pm 0.58^*$
Chloroform fraction of <i>P. barbata</i> (500 mg/kg)	$77.60 \pm 1.15^*$	$5.33 \pm 0.58^*$

Values are mean \pm SEM, (n = 5); * $P < 0.05$ when compared to the control group

All the fractions at 500 mg/kg b.w. remarkably ($P < 0.05$) diminished hyperthermia in animals. In animals, the n-hexane fraction reached basement body temperature at the 3rd hour, while the hydro-ethanol fraction stabilized at the 4th hour. The chloroform fraction did not stabilize mouse body temperature even at the 4th hour of observation. Paracetamol, on the other hand, stabilized mouse body temperature within the first hour of treatment (Table 7).

Table 7. Hyperthermia reduction by *P. barbata* and control groups in mice

Test groups (10 mL/kg)	Rectal temperature in °F					
	-18 hr	0 hr	1 hr	2 hr	3 hr	4 hr
Control (1% Tween-80 dissolved in normal saline)	98.40	100.30	100.53	100.47	100.50	100.36
Standard (Paracetamol) (150 mg/kg)	98.40	100.30	98.40	98.40	98.50	98.40
Hydro-ethanol fraction of <i>P. barbata</i> (500 mg/kg)	98.40	100.80	98.83	98.77	98.69	98.46
n-Hexane fraction of <i>P. barbata</i> (500 mg/kg)	98.50	100.70	99.50	98.70	98.50	98.50

Chloroform fraction of <i>P. barbata</i> (500 mg/kg)	98.60	100.50	99.90	99.60	99.40	99.80
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4. DISCUSSION

Antioxidant activity is one of the most crucial pharmacological characteristics of this plant. This assay, which reveals details about the test compound's reactivity with a stable free radical, is frequently employed as a preliminary examination. Reactive oxygen species (ROS) are several types of activated oxygen, including hydroxyl ions, superoxide ions, and hydrogen peroxide, among others. These ROS have a potent therapeutic effect on various dangerous conditions like atherosclerosis, cataracts, cancer, inflammation, coronary heart disease, and aging. By using antioxidant defense mechanisms, these antioxidant compounds scavenge ROS and safeguard cells [23].

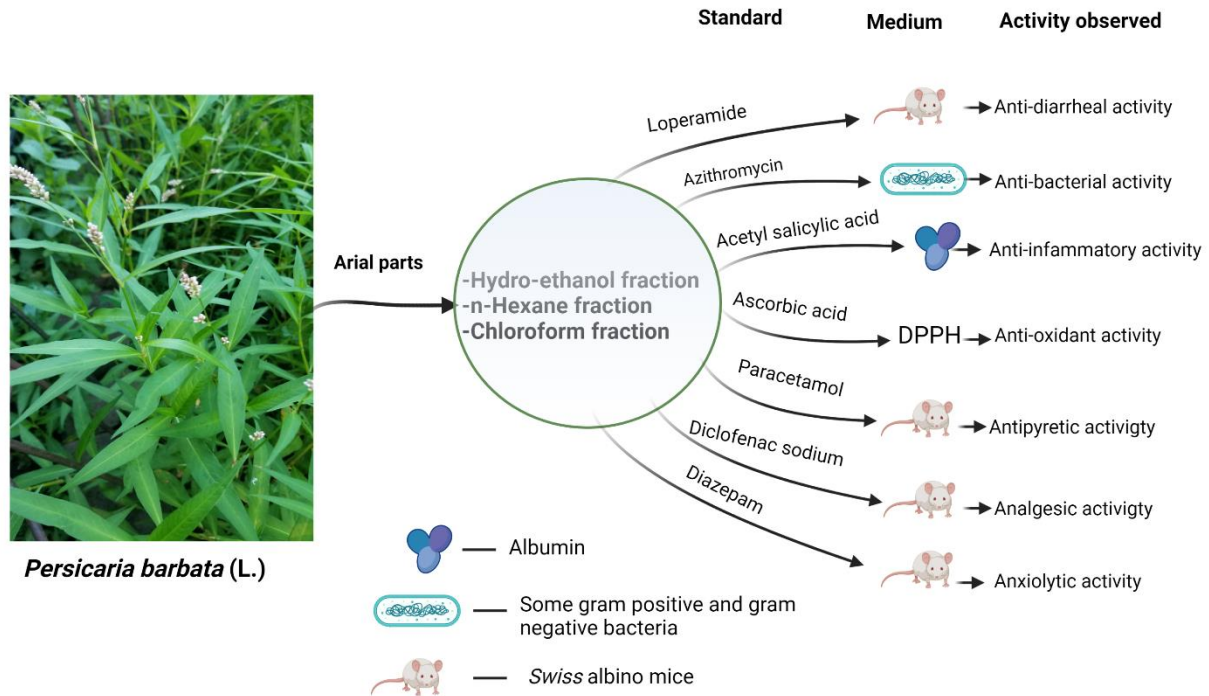


Figure 1. Different pharmacological activity test with different solvent fractions of *P. barbata*

An ethanolic extract of *P. barbata* showed DPPH scavenging capacity comparable to the standard drug. The greatest effect was seen with the highest concentration tested, and it was concentration-dependent. Our findings are in agreement with a recent report by Sukrila [24]. The RSM

methodology was utilized to estimate the antioxidant capability of *P. barbata* [25]. Additionally, the anti-inflammatory effect was studied by protein denaturation inhibition, and it was significantly inhibited by *P. barbata* and showed a higher result than standard acetyl salicylic acid at its highest concentration tested. In this case, the hydro-ethanol fraction of the plant showed a greater result when compared with the other two concentrations of n-hexane and chloroform.

An anti-bacterial effect was found by studying the zone of inhibition where the hydro-ethanol fraction was effective against all tested gram-positive and gram-negative bacteria except *V. cholera*. where the n-hexane fraction showed only limited effectiveness against *E. coli* and *K. pneumonia* and the chloroform fraction showed effectiveness against *S. aureus*, *P. azotoformis*, *E. coli*, and *P. aeruginosa*. In a study, 70% ethyl acetate, ethanolic, and methanol-water extractions of *P. barbata* were found effective against *S. epidermis*, *S. aureus*, and *P. acnes* [24].

The anti-diarrheal effect was found mostly in the chloroform fraction, which is close to the standard drug loperamide, and the other two concentrations, hydro-ethanol and n-hexane, showed defecation results that were half those of the control group. The analgesic effect of *P. barbata* with standard diclofenac sodium was observed *in vivo*, and it was discovered that the chloroform fraction is more effective than n-hexane and hydro-ethanol, but the standard group showed a greater effect than the extracts.

We feel anxiety to some extent in our regular lives. It is a feeling of uneasiness. It includes being worried or feeling fear within the range of mild or severe. To date, many anxiolytic drugs have been introduced. Unfortunately, many of them are associated with mild to severe side effects [26,27]. The overall fact stimulates medicinal scientists to search for new and safer anxiolytics, especially from plant sources. In this study, the anxiolytic effects of the organic extracts of *P. barbata* were observed using two different models, and the results suggest that the herb has significant anxiolytic effects on the experimental animals.

Fever is recognized as a common medical sign in humans. During a fever, our body temperature ranges from 36.5 to 37.5 °C. Fever may occur due to a pathogenic attack (primary or secondary), tissue damage, mild to severe inflammation, malignancy, graft rejection, and many other inflammatory conditions [28]. To date, a number of antipyretic drugs have been discovered, many of which have been identified as risky drugs. It is because the safety profiles of these drugs are still controversial. For example, paracetamol is widely used as an anti-pyretic analgesic drug [29]. It is to be mentioned that if fever is left unmanaged or mismanaged, it may cause serious health problems, even death [30]. In this study, we have seen that the ethanol and n-hexane fractions of *P. barbata* exerted significant antipyretic effects on yeast-induced hyperthermia mice. Figure 1 shows the overall study protocol and biological activity observed in this research.

5. CONCLUSION

Our preliminary phytochemical report suggests that *P. barbata* possesses many important secondary chemical groups, including alkaloids, glycosides, flavonoids, and tannins. *P. barbata*

organic extracts showed concentration-dependent DPPH radical scavenging, inhibition of protein denaturation capacity, moderate but broad-spectrum antibacterial activity, and significant anti-diarrheal, analgesic, anxiolytic, and antipyretic effects in Swiss albino mice. Its antioxidant, anti-inflammatory and antimicrobial capacities might be linked with its neuroprotective and antipyretic effects in test systems. More studies are required to isolate and characterize the active phytochemicals responsible for the above-mentioned pharmacological effects of this hopeful herb.

Conflict of interest

None declared.

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