

Chemical, Biological & Pharmacological Investigations of Different Fractional Extracts of *Passiflora edulis* Sims. (Family: Passifloraceae)

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Abstract

In the evaluation of antimicrobial activity, antibacterial activity of ethanol, chloroform & n-hexane extracts of *P. edulis* was tested against 10 bacteria at concentrations of 500µg/disc. Standard antibiotic disc of Azithromycin (30 µg/disc) was used for the comparison. The extracts showed no activity against gm (+ve) & gm (-ve) bacteria at a concentration of 500 µg/disc. Antifungal activity of ethanol, chloroform & n-hexane extracts of *P. edulis* was tested against 7 fungi at concentrations of 500µg/disc. Standard antifungal disc of Fluconazole (30 µg/disc) was used for the comparison. In antifungal screening the chloroform extract showed activity against *A. fumigatus* (15nm). In DPPH scavenging assay the ethanol extract of leaves showed maximum % inhibition of 73.41% at 100µg/ml while the standard ascorbic acid showed % inhibition of 82.14% at the same concentration. The DPPH radical scavenging activity was increased by increasing the concentration of the sample extract. The extract exhibited considerable DPPH free radical scavenging activity as indicated by their IC50 values which indicate the potency of scavenging activity. Standard ascorbic acid was found to have an IC50 of 1.66µg/ml. In comparison to standard ascorbic acid, ethanol extract of *P. edulis* showed of an IC50 of 2.57µg/ml. In the evaluation of anti-inflammatory activity by albumin denaturation assay method, the percent inhibition of protein denaturation in the experiment of ethanol extract of leaves of *P. edulis* was found to be 55.454% at 500µg/ml, 49.818% at 250µg/ml and 41.818% at 125µg/ml. The extract possesses significant activity comparable with that of the standard acetyl salicylic acid which showed percent inhibition of protein denaturation of 91.945%, 88.181% and 45.454% in the same concentration range. In this analgesic activity study using Eddy's hot plate method in mice, the ethanol extract of *P. edulis* showed mild analgesic activity. After 30 minutes of administration of standard drug (Diclofenac 9 mg/kg), mean latency was found to be 17.34sec. While the extract at concentration of 400 mg/kg exposed mean latency of 6.67sec. Respectively. During antipyretic activity by Brewer's yeast induced pyrexia method in mice, the ethanol extract of leaves of *P. edulis* produced moderate antipyretic activity. In this test, the extract reduced temperature from 102.4°F to 99.84°F (p=0.151188), 98.74°F (p=0.070485), 98.6°F (p=0.056108), 98.37°F (p=0.039072) and 98.27°F (p=0.110528) in 1st, 2nd, 3rd, 4th and 5th hour respectively and caused maximum reduction of temperature in 1st hour. During antidiarrhoeal activity by castor oil induced test in mice, the ethanol extract of *P. edulis* produced moderate antidiarrheal episode. The latent period and the total diarrheic faces were found to be 0.978 hr & 8.34 at 500 mg/kg for *P. edulis* as compared to standard (Loperamide 3mg/kg) 2.845 hr & 5.34.

In the sedative and hypnotic activity by using open field box, hole cross box and swing Test method on swiss albino mice ethanol extract of *P. edulis* showed moderate sedative and hypnotic activity.

During the open field test, squares crossed by the group receiving standard Diazepam and ethanol extract of leaves of *P. edulis* were 63 and 84.34 respectively. During the swing test, number of swings observed after administration of standard Diazepam and ethanol extract of leaves of *P. edulis* were 3.67 and 8.67 respectively. During diuretic activity by using the Lipschitz test in mice, ethanol, n-hexane and chloroform extracts of *P. edulis* (500mg/kg body wt.) showed no activity compared to standard drug Furosemide (20mg/kg body wt.).

Key words: passiflora edulis sims, antimicrobial activity, antibacterial activity

Introduction

On earth, each and every human nation has been surrounded in the biodiversity of nature. Through natural ecosystem function and stability biodiversity provides humankind huge direct benefits and indirect essential services. It comprises about 5 to more than 50 millions of species of biodiversity 270,000 are plant species [1]. Chemical constituents such as tannins, flavonoids, steroids, saponins, glycosides, phenolics, terpenes, alkaloids, waxes, essential oils, carbohydrates, amino acids, proteins etc. are contained in plants. About 74% of drugs in the USA are based on plants and in developing countries a large portion of the world population depends directly on the traditional medicine system for a variety of diseases. In the world, approximately 20% of the plants have been used for pharmacological and biological screening. In the original system of medicine in India, several thousands of plants are used medicinally, mainly as herbal preparations. The sources of very potent and powerful drugs which have stood the test of time and modern chemistry have not been able to replace most of them [2]. For all time, man has used plants and herbs to treat disease and heal injuries. Now-a-days, to explain some of the medicinal phenomena associated with traditional herbal remedies scientific studies are began and renewed interest has been shown. Governments and the scientific and medical communities have created awareness about the importance of medicinal plants in health care systems in many developing countries [3]. Since ancient times, plants have also been used in the production of stimulant beverages (e.g. tea, coffee, cocoa, and cola) and inebriants or intoxicants (e.g., wine, beer, and kava) in many cultures and till today this approach continues. In the 9th century, coffee (*Coffea arabica*) was initially cultivated in Yemen but tea (*Thea sinensis*) was first added in ancient China for commercial purposes. Bitter beverages containing raw cocoa beans (*Theobroma cacao*), red peppers, and various herbs are consumed by dignity using the Aztec. At the present time, tea, coffee, and cocoa are important property and their utilization has spread worldwide. Methylated xanthine derivatives, namely caffeine, theophylline, and theobromine, are the active components of these stimulants and these are the main constituents of coffee, tea, and cocoa, respectively [4]. To discover all medicinal plants that exist in the world, the World Health Organization made a challenge several years ago. Since botanical confirmation was not attempted it was admitted that the collection of names of medicinal plants certainly contained many replicates. Additional, excluding data indicating what the plants were used for, the list including more than 20,000 species only provided Latin binomials and the countries where the plants were used [5].

Materials and Methods

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens using the standard procedures as described below:

Phytochemical studies in ethanol extract of leaves of *P. edulis*

The results of the phytochemical investigation of the ethanol extract of leaves of *P. edulis* has shown a remarkable variation in the presence of the above studied phytochemical compounds in the studied taxa. The study revealed that the ethanol extract of leaves of *P. edulis* are showing maximum presence of alkaloids, glycosides, flavonoids and phenols in ethanol solvent extracts.

Evaluation of Antimicrobial Activity

Experimental design (6)

The assigned assay method is based on the ability of antibiotics to diffuse from a confined source through the gel media and create a concentration gradient. If the agar seeded or streaked with a sensitive organism, a zone of inhibition will result. In this method, a definite amount of the test sample was dissolved in definite amount of solvent to give solution of given concentration ($\mu\text{g}/\mu\text{l}$). Then the sterile filter paper discs having 5mm in diameter were impregnated aseptically with known amount of the test substances and dried. Such discs and standard drugs discs were placed on plate containing a suitable medium seeded with the test organisms. These plates were kept at low temperature (4°C) for 24h to allow maximum diffusion. The dried discs absorb water from the agar medium and the material under test was dissolved. The plates for antibacterial test were then kept in an incubator (37°C) for 24h and the plates for antifungal test were kept at room temperature for 72 hours to allow sufficient growth of organisms. If the test material has antimicrobial activity, it will inhibit the growth of microorganism having a clear distinct zone called "Zone of Inhibition" [7]. The antimicrobial activity of the test agent was determined by measuring the diameter of the zone of inhibition in terms of mm.

Evaluation of Antioxidant Activity

Determination of quantitative antioxidant activity

Quantitative antioxidant activity was determined by determining the half maximal inhibitory concentration (IC_{50}) of ethanol extract of *P. edulis* graphically in comparison to standard ascorbic acid (8).

% scavenging of the DPPH free radical was measured by using the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity was determined by calculating the percent inhibition of protein denaturation. The percent inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The percent mean inhibition protein denaturation of ethanol extract of leaves of *P. edulis* was calculated and noted down in the following table and results were graphically presented and compared with the standard drug Acetyl salicylic acid (9).

Evaluation of Analgesic Activity

Experimental design (10)

In this experiment, the analgesic activity of ethanol extract of leaves of *P. edulis* was investigated in swiss albino mice, as per the method described by Eddy and Leimbach. Overnight fasted mice were placed individually on a thermostatically controlled heated beaker and the reaction time of each mouse was recorded. The temperature of the hot plate was maintained at $45 \pm 1^{\circ}\text{C}$. The reaction time was considered as the time elapsed between placing of the mouse on the hot plate and appearance of signs of acute discomfort, characterized by flicking or licking of the hind paw, forepaw or jumping in an attempt to escape from the pain. The mice showing initial reaction time of 1 sec or less were selected for this study.

The animals were then, divided into control, standard and one test group containing three mice in each group. Control group received vehicle (1% Tween-80 in water) at a dose of 10ml/kg body weight orally. The standard group received Diclofenac-Na at the dose of 9mg/kg body weight intraperitoneally; test group received the ethanol extract of *P. edulis leaves* at the dose of 400 mg/kg body weight orally. Thereafter, the initial latent reaction time of each mouse in heated beaker was recorded on "0" minute (just after treating with respective substances) and finally 30 minutes later the latent reaction time of each mouse was recorded.

Evaluation of Antipyretic Activity

This study was conducted by slightly modifying the method described by Adams (11). Animals of either sex were divided into control, standard and one test group containing 3 mice in each group for this experiment. The normal body temperature of each mouse was measured from rectum at one hour interval on a thermometer and recorded. The antipyretic activities of extract were evaluated using Brewer's yeast induced pyrexia in Swiss albino mice. Before yeast injection, rectal temperature of mice were recorded and after recording animals were given subcutaneous injection of 10ml/kg of 10 % w/v yeast solution for elevation of body temperature of mice. At the 24hrs after yeast injection, the vehicle, standard drug and extract were administered into different groups. Distilled water at dose of 10ml/kg was administered orally to the control groups of animals and Paracetamol at dose of 150mg/kg was administered orally to standard group of animals. The ethanol extract of *P. edulis* plant was administered orally at a dose of 500mg/kg of body weight. Rectal temperature was recorded by digital thermometer at 1st, 2nd, 3rd, 4th, and 5th hours after drug administration.

Evaluation of Antidiarrheal Activity

Experimental design (12)

The animals were all screened initially by giving 0.4 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were then, divided into control, standard and one test group containing three mice in each group. Control group received vehicle (1% Tween-80 in water) at a dose of 10ml/kg body weight orally. The standard group received Loperamide at the dose of 3mg/kg orally; test group received the ethanol extract of *P. edulis* at the dose of 500 mg/kg body weight orally. Each animal is placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhea was induced by oral administration of 0.4 ml castor oil to each mouse, 30 minutes after the above treatments. During the observation period (4hrs), the total latency periods (first diarrheal stool after the administration of castor oil) and the number of diarrheic feces excreted by the animals were recorded. A numerical score based on stool consistency was assigned (normal stool =1 and watery stool = 2).

Pharmacological Evaluation of Sedative and Hypnotic Activities

Preparation of samples for the test, standard and control groups (13)

In the present work, 500mg/kg dose was selected for plant species and for this 3mg ethanol extract of leaves of *P. edulis* was dissolved in 1.2ml of distilled water for three mice.

For standard, 4mg/kg dose of Diazepam was selected. For this 0.08mg Diazepam was dissolved in 1.2ml of distilled water for three mice and for control group 10ml/kg dose of distilled water was selected.

Experimental design

S/N	Test Samples	Group	Dose (/unit)	Routes of administration
01	Double distilled water	Control	10ml/kg of body wt.	Orally
02	Diazepam	Standard	4mg/kg of body wt.	
03	Ethanol extract of <i>P. edulis</i> Sims.	Test Sample	500mg/kg of body wt.	

Table 1: Test materials used in the evaluation of sedative and hypnotic activity

Results and Discussions

Determination of the antifungal activity

The antifungal activity of the ethanol, n-hexane and chloroform extracts of leaves of *P. edulis* were determined by covering the zone of inhibition (mm) of the 10 bacteria with the percent zone of inhibition caused by the test groups in comparison to the standard Fluconazole and control groups.

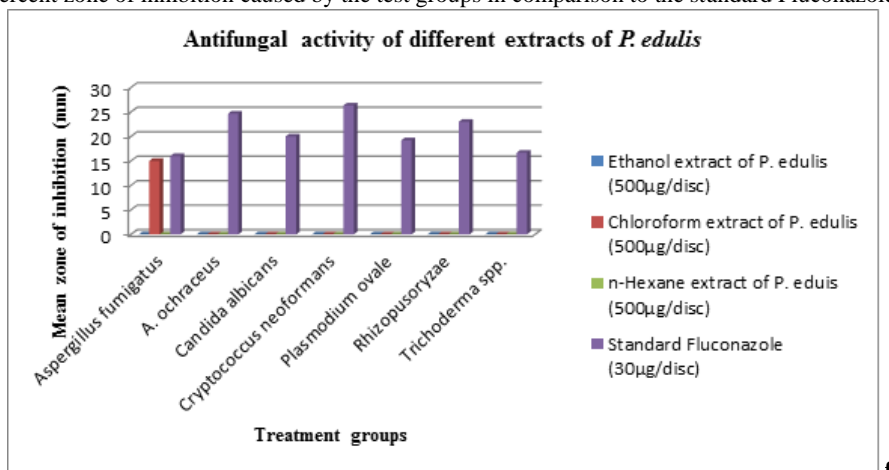
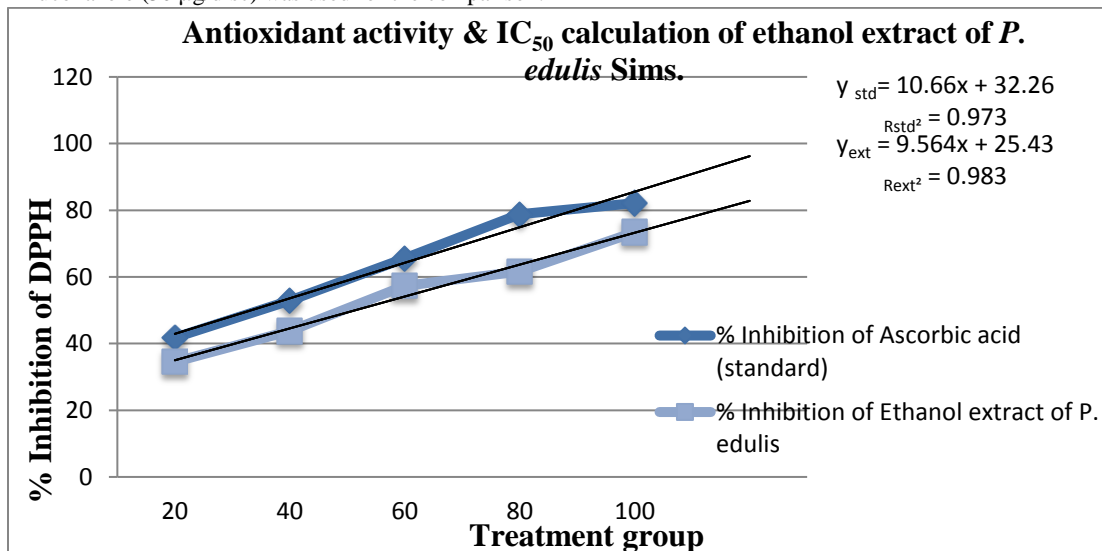


Figure 1: Antifungal activities of different extracts of *P. edulis* Sims. & standard**Results of the antifungal activity**

Antifungal activity of ethanol, chloroform & n-hexane extracts of *P. edulis* was tested against fungi at concentrations of 500µg/disc. Standard antifungal disc of Fluconazole (30 µg/disc) was used for the comparison.

In antifungal screening the chloroform extract showed activity against *A. fumigatus* (15nm).

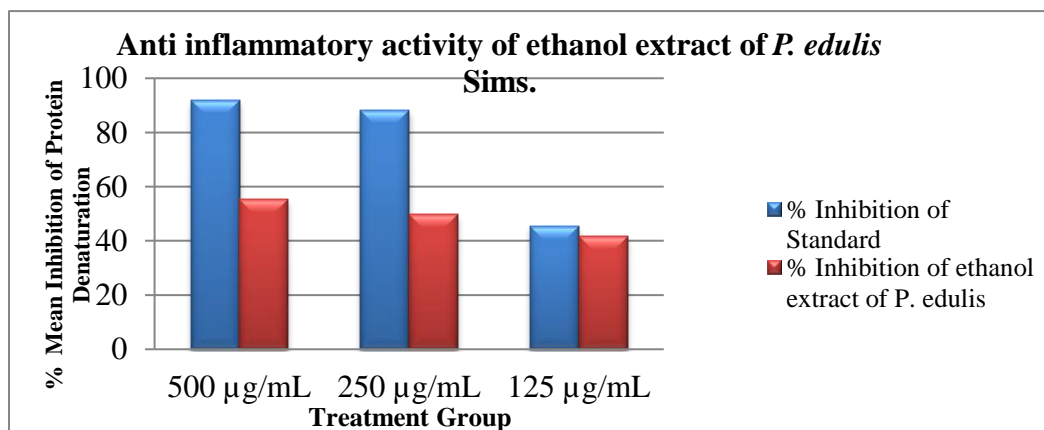
Quantitative antioxidant activity**Figure 2: Antioxidant activity & IC₅₀ calculation of ethanol extract of *P. edulis* Sims.****IC₅₀ calculation from graph**

Sample	IC ₅₀
Standard (ascorbic acid)	1.66 µg/ml
Ethanol extract of <i>P. edulis</i>	2.57 µg/ml

Result of quantitative antioxidant assay

In DPPH scavenging assay the ethanol extract of leaves showed maximum % inhibition of 73.41% at 100µg/ml while the standard ascorbic acid showed % inhibition of 82.14% at the same concentration (Table 6.1) The DPPH radical scavenging activity was increased by increasing the concentration of the sample extract. The extract exhibited

considerable DPPH free radical scavenging activity as indicated by their IC₅₀ values which indicate the potency of scavenging activity. Standard ascorbic acid was found to have an IC₅₀ of 1.66µg/ml. In comparison to standard ascorbic acid, ethanol extract of *P. edulis* showed of an IC₅₀ of 2.57µg/ml.

Anti-inflammatory activity**Figure 3: Anti-inflammatory activity of ethanol extract of *P. edulis* Sims**

Results of anti-inflammatory activity

In the present investigation, the percent inhibition of protein denaturation in the experiment of ethanol extract of leaves of *P. edulis* was found to be 55.454% at 500µg/ml, 49.818% at 250µg/ml and 41.818% at 125µg/ml (Table 7.1). The extract possesses significant activity comparable with

that of the standard acetyl salicylic acid which showed percent inhibition of protein denaturation of 91.945%, 88.181% and 45.454% in the same concentration range.

Analgesic activity:

S/N	Test Samples	Group	Dose (/unit)	Routes of administration
01	1% Tween-80 in distilled water	Control	10ml/kg of body wt.	Orally
02	Diclofenac-Na	Standard	9mg/kg of body wt.	Intraperitoneally
03	Ethanol extract of <i>P. edulis</i> Sims.	Test Sample	400mg/kg of body wt.	Orally

Table 2: Test materials used in the evaluation of analgesic activity

Determination of analgesic activity

Analgesic activity was determined by evaluating the latent period by recording the reaction time of the each mouse of the test group on a

thermostatically controlled heated beaker jumping in an attempt to escape from the pain in comparison to the control and standard drug Diclofenac-Na.

Test groups	Mice No.	BW (g)	Latent period (Sec.)				SD		SE	
			Initial	Mean	After 30 minutes	Mean	Initial	After 30 minutes	Initial	After 30 minutes
Control (1% Tween-80, 10ml/kg)	M1	20	2	2.67	4	4.0	1.154	1	0.816	0.707
	M2	21	2		5					
	M3	23	4		3					
Standard (Diclofenac-Na 9mg/kg)	M1	22	2	2.34	17	17.34	0.577	1.527	0.408	1.080
	M2	22	3		16					
	M3	20	2		19					
Ethanol extract of <i>P. edulis</i> Sims. (400mg/kg)	M1	20	2	3.0	6	6.67	1	0.577	0.707	0.408
	M2	23	3		7					
	M3	20	4		7					

[BW=Body weight, SD=Standard deviation, SE=Standard error]

Table 3: Effect of ethanol extract of *P. edulis* on the latent period of Eddy's hot plate test in mice

Test groups	MLP (Sec.)		t-test (p-value)	
	Initial	After 30 minutes	Initial	After 30 minutes
Control (1% Tween-80, 10ml/kg)	2.67±0.816	4±0.707	0	0
Standard (Diclofenac-Na 9mg/kg)	2.34±0.408	17.34±1.080	>0.05	<0.05
Ethanol extract of <i>P. edulis</i> Sims. (400mg/kg)	3±0.707	6.67±0.408	>0.05	<0.05

[MLP=mean±SE, SE=Standard error, MLP=Mean latent period]

Table 4: Results of the treatment groups in comparison to the control latency period

Results of analgesic activity

The ethanol extract of *P. edulis* showed significant analgesic activity. After 30 minutes of administration of standard drug (Diclofenac-Na 9 mg/kg), mean latency was found to be 17.34sec. while the extract at

concentration of 400 mg/kg exposed mean latency of 6.67sec. respectively.

Antipyretic activity

S/N	Test Samples	Group	Dose (/unit)	Routes of administration
01	Double distilled water	Control	10ml/kg of body wt.	Orally
02	Paracetamol	Standard	150mg/kg of body wt.	
03	Ethanol extract of <i>P. edulis</i>	Test Sample	500mg/kg of body wt.	
04	Brewer's yeast (10% w/v)	For induce fever	10ml/kg of body wt.	Subcutaneous

Table 5: Test materials used in the evaluation of antipyretic activity**Determination of antipyretic activity**

Antipyretic activity was determined by determining the rectal temperature of the mice of the test group in comparison to the control and standard drug Paracetamol.

Test groups	Mice No.	BW (g)	Rectal temperature in °F at different hours											
			-24 hr	MT	1 st hr	MT	2 nd hr	MT	3 rd hr	MT	4 th hr	MT	5 th hr	MT
Control (Double distilled water, 10ml/kg)	M1	20	103		101.2		101.2		101.3		101		100.8	99.8
	M2	22	101.3		100.5		100.4		100.2		100		99.9	
	M3	20	102.1	102.14	99.9	100.54	99.8	100.47	100	100.5	100.1	100.37	98.7	
Standard (Paracetamol 150mg/kg)	M1	21	101.8		99.7		98.5		98.2		98.2		98.0	97.8
	M2	23	103.3		99.0		98.3		98.3		98.0		97.8	
	M3	23	101.6	102.24	99.6	99.44	98.6	98.47	98.0	98.17	97.8	98.0	97.6	
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	M1	20	101.2		100.1		99.6		99.4		99.1		99.0	98.27
	M2	23	102.8	102.4	99.9	99.84	98.2	98.74	98.2	98.6	98.0	98.37	97.8	
	M3	21	103.2		99.5		98.4		98.2		98.0		98.0	

[BW=Body weight, MT= Mean temperature]

Table 6: Effect of ethanol extract of leaves of *P. edulis* on the temperature of Brewer's yeast induced pyrexia in mice

Test groups	SD						SE					
	-24 hr	1 st hr	2 nd hr	3 rd hr	4 th hr	5 th hr	-24 hr	1 st hr	2 nd hr	3 rd hr	4 th hr	5 th hr
Control (Double distilled water, 10ml/kg)	0.850	0.650	0.702	0.7	0.550	1.053	0.601	0.460	0.497	0.495	0.389	0.745
Standard (Paracetamol 150mg/kg)	0.929	0.378	0.153	0.152	0.2	0.2	0.657	0.268	0.108	0.108	0.142	0.142
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	1.058	0.306	0.757	0.7	0.635	0.642	0.748	0.216	0.535	0.49	0.45	0.455

[SD=Standard deviation, SE=Standard error]

Table 7: Effect of ethanol extract of leaves of *P. edulis* on the temperature (After 5 hours) of Brewer's yeast induced pyrexia in mice

Time interval	Test groups	Rectal temperature in °F	t-test (p-value)
-24 hr	Control (Double distilled water, 10ml/kg)	102.14±0.601	0
	Standard (Paracetamol 150mg/kg)	102.24±0.657	>0.05
	Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	102.4±0.216	>0.05
1 st hr	Control (Double distilled water, 10ml/kg)	100.54±0.460	0
	Standard (Paracetamol 150mg/kg)	99.44±0.268	>0.05
	Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	99.84±0.216	>0.05
2 nd hr	Control (Double distilled water, 10ml/kg)	100.47±0.497	0
	Standard (Paracetamol 150mg/kg)	98.47±0.108	<0.05
	Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	98.74±0.535	>0.05
3 rd hr	Control (Double distilled water, 10ml/kg)	100.5±0.495	0
	Standard (Paracetamol 150mg/kg)	98.17±0.108	<0.05
	Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	98.6±0.49	>0.05
4 th hr	Control (Double distilled water, 10ml/kg)	100.37±0.389	0
	Standard (Paracetamol 150mg/kg)	98±0.142	<0.05
	Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	98.37±0.45	<0.05
5 th hr	Control (Double distilled water, 10ml/kg)	99.8±0.745	0
	Standard (Paracetamol 150mg/kg)	97.8±0.142	>0.05
	Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	98.27±0.455	>0.05

[Values are expressed as , mean temperature ± SE (n = 3); SE=Standard error]

Table 8: Results of standard and test groups in comparison to control group**Results of antipyretic activity**

During antipyretic activity by Brewer's yeast induced pyrexia method in mice, the ethanol extract of leaves of *P. edulis* produced moderate antipyretic activity (Table 9.1, 9.2 & Table 9.3). In this test, the extract reduced temperature from 102.4°F to 99.84°F (p>0.05), 98.74°F

(p>0.05), 98.6°F (p>0.05), 98.37°F (p<0.05) and 98.27°F (p>0.05) in 1st, 2nd, 3rd, 4th and 5th hour respectively and caused maximum reduction of temperature in 1st hour.

Antidiarrheal activity

S/N	Test Samples	Group	Dose (/unit)	Routes of administration
01	1% Tween-80 in distilled water	Control	10ml/kg of body wt.	Orally
02	Loperamide	Standard	3mg/kg of body wt.	
03	Ethanol extract of <i>P. edulis</i> Sims.	Test Sample	500mg/kg of body wt.	
04	Castor oil	For induce diarrhea	0.4ml/mice	

Table 9: Test materials used in the evaluation of antidiarrhoeal activity**Determination of anti-diarrheal activity**

Antidiarrheal activity was determined by evaluating the latency period and diarrheal frequency by counting the feces number of the test group in comparison to the control and standard drug Loperamide.

Test groups	Mice No.	BW (g)	LP (mins.)	MLP (mins.)	SD	SE	t-test (p-value)
Control (1% Tween-80, 10ml/kg)	M1	21	45	49.34	6.66	4.71	0
	M2	22	57				
	M3	21	46				
Standard (Loperamide 3mg/kg)	M1	20	170	170.67	2.08	1.47	<0.05
	M2	22	169				
	M3	21	173				
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	M1	20	56	58.67	2.52	1.78	>0.05
	M2	23	59				
	M3	21	61				

BW=Body weight, LP=Latent period, MLP=Mean latent period, SD=Standard deviation, SE=Standard error

Table 10: Effect of ethanol extract of leaves of *P. edulis* Sims. on the latent period of castor oil induced diarrheal episodes in mice

Test groups	MLP (h)	% MLP	TLP
Control (1% Tween-80, 10ml/kg)	0.823	-	2.469±4.71
Standard (Loperamide 3mg/kg)	2.845	345.686	8.535±1.47
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	0.978	118.84	2.934±1.78

% MLP = $\frac{\text{Test group MLP} \times 100}{\text{Control MLP}}$, TLP = (MLP×3±SE), TLP= Total latent period

Table 11: Results of the treatment groups in comparison to the control latency period

Test groups	Mice No.	BW (g)	No. of faeces (240 min)	MD (240 min)	SD	SE	t-test (p-value)
Control (1% Tween-80, 10ml/kg)	M1	21	14	12.34	2.08	1.47	0
	M2	22	13				
	M3	21	10				
Standard (Loperamide 3mg/kg)	M1	20	5	5.34	0.58	0.41	<0.05
	M2	22	6				
	M3	21	5				
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	M1	20	8	8.34	1.53	1.08	>0.05
	M2	23	10				
	M3	21	7				

[BW = body weight, MD = Mean defecation, SD=Standard deviation, SE=Standard error]

Table 12: Effect of ethanol extract of leaves of *P. edulis* Sims. on castor oil (0.4ml each mouse at 240min.) induced diarrhea in mice

Test groups	% Defecation-	% Inhibition of Defecation	TNF (240min)
Control (1% Tween-80, 10ml/kg)	100	0	37±1.472
Standard (Loperamide 3mg/kg)	43.09	56.91	16±0.408

Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	67.80	32.2	25±1.08
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*[TNF = Total number of faeces (MD×3 ± SE)], MD= Mean defecation, SE= Standard error]

Table 13: Results of the treatment groups in comparison to the control 4hrs defecation period

Results of antidiarrheal activity

During antidiarrheal activity by castor oil induced test in mice, the ethanol extract of *P. edulis* produced moderate antidiarrheal episode. The latent period and the total diarrheic faces were found to be 0.978 hr & 8.34 at

500 mg/kg for *P. edulis* as compared to standard (Loperamide 3mg/kg) 2.845 hr & 5.34.

Sedative and hypnotic activity

Test groups	Mice No.	BW (g)	No. of field cross	MNFC	SD	SE	t-test (p-value)
Control (Double distilled water, 10ml/kg)	M1	22	101	101.34	1.53	1.08	0
	M2	21	100				
	M3	21	103				
Standard (Diazepam 4mg/kg)	M1	21	64	63	1	0.707	<0.05
	M2	20	62				
	M3	23	63				
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	M1	21	84	84.34	1.53	1.08	<0.05
	M2	21	86				
	M3	23	83				

[BW=Body weight, MNFC=Mean number of field cross, SD=Standard deviation, SE=Standard error]

Table 14: Effect of ethanol extract of leaves of *P. edulis* Sims. on open field test in mice

Test groups	MNFC±SD
Control (Double distilled water, 10ml/kg)	101.34±1.53
Standard (Diazepam 4mg/kg)	63±1
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	84.34±1.53

[MNFC=Mean number of field cross, SD=Standard deviation]

Table 15: Sedative and hypnotic activity by open-field test

Test groups	Mice No.	BW (g)	No. of swing	MNS	SD	SE	t-test (p-value)
Control (Double distilled water, 10ml/kg)	M1	22	12	13.34	1.155	0.817	0
	M2	21	14				
	M3	21	14				
Standard (Diazepam 4mg/kg)	M1	21	4	3.67	0.58	0.408	<0.05
	M2	20	4				
	M3	23	3				
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	M1	21	8	8.67	0.58	0.408	<0.05
	M2	21	9				
	M3	23	9				

[BW=Body weight, MNS=Mean number of swing, SD=Standard deviation, SE=Standard error]
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Table 16: Effect of ethanol extract of leaves of *P. edulis* Sims. on swing test in mice

Test groups	MNS±SD
Control (Double distilled water, 10ml/kg)	13.34±1.155
Standard (Diazepam 4mg/kg)	3.67±0.58
Ethanol extract of <i>P. edulis</i> Sims. (500mg/kg)	8.67±0.58
[MNS=Mean number of swing, SD=Standard deviation]	

Table 17: Sedative and hypnotic activity by swing test

Results of sedative and hypnotic activity

In open field box, hole cross box and swing test method on swiss albino mice ethanol extract of *P. edulis* showed moderate sedative and hypnotic activity. During the open field test, squares crossed by the group receiving standard Diazepam and ethanol extract of leaves of *P. edulis* were 63 and 84.34 respectively. During the swing test, number of swings observed after administration of standard Diazepam and ethanol extract of leaves of *P. edulis* were 3.67 and 8.67 respectively.

Conclusion

The results of the phytochemical investigation of the ethanol extract of leaves of *P. edulis* has shown a remarkable variation in the presence of the above studied phytochemical compounds in the studied taxa. The study revealed that the ethanol extract of leaves of *P. edulis* are showing maximum presence of alkaloids, glycosides, flavonoids and phenols in ethanol solvent extracts. Pathogenic microbial infectious agents exhibit the increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs, problems with multiple dosage regimens and antibiotic resistance. All over the world, for their possible activity, appearance of new diseases has led to the screening of medicinal plants. A large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant produce drugs and medicines are imported in Bangladesh. If the manufacturers, to satisfy their needs, utilize the native medicinal plants or their semi processed products huge foreign exchanges can be saved.

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