

Evaluation of antioxidant, analgesic and antidiarrheal activity of *Phoenix paludosa roxb* Leaves

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Abstract – In the present study, the ethanol extract of *Phoenix paludosa Roxb*'s leaves were investigated for evaluating antioxidant, analgesic and antidiarrheal potential. Scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, total phenolic and flavonoid content and assessment of reducing power was used to investigate antioxidant potential of the leaves. Acetic acid induced writhing and hot-plate test were utilized to evaluate analgesic activity. Castor oil and magnesium sulfate induced diarrheal model and gastrointestinal motility test were used to evaluate antidiarrheal potential of the leaves. The extract showed IC₅₀ value of 13.88 µg/ml in DPPH scavenging assay. Total phenolic content was determined by Folin-Ciocalteu's reagent and the value was 8.29 mg GAE/g of dried plant material. Total flavonoid content was 11.42 mg QE/g of dried plant material. The extract also showed reducing power in concentration dependent manner. In dose dependent manner, the extract significantly ($P < 0.001$) exhibited inhibition of writhing in acetic acid induced writhing test. In hot-plate test, pain threshold was raised significantly ($P < 0.001$) at both doses in same manner. The extract significantly ($P < 0.001$) and dose dependently decreased the frequency of diarrhea and increased the latent period in both models. The extract also delayed the intestinal transit of charcoal meal in mice and the results were statistically significant ($P < 0.001$). The results suggest that the ethanol leaves extract of *P. paludosa* could be used as potential antioxidant, analgesic and antidiarrheal agent and demands further experimental analysis to clarify the underlying mechanism.

Keywords –: DPPH assay, phenolic content, flavonoid content, reducing power, writhing test, hot plate test, castor oil, magnesium sulfate.

1. Introduction

Phoenix paludosa Roxb. (Arecaceae) is a flowering plant belongs to the palm family, indigenous to coastal regions of Bangladesh, India, Thailand and Myanmar. It is small to medium sized date palm, up to 5 m tall, arching leaves with narrow and dropping leaflets. The leaves are 3-5 m long with spines on the petiole and leaflets are 30 cm long and 2 cm wide. The flowers are yellowish-brown and about 1 cm wide with large multi-branched panicles of 30-90 cm long. The fruits are single seeded drupe, fleshy and smooth with blackish brown exocarp. It grows all over the Sundarban in Bangladesh and commonly known as Hantal and Hital. Traditionally the plant is used as antipyretic and anti-inflammatory agent in the early days in various regions of Bangladesh. Seed is used as animal feed and coffee additive. It is also used in soap and cosmetic industries.

In previous study, DPPH scavenging activity, lipid peroxidation inhibition and quinine reductase induction activity of twig of *P. paludosa* were investigated and reported [1]. Lupeol, epilupeol and β -sitosterol were isolated from the methanol leaves extract of *P. paludosa* and several fractions were subjected to bioactivity studies to reveal antimicrobial and cytotoxic property [2].

Upon literature survey and based on traditional uses, the present study was designed to investigate antioxidant, analgesic and antidiarrheal activities of the ethanol leaves extract of *P. paludosa*.

2. Materials and Methods

2.1. Chemicals and Reagents

Ascorbic acid, potassium ferricyanide, trichloroacetic acid, gallic acid, sodium carbonate, ferric chloride, acetic acid and magnesium sulfate were purchased from Merck, Germany. Folin-Ciocalteu's reagent, quercetin and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Tween-80 and castor oil were purchased from Loba Chemie Pvt Ltd, India. Solvents and all other reagents were of analytical grade.

2.2. Standard Drugs

Diclofenac sodium and loperamide were obtained from Beximco Pharmaceuticals Ltd, Bangladesh. Morphine sulfate was obtained from Popular Pharmaceuticals Ltd, Bangladesh.

2.3. Phytochemical Screening

The ethanol leaves extract *P. paludosa* was subjected to different preliminary phytochemical tests to identify major functional groups [4-5].

2.4. Plant Materials

For the present investigation, the fresh leaves of *P. paludosa* were collected from Sundarban, Khulna, Bangladesh in January' 2011 and were taxonomically identified by the experts at Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen (Accession no. DACB-34176) was also deposited there for further study.

2.5. Preparation of Plant Extract

The fresh leaves of *P. paludosa* were shade dried and powdered by grinder. About 250 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of ethanol. The container was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The mixture was filtered with clear cotton plug to remove all debris. Then it was filtered through Whitman filter paper. Dried extract was stored at 4 °C in air tight container and the yield was 1.68% of dried plant material.

2.6. Experimental Animals

Young Swiss-Albino mice aged 4-5 weeks; appropriate average weights (20-25 gm) were procured from International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B) and kept in standard environmental condition for one week in the animal house of Pharmacy Discipline, Khulna University for adaptation. They were housed in standard plastic polypropylene cages and maintained under standard conditions at 25 ± 0.5 °C and relative humidity of 55-60% and 10 h light: 14 h dark cycle each day for one week before and during the investigations. All animals were provided with the standard rodent pellet diet and water *ad libitum*. Experiments were performed according to animal ethics guidelines [3].

2.7. In Vitro Antioxidant Activity

2.7.1. DPPH Scavenging Assay

Free radical scavenging activity of the ethanol leaves extract was determined by DPPH scavenging assay [6]. Sample was prepared in ethanol at different concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml. Sample of 1 ml of each concentration was added to 3 ml of 0.004% ethanol solution of DPPH. After incubation of 30 min at room temperature, absorbance was measured at 570 nm against blank. Ascorbic acid was used as standard free radical scavenger and activity of the extract was compared with it. The percent inhibition was calculated from the formula: $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance of extract or standard. IC₅₀ was calculated from the graph obtained by plotting % inhibition versus concentration (µg/ml).

2.7.2. Total Phenolic and Flavonoid Content Determination

2.7.2.1. Sample Preparation

The extract of 0.5 g was weighted and dissolved in 50 ml of 80% aqueous methanol. Then the mixture was sonicated for 20 min in ultrasonic bath to extract phenolic and flavonoid compounds. Then 2 ml of the sonicated mixture was centrifuged for 5 min at 14000 rpm.

2.7.2.2. Total Phenolic Assay

Total phenolic content of the ethanol leaves extract was determined by using Folin Ciocalteu's reagent [7]. An aliquot (1 ml) of extract or standard methanol solution of gallic acid (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml) was added to 9 ml of distilled water in 25 ml volumetric flask. Diluted (10 times with distilled water) Folin Ciocalteu's reagent of 1 ml was added to the mixture with continuous shaking. After the interval of 5 min, 10 ml of 7% Na₂CO₃ was added to the

mixture and adjusted with distilled water to the volume of 25 ml. After incubation of 30 min at room temperature, the absorbance was measured at 750 nm against blank. Standard calibration curve of gallic acid was prepared by plotting absorbance versus concentration (µg/ml). Total phenolic content of the extract was expressed as mg gallic acid equivalent (GAE)/g of dried plant material.

2.7.2.3. Total Flavonoid Assay

Total flavonoid content of the ethanol leaves extract was determined by the aluminum chloride colorimetric assay [7]. An aliquot (1 ml) of extract or standard methanol solution of quercetin (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml) was added to 4 ml of distilled water in 10 ml volumetric flask. Then 0.3 ml of 5 % NaNO₂ was added to the mixture. After the interval of 5 min, 0.3 ml of 10 % AlCl₃ was added to the mixture. Then 2 ml of 1M NaOH was added to the mixture and adjusted to 10 ml with distilled water. The solution was mixed with continuous shaking. Then absorbance was measured at 510 nm against blank. Standard calibration curve of quercetin was prepared by plotting absorbance versus concentration (µg/ml). Total flavonoid content of the extract was expressed as mg quercetin equivalent (QE)/g of dried plant material.

2.7.3. Reducing Power Assay

Method described by Oyaizu, 1986 [8]. Extract was prepared at the different concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml. An aliquot (1 ml) of extract of each concentration was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K₃Fe(CN)₆]. Then the mixture was incubated for 30 min at 50 °C. After that, 2.5 ml of trichloroacetic acid (TCA) (10%) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of Ferric chloride (FeCl₃) (0.1%) with continuous shaking. After 5 min, absorbance was measured at 700 nm. Ascorbic acid was used as standard and reducing power of the extract was compared with it.

2.8. Analgesic Activity

2.8.1. Acetic Acid Induced Writhing Test

Acetic acid induced writhing test was conducted according to the method of Koster et al., 1959 [9]. Randomly screened experimental animals were divided into four groups ($n = 6$) to carry out the present investigation. Test groups received the ethanol leaves extract at the doses of 250 and 500 mg/kg in oral route. Standard analgesic drug diclofenac sodium (25 mg/kg, p.o.) was administered to the positive control group. Control group orally received 1% tween-80 in distilled water at the dose of 10 mL/kg. After the interval of 30 min, each animal was given an intraperitoneal (i.p.) injection of 0.6% v/v acetic acid at the dose of 10 mL/kg to induce the characteristic writhing. After 5 min, the number of writhing was recorded for the period of 10 min for each mouse. The percent writhing inhibition was calculated and compared with control to assess analgesic activity.

2.8.2. Hot-Plate Test

Experimental animals were selected based on their reaction time between 3-5 sec when placed in hot-plate maintained at

the temperature of 55 ± 0.5 °C and animals not responding in this period were rejected. Selected animals were divided into four groups ($n = 6$) and subjected to different treatments. Leaves extract (250 and 500 mg/kg, p.o.) was given to the test groups. Standard morphine sulfate (5 mg/kg, i.p.) was administered to the positive control group. Control group received 1% tween-80 in distilled water (10 mL/kg, p.o.). Each mouse was placed in the hot-plate to record the reaction time at before (0), 30, 60, 90 and 120 min. analgesic activity was assessed by comparing the reaction time with control group [10].

2.9. In-Vivo Antidiarrheal Activity

2.9.1. Castor Oil-Induced Diarrhea

The experiment was carried out according to the method described by Abdullahi et al., 2001 [11]. Experimental animals were selected initially based on their sensitivity to castor oil-induced diarrhea and divided into four groups ($n = 6$). Test groups were provided with the leaves extract (250 and 500 mg/kg, p.o.) and positive control group was supplied with standard antidiarrheal drug loperamide (3 mg/kg, p.o.) in suspension. Control group was supplied with 1% tween-80 in distilled water (10 mL/kg, p.o.). After the interval of 60 min, each animal was given 0.5 mL of castor oil in oral route to induce diarrhea. Each animal was placed in individual plastic transparent cage and floor was lined with white blotting paper which was changed in every hour throughout the observation period of 4 h. Onset of diarrhea and total number of faces for each animal was recorded. Latent period and percent inhibition of defecation were compared with control group to assess antidiarrheal activity.

2.9.2. Magnesium Sulfate-Induced Diarrhea

Magnesium sulfate was administered orally at the dose of 2 g/kg to the animals after 30 min of the administration of different treatments [12]. Control group received 1% tween-80 in distilled water (10 mL/kg, p.o.); positive control group received loperamide (3 mg/kg, p.o.) in suspension and test groups received ethanol leaves extract (250 and 500 mg/kg, p.o.). Onset of diarrhea and total number of stools were recorded for each mouse. To reveal antidiarrheal activity latent period and percent inhibition of defecation were compared with control group.

2.9.3. Effect on Gastrointestinal Motility

Experimental animals were divided into four groups ($n = 6$) to conduct the current investigation. Each animal was given 1 mL charcoal meal (5% activated charcoal suspended in 1% tween-80) orally after 60 min of the administration of all the treatments. Test groups received ethanol leaves extract (250 and 500 mg/kg, p.o.); positive control group received standard antimotility drug loperamide (3 mg/kg, p.o.) and control group received 1% tween-80 in distilled water (10 mL/kg, p.o.). After the interval of 30 min, each animal was anaesthetized by chloroform and dissected. Intestine was removed and placed on moist filter paper. The intestinal transit was calculated as the percent distance travelled by charcoal compared to the length of the respective intestine [12].

2.10. Statistical Analysis

Results are expressed as Mean $\pm$ S.E.M. The difference between experimental and control group was determined by Student's t-test. The results were considered statistically significant when $P < 0.001$.

3. Results

3.1. Phytochemical Screening

In phytochemical screening the ethanol leaves extract of *P. paludosa* revealed the presence of reducing sugars, glycosides, steroids, gums, flavonoids and tannins (Table 1).

Table 1: Phytochemical screening of *P. paludosa* leaves

Phytochemical Result	constituents
Reducing sugars	+
Alkaloids	-
Glycosides	+
Steroids	+
Gums	+
Saponins	-
Flavonoids	+
Tannins	+

+ = Presence - = Absence

3.2. In Vitro Antioxidant Activity

3.2.1. DPPH Scavenging Activity

The scavenging of DPPH free radical was in concentration dependent manner. Activity was increased with the gradual increase of concentration. The ethanol leaves extract of *P. paludosa* showed IC_{50} value of 13.88 μ g/ml whereas standard ascorbic acid showed IC_{50} value of 7.55 μ g/ml (Figure 1).

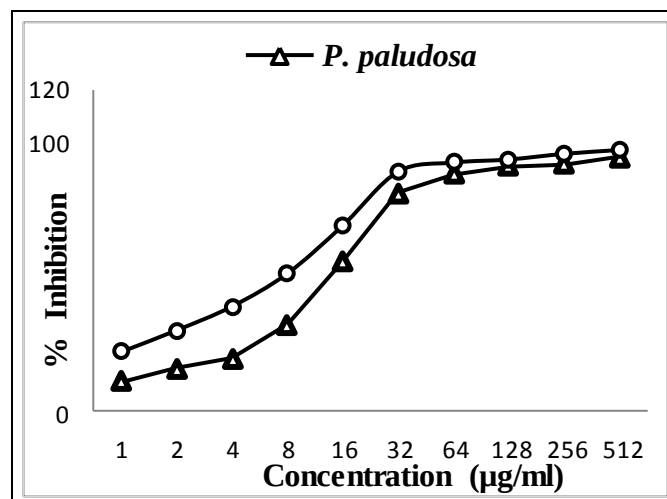


Figure 1. DPPH scavenging activity of *P. paludosa* leaves

3.2.2. Total Phenolic and Flavonoid Content

The total phenolic content was found to be 8.29 mg GAE/g of dried plant material (Figure 2) and total flavonoid content was found to be 11.42 mg QE/g of dried plant material (Figure 3).

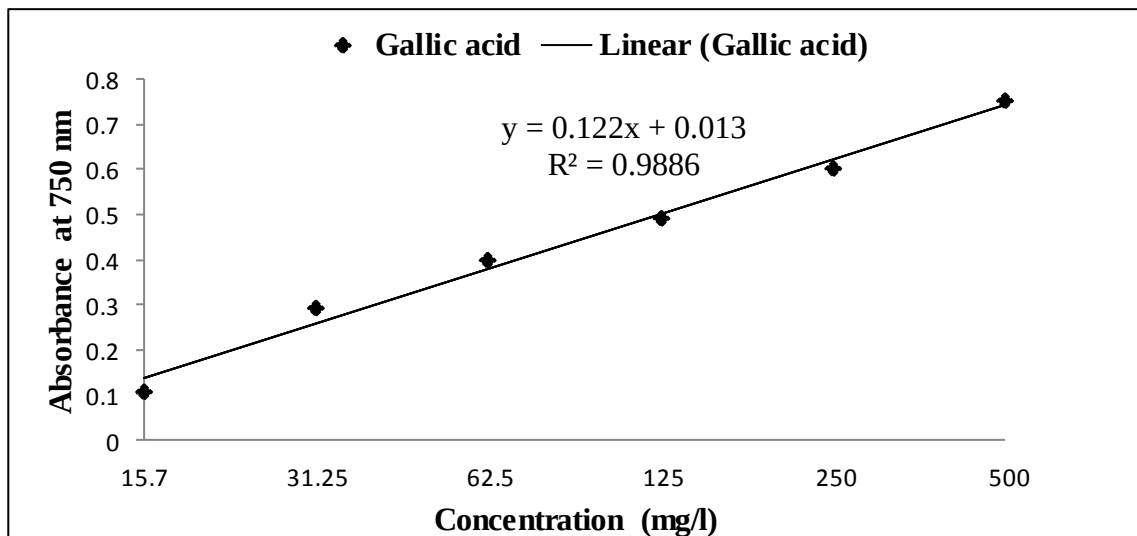


Figure 2. Standard calibration curve of Gallic acid

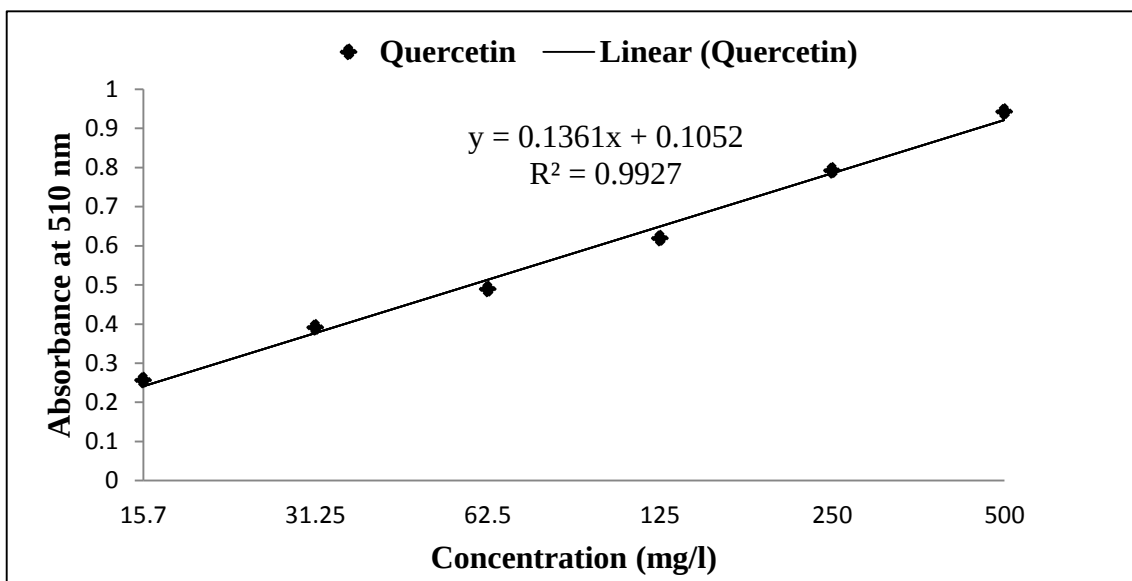


Figure 3. Standard calibration curve of Quercetin

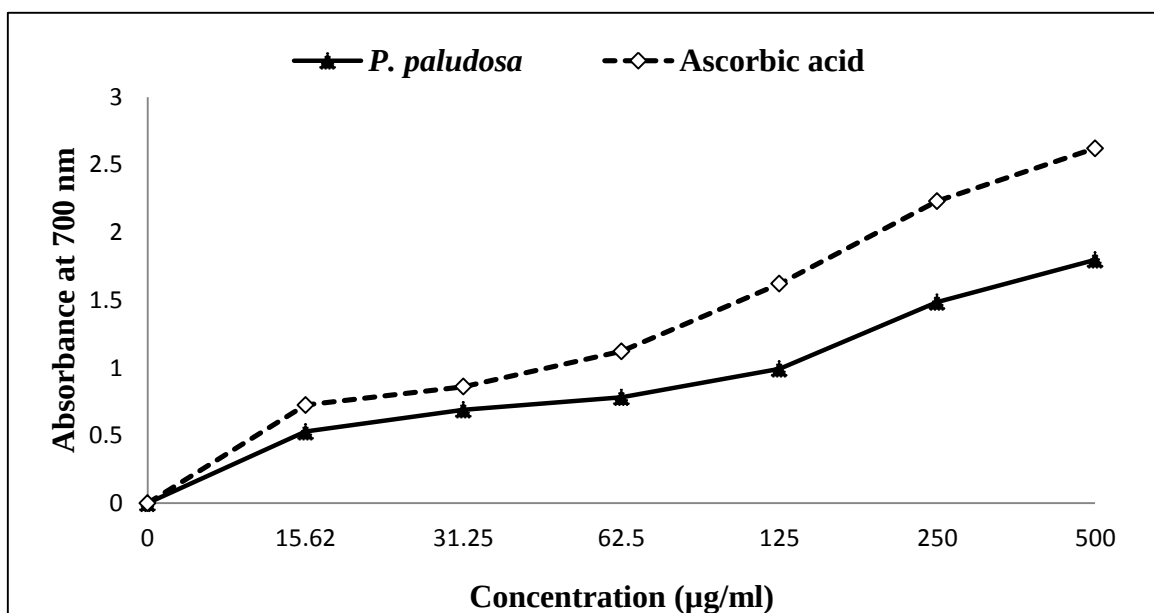


Figure 4. Reducing power of *P. paludosa* leaves

3.2.3. Reducing power

Reducing power of the ethanol leaves extract of *P. paludosa* was increased with the increase of concentration and compared with standard ascorbic acid (Figure 4). Absorbance was increased with the increase of concentration which substantiated strong reducing ability.

3.3. Analgesic Activity

3.3.1. Activity in Acetic Acid-Induced Writhing Test

The ethanol leaves extract of *P. paludosa* showed dose dependent inhibition of writhing. The extract exhibited 47.79 and 65.44% inhibition of writhing at the doses of 250 and 500 mg/kg, respectively and results were statistically significant ($P < 0.001$). Standard diclofenac sodium also showed strong analgesic activity with 76.10% inhibition of writhing as compared with control. Activity of the extract was strongly comparable with the standard (Table 2).

Table 2: Effect of *P. paludosa* leaves on acetic acid induced writhing in mice

Treatment <i>n</i> = 6	Dose (mg/kg)	No. of writhes	% inhibition
Control	---	27.2±1.36	---
Diclofenac sodium	25	6.5±0.47*	76.10
Extract 250	250	14.2±0.61*	47.79
Extract 500	500	9.4±0.52*	65.44

Results are expressed as mean ± SEM, SEM= Standard error for mean, * $P < 0.001$ versus control, Student's t-test.

3.3.2. Activity in hot-plate test

The ethanol leaves extract *P. paludosa* significantly ($P < 0.001$) raised pain threshold in dose dependent manner and maximum reaction time was observed at 60 min for both doses (250 and 500 mg/kg). Standard morphine sulfate (5 mg/kg) also raised pain threshold and maximum reaction time was observed at 90 min (Table 3).

3.4. In Vivo Antidiarrheal Activity

3.4.1. Effect On Castor Oil-Induced Diarrhea

In castor oil induced diarrhea, the ethanol leaves extract *P. paludosa* significantly and dose dependently increased onset of diarrhea as compared with control ($P < 0.001$). The extract showed 46.11 and 61.23% inhibition of defecation at the doses of 250 and 500 mg/kg, respectively (Table 4). Standard drug loperamide (3 mg/kg) also increased onset of diarrhea and exhibited 88.58% inhibition of defecation.

3.4.2. Effect on Magnesium Sulfate-Induced Diarrhea

The ethanol leaves extract *P. paludosa* showed significant antidiarrheal activity in magnesium sulfate-induced diarrhea in dose dependent manner (Table 5).

The extract prolonged the onset of diarrhea at both doses and also reduced the total number of faces as compared with control. The extract showed 69.63 and 63.92% of inhibition at the doses of 250 and 500 mg/kg, respectively. Standard drug loperamide (3mg/kg) showed 82.68% inhibition of defecation as well as prolonged the onset of diarrhea.

3.4.3. Effect on Gastrointestinal Motility

The ethanol leaves extract *P. paludosa* significantly ($P < 0.001$) and dose dependently retarded the intestinal transit charcoal meal in experimental mice as compared with control (Table 6). Standard antimotility drug loperamide also retarded intestinal transit of charcoal meal.

Table 3. Effect of *P. paludosa* leaves in hot-plate test in mice

Treatment <i>n</i> = 6	Dose (mg/kg)	Reaction time (sec)				
		0 min	30 min	60 min	90 min	120 min
Control	---	4.28±0.05	4.29±0.04	4.37±0.05	4.13±0.08	4.35±0.09
Morphine	5	4.51±0.05*	7.68±0.12*	8.18±0.19*	10.11±0.18*	9.39±0.12*
Extract 250	250	4.34±0.09*	5.86±0.05*	6.90±0.12*	6.05±0.09*	5.55±0.18*
Extract 500	500	4.52±0.11*	6.69±0.07*	7.81±0.09*	7.02±0.14*	6.15±0.19*

Results are expressed as mean ± SEM, SEM= Standard error for mean, * $P < 0.001$ versus control, Student's t-test.

Table 4. Effect of *P. paludosa* leaves on castor oil-induced diarrhea in mice

Treatment n=6	Dose (mg/kg)	Onset of diarrhea (min)	No. of stools after 4 h	% Inhibition of defecation
Control	---	45.68±2.18*	21.9±1.22*	---
Loperamide	3	175.28±1.26*	2.5±0.57*	88.58
Extract 250	250	90.25±4.16*	11.8±0.25*	46.11
Extract 500	500	112.29±3.66*	8.49±0.42*	61.23

Results are expressed as mean ± SEM, SEM= Standard error for mean,
*P < 0.001 versus control, Student's t-test.

Table 5. Effect of *P. paludosa* leaves on magnesium sulfate-induced diarrhea in mice

Treatment n=6	Dose (mg/kg)	Onset of diarrhea (min)	No. of stools after 4 h	% Inhibition of defecation
Control	---	43.12±1.12*	20.21±1.06*	---
Loperamide	3	185.28±1.06*	3.5±0.67*	82.68
Extract 250	250	70.59±3.23*	12.2±0.53*	39.63
Extract 500	500	101.69±3.45*	7.29±0.61*	63.92

Results are expressed as mean ± SEM, SEM= Standard error for mean,
*P < 0.001 versus control, Student's t-test.

Table 6: Effect of *P. paludosa* leaves on charcoal meal stimulated gastrointestinal transit in mice

Treatment n = 6	Dose (mg/kg)	Mean intestinal length (cm)	Mean distance traveled by charcoal (cm)	% GI transit
Control	---	63.58±1.69*	48.03±0.96*	75.54
Loperamide	3	65.88±1.51*	19.59±1.17*	29.73
Extract 250	250	61.29±2.03*	32.54±0.81*	53.09
Extract 500	500	69.21±2.09*	23.03±0.89*	33.27

Results are expressed as mean ± SEM, SEM= Standard error for mean,
*P < 0.001 versus control, Student's t-test.

4. Discussion

The antioxidant potential of the ethanol leaves extract of *P. paludosa* was substantiated by several established *in vitro* models. DPPH is a stable free radical converts into stable diamagnetic molecule by accepting electron or hydrogen radical and most widely used in free radical scavenging assay to assess the ability of extract to scavenge reactive free radical. The extract showed potential DPPH scavenging activity, which was strongly comparable to the standard antioxidant ascorbic acid.

Phenols and flavonoids are considered as most responsible functional groups having antioxidant potential in plant source. There is a strong positive relationship between the phenolic content and antioxidant potential of different plant species because of the scavenging ability of hydroxyl groups attached with phenols [13]. It is also established

that phenolic groups are effective hydrogen donors, which lead to the attribute of good antioxidants [14]. Flavonoids act as scavengers of various "reactive oxygen species (ROS)" like superoxide anion, hydroxyl, peroxy, hydrogen peroxide etc. They are also reported as quenchers of singlet oxygen [15]. The extract revealed good amount of flavonoids which justifies radical scavenging activity showed in DPPH assay. It is well known that phenols and flavonoids constituents of plants exhibit antioxidant potential due to their redox properties with metal chelating potential [16].

Reducing power assay was also utilized to evaluate antioxidant activity of the extract because antioxidants act through reducing reactive free radicals. Reducing power was determined by evaluating the ability of reducing ferricyanide $[\text{Fe}(\text{CN})_6]^{3-}$ to ferrocyanide $[\text{Fe}(\text{CN})_6]^{4-}$ through electron donation. Resulting product was estimated by adding Fe^{3+} ions which lead to the formation of Prussian blue complex $(\text{Fe}^{3+})_4[\text{Fe}^{2+}(\text{CN})_6]^{3-}$, which shows absorbance at 700 nm [17]. The extract showed potential reducing power exhibited by the increase of absorbance with the increase of concentration and activity was highly comparable with standard ascorbic acid.

Analgesic activity of the extract was evaluated by two widely used models namely acetic acid-induced writhing and hot-plate method. Acetic acid-induced writhing method was used to assess peripherally acting analgesic activity of the plant extract in which writhing results from the sensitization of pain receptors by prostaglandins release [18-19]. Acetic acid-induced abdominal contraction is most sensitive and established model to evaluate peripherally acting analgesia. Local peritoneal receptors are involved to this response [20]. The extract showed potential analgesic activity exhibited by dose dependent inhibition of writhing as compared to control group. The probable mechanism may be the inhibition of prostaglandins (PGE_2 and PGE_{20}) synthesis. Hot-plate method was utilized to assess centrally acting analgesic activity. The extract raised pain threshold in dose dependent manner which substantiated central analgesia. Opioid and steroid analgesic drugs act in the spinal cord level by binding with different receptors like μ , δ and κ in pre and post synaptic membrane as well as inhibiting neurotransmitter release and transmission. Probable mechanism of the extract may be same as opioid analgesics like standard morphine [21-23].

Castor oil induced diarrheal model was utilized to evaluate antidiarrheal effect of the extract and the model was very much logical because prostaglandins are involved in causation of diarrhea by castor oil through the release of ricinoleic acid which causes irritation of the intestinal mucosa [24]. Several mechanism are already reported in previous reports to illustrate the causes of castor oil induced diarrhea like inhibition of intestinal Na^+ , K^+ -ATPase activity [25], stimulation of prostaglandins formation through irritation of the intestinal mucosa [26], activation of adenylate cyclase mediated active secretion [27] and contribution of nitric oxide [28]. The leaves extract also showed remarkable diarrheal activity in magnesium sulfate-induced diarrhea in mice. It has been reported that magnesium sulfate causes diarrhea by

increasing the volume of intestinal content through inhibition of reabsorption of fluids and watery materials. It is also established that magnesium sulfate causes the liberation of cholecystokinin from the duodenal mucosa; which elevates the secretion and intestinal motility resulting the inhibition of reabsorption of salts and fluids [29]. So that we can definitely say that the extract might have ability to increase reabsorption of water and electrolyte. Moreover, this antisecretory effect was also supported by the results of the gastrointestinal motility test. The extract significantly delayed gastrointestinal transit of charcoal meal as compared with control; which ensures that the extract might have ability in greater extent to decrease gastrointestinal motility as well as to increase reabsorption of fluids and electrolytes.

5. Conclusion

The results obtained in the present study indicated that the ethanol leaves extract of *P. paludosa* possesses potential antioxidant, analgesic and antidiarrheal activities, which rationales its uses in folk medicine and demands further investigations like LC-MS to screen bioactive compounds responsible for these bioactivities as well as to illustrate actual mechanisms.

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