ISSN: 2279 - 0594

Available Online at www.jbpr.in

Journal of Biomedical and Pharmaceutical Research 2 (3) 2013, 52-63

RESEARCH ARTICLE

STUDY OF PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTIDIARRHOEAL AND ANALGESIC ACTIVITIES OF HYDROMETHANOL EXTRACTS OF *POLYALTHIA SUBEROSA* (ROXB.)

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Received 18/04/2013; Revised 23 April 2013; Accepted 28 April 2013

ABSTRACT

Hydromethanol extracts of three different parts namely leaves, bark and fruits of the *Polyalthia suberosa* (Roxb.) was screen for it's antioxidant, analgesic and antidiarrheal activity. Bark extracts showed the maximum reducing activity in both model and higher than the reference standard ascorbic acid. Fruits extract was also found to have strong reducing capacity but slightly lower compared to the ascorbic acid. The analgesic activity of all parts of *P. suberosa* at all doses, produced significant (p<0.05-0.01) inhibition of writhing reaction in a dose dependent manner. Maximum inhibition of writhing (55.15% inhibition) was produced by the bark extract at 400 mg/kg dose whereas fruits and leaves extracts at the same dose displayed a maximum of 53.09% and 38.92% inhibition of writhing response respectively and at the dose of 200 and 400 mg/kg, produced a significant (p<0.05-0.001) increase in pain threshold in tail immersion methods in a dose dependent manner after 60 and 90 min of drug administration. In castor oil-induced diarrhoea statistically significant (p<0.05) inhibition of frequency of diarrhoeal was observed with bark (69.52% inhibition) and fruits (63.81% inhibition) of the plant at 400 mg/kg dose level. Similarly in MgSO4-induced diarrhoeal model, the antidiarrhoeal actions exhibited by the hydromethanol extracts of the three parts of the plant, at 400 mg/kg dose, were found to be statistically significant (p<0.05) and was comparable to the reference drug Loperamide.The overall results were bark and fruits extracts of *Polyalthia suberosa* possess strong antioxidant potential along with excellent analgesic and antidiarrheal activity while leaves extract possess moderate activity.

KEYWORDS: *Polyalthia suberosa,* antioxidant, analgesic and antidiarrheal activity

INTRODUCTION:

Polyalthia suberosa Roxb. (Synonym: *Uvaria suberosa* Roxb.Family: Annonceae), locally known as Jam Debdaru, ham jam, is a short small tree widely distributed in Bangladesh, West Indies, Philippine, India, Sri-Lanka, Malaysia and Myanmar . Fruits are used to stop diarrhea. Fruits and flowers are used to relieve pulmonary complaints. Leaves are used as a remedy for coughs, colds and diarrhea. It is also used in flatulence and as Anti-HIV agent. Bark is regarded as a febrifuge and is said to halt diarrhea and dysentery. It is powerful astringent and also used as analgesic and laxative. Seed have a diuretic action and is a sedative and soporific. The latex is used in the tropics as a crude filling for tooth cavities.the aim of the study was to investigate the analgesic, antidiarrhoeal and antioxidant activities of Hydromethanol extracts of three different parts namely leaves, bark and fruits of the *Polyalthia suberosa* (Roxb.)

MATERIAL AND METHOD:

COLLECTION AND IDENTIFICATION:

Various parts of the plants leaves, bark and fruits (*Polyalthia suberosa)* were collected from the village Roypur of Chuadanga district and identified by the expert of Bangladesh National Herbarium Mirpur, Dhaka.

DRYING OF THE PLANT SAMPLE:

The flowering tops and other adulterants were removed to get fresh sample. Then the collected samples were dried for two days in the laboratory under electric fan. Finally the leaves, bark and fruits were dried in hot air woven at 55^0C for 2 days and at 40^0C for the next 2 days. Before drying the samples were cut and sliced where necessary.

GRINDING OF THE DRIED SAMPLES:

The dried samples were grounded to coarse powder with a mechanical grinder (Grinding Mill) and

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powdered samples were kept in clean closed glass containers pending extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously grounded material or other foreign matters deposited on the grinder.

EXTRACTION OF THE DRIED POWDERED SAMPLES:

The dried leaves, barks and fruits of *Polyalthia suberosa* were coarsely powdered by a milling machine and extracted with a mixture of methanol:water (7:3, v/v) by a Soxhlet apparatus at 50°C. After completion of the extraction from different parts of plants, the extracts were filtered using a sterilized cotton filter. Then solvent was completely removed and obtained dried crude extract which were used for investigation.

PHYTOCHEMICAL SCREENING:

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

Table 1: Results of phytochemical screening

Test for	Leaves	Bark	Fruits
Carbohydrates	+	+	+
Reducing sugar	+	+	+
Glycosides	+	_	_
Alkaloids	+	+	+
Saponins	+	_	_
Flavonoids	+	+	+
Tannins	+	+	+

"+" indicates present, "-" Indicates absent

IN VITRO ANTIOXIDANT ACTIVITY SCREENING OF P. SUBEROSA:

DETERMINATION OF TOTAL PHENOL:

To measure total phenol content of plant extract, extract (100 µl) was mixed with 500 µL of the Folin–Ciocalteu reagent and 1.5 mL of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. It was allowed to stand for 2 h. Then the absorbance at 765 nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid (Yu *et al*., 2002).

DETERMINATION OF FLAVONOID CONTENT:

The total flavonoid content was estimated using a method previously described by Kumaran and Karunakaran (2007) using quercetin as a reference compound. 1ml of plant extract in methanol (50-250 µg/ml) was be mixed with 1ml aluminium trichloride in ethanol (20 mg/ml and a drop of acetic acid, and then diluted with ethanol to 25 ml. The absorption at 415nm was read after 40 min. Blank samples was prepared from 1ml of plant extract and a drop of acetic acid, and then diluted to 25 ml with ethanol. The absorption of standard quercetin solution (0.5 mg/ml) in ethanol was measured under the same conditions. These data were used to determine the flavonoid content using a standard curve obtained from various concentration of quercetin.

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY:

The total antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). 0.3 ml extracts was mixed with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. The total antioxidant activity was expressed as the number of equivalents of ascorbic acid.

DPPH FREE RADICAL SCAVENGING ASSAY:

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm determined after 30 min, and the percentage inhibition activity was calculated.

NITRIC OXIDE SCAVENGING ASSAY:

Nitric oxide radical scavenging was estimated on the basis of Griess Illosvoy reaction using method followed by Govindarajan et al (2003). In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%).

REDUCING POWER CAPACITY ASSESSMENT:

The reducing power of the plants extracts was determined according to the method previously described (Oyaizu, 1986). Different concentrations of plants extracts (100 µg – 1,000 µg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was then incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml. 0.1%) and the absorbance was measured at 700 nm.

CUPRIC REDUCING ANTIOXIDANT CAPACITY (CUPRAC):

In the experiment, 500µl of each fraction and standard (ascorbic acid) in different concentrations were taken in test tubes. 1.0 ml of 0.01M $CuCl_2.2H_2O$ solution was added into the test tubes. 1.0 ml of ammonium acetate buffer (pH 7.0) was added into the test tubes. Then 1.0 ml of 0.0075 ml of neocaproin solution was added into the test tubes. After addition of 600 µl of distilled water the final volume of the mixture was adjusted to 4.1 ml. The total mixture was incubated for 1 hour at room temperature. Then the absorbance of the solution was measured at 450 nm (Resat et al., 2004).

TOTAL ANTIOXIDANT CAPACITY:

Total antioxidant activity of the hydromethanolic extracts of the leaves, bark and fruit of *P. suberosa* was evaluated by the phosphomolybdenum method and expressed as ascorbic acid equivalent (AAE) per gram of plant extract. Total antioxidant contents were calculated using the standard curve of ascorbic acid (y = 0.0043x + 0.1503; R^2 = 0.887). The total antioxidant contents were found in the following order: Bark>Fruits>Leaves.

Table 2: Total antioxidant contents of the hydromethanolic extracts of different parts of *P.suberosa*

Plant parts	Total antioxidant mg/g plant extract (in AAE)
Leaves extract	455.465 ± 4.933
Bark extract	862.441 ± 16.444
Fruits extract	511.279 ± 11.511

Results are expressed as mean ± SD (n=2)

TOTAL PHENOLIC CONTENT:

Total phenolic contents were determined by using the Folin-Ciocalteu reagent and expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of leaves, bark and fruits of *P. suberosa* were calculated using the standard curve of Gallic acid (y= 0.0138x + 0.1275; R^2 = 0.9881). Highest phenolic contents were found in the bark extract while leaves extract showed lowest phenolic contents.

Table 3: Total phenolic contents of the hydromethanolic extracts of different parts of *P. suberosa*

Plant parts	Total phenol mg/g plant extract (in GAE)
Leaves extract	45.290 ± 2.306
Bark extract	249.638 ± 4.867
Fruits extract	134.782 ± 3.330

Results are expressed as mean ± SD (n=2)

TOTAL FLAVONOID CONTENT:

Aluminum chloride colorimetric method was used to determine the total flavonoids content in the plant extract of different parts of the *P. suberosa.* The total flavonoids contents of leaves, bark and fruits of extract were calculated using the standard curve of quercetin (y = 0.0098x - 0.0364; R^2 = 0.9724) and expressed as quercetin equivalents (QAE) per gram of the plant extract.

Table 4: Total flavonoids contents of the hydromethanolic extracts of different parts of *P. suberosa*

Plant parts	Total flavonoids mg/g plant extract (in QAE)
Leaves extract	109.235 ± 3.968
Bark extract	166.378 ± 6.133
Fruits extract	60.765 ± 1.804

Results are expressed as mean ± SD (n=2)

REDUCING POWER ASSESSMENT:

The reductive capacity of the extracts were assessed using ferric to ferrous reductive activity as determined spectrophotometrically from the formation of Perl's Prussian blue color complex (Yildirim et al., 2000). The extracts were found to exhibit strong reducing power which was comparable to the ascorbic acid. Reducing power was found to increase with the concentration of the extracts in all cases. Bark extract was found to be the most potent as in other models of antioxidant test (Figure 1).

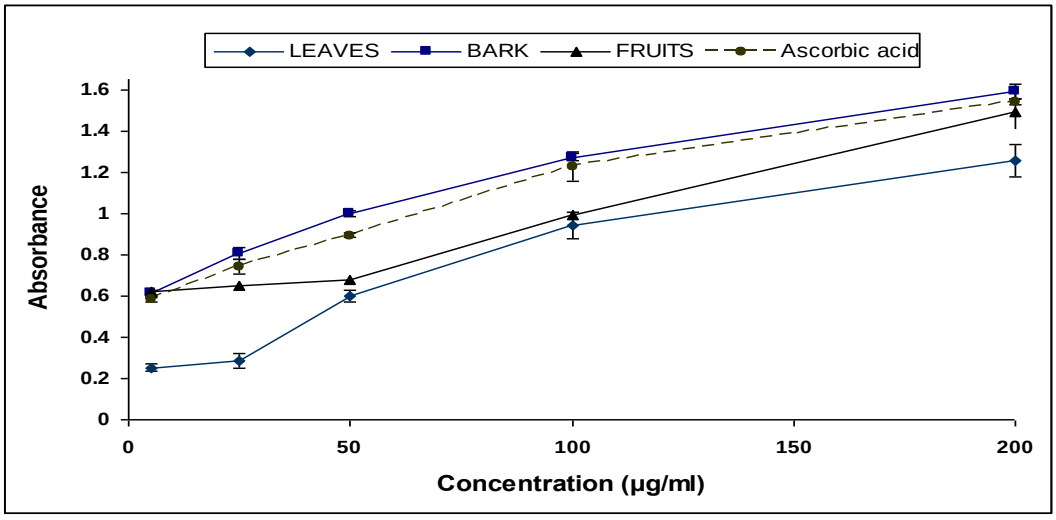

Figure 1: Ferric ion reducing capacity of the hydromethanol extracts of three parts of at different conc.

CUPRIC ION REDUCING CAPABILITY:

Reduction of Cu^{2+} ions was found to raise as the concentration of the hydromethanol extracts of leaves, fruits and barks increased. All extracts produced a dose dependent reduction of Cu^{2+} in a way similar to the reference antioxidant ascorbic acid. Bark extract showed strong reducing power than the reference standard ascorbic acid. Fruits extract has been found to be almost similar reducing activity to ascorbic acid in this method (Figure 2).

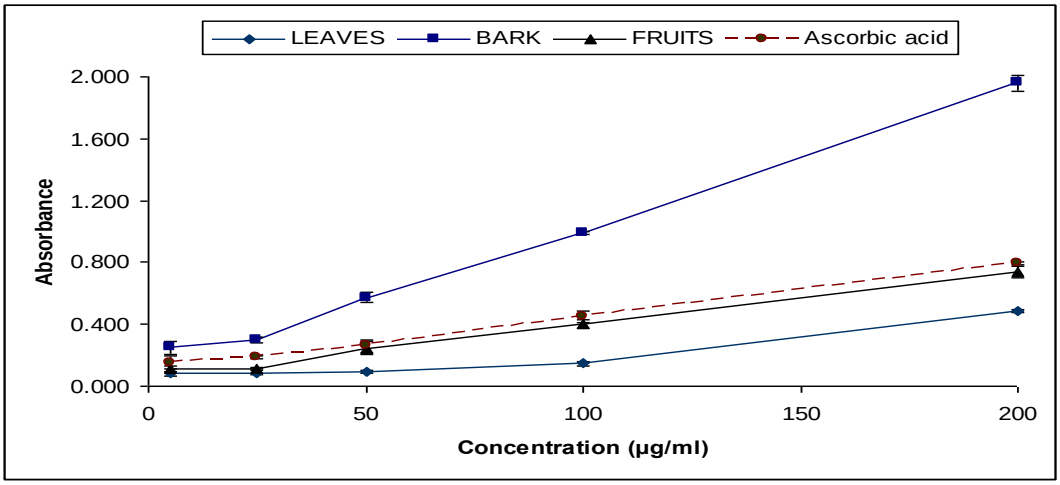

Figure 2: Cupric ion reducing capacity of the hydromethanol extracts of three parts of *P. suberosa* at different concentration

DPPH FREE RADICAL SCAVENGING ACTIVITY:

The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. Practically, the reaction brings about the reduction of DPPH radicals to the corresponding hydrazine, which is manifested by a color change from violet to yellow, which is monitored spectrophotometrically. % scavenging of DPPH radical was found to rise with increasing concentration of the different parts with highest scavenging displayed by bark extract of the plant (Figure 3). The IC_{50} values for leaves bark and fruits were 275.790, 111.544, and 174.359 respectively whereas the IC_{50} value for ascorbic acid was 26.491.

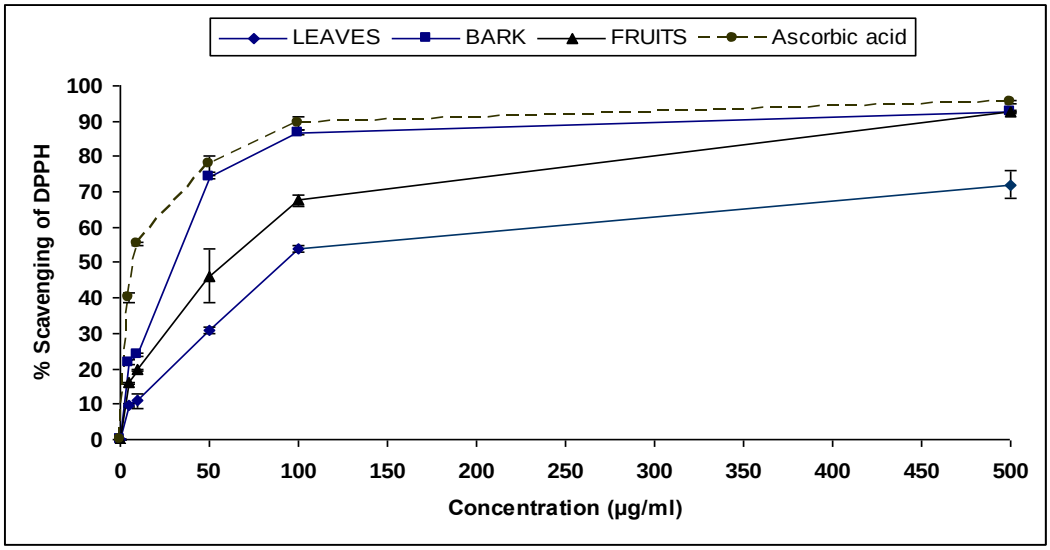

Figure 3: DPPH free radical scavenging activity of the hydromethanol extracts of three parts of *P. suberosa* at different concentration

NO FREE RADICAL SCAVENGING ACTIVITY:

The scavenging capability of the NO is determined by the decrease in its absorbance at 550 nm, induced by antioxidants. In order to evaluate the antioxidant potency through NO scavenging by the test samples, the change of optical density of NO was monitored. Figure 4 showed the decrease in absorbance of NO due to the scavenging ability in different concentrations of experimental plant extract and standard ascorbic acid. The extracts also showed dose dependent scavenging of NO similar to the standard ascorbic acid. However, maximum scavenging of NO was found with bark extract. In this assay the IC_{50} values for leaves, bark and fruits were calculated as 111.224, 37.560, 71.055 µg/mL respectively whereas the IC_{50} value for ascorbic acid was 59.327 µg/mL.

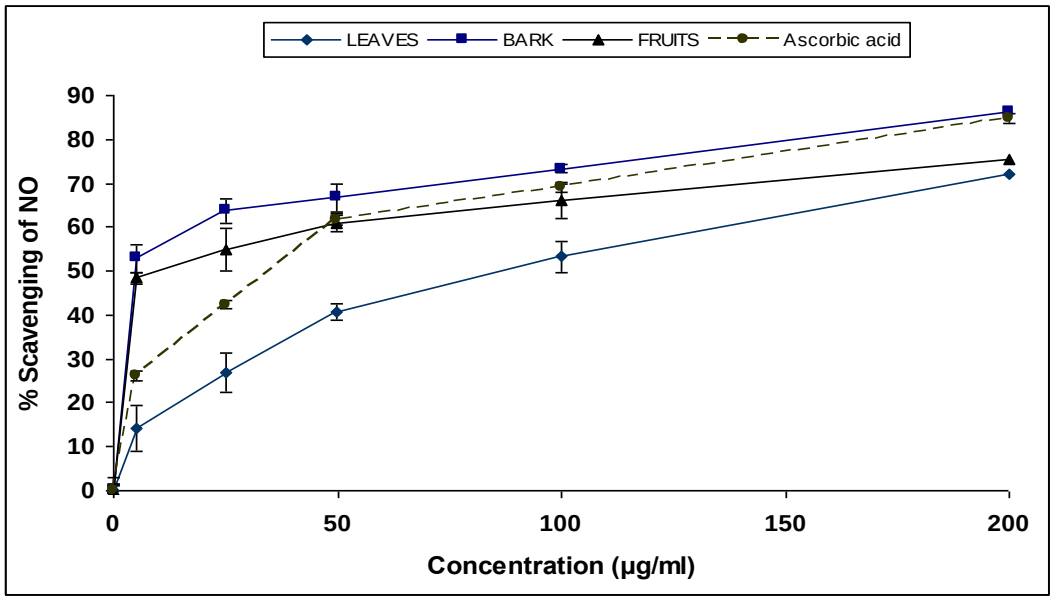

Figure 4: NO free radical scavenging activity of the hydromethanol extracts of three parts at different conc.

DISCUSSION:

In the DPPH and NO free radical scavenging assay, it is observed that different parts of the plant showed concentration dependent free radical scavenging activity (Figure 3 and Figure 4). This free radical scavenging activity may be due to the presence of phenolic compounds and flavonoids in the plant extracts. Bark extract has been found to be the most potent free radical scavenger compared to the fruits and leaves extracts. In case of NO scavenging assay, bark extract showed higher radical scavenging activity (IC_{50} 37.560 µg/mL) than the reference ascorbic acid (IC_{50} 59.327 µg/mL). % free radical scavenging activity and the IC_{50} values are significantly varied among the tested extracts. Bark extracts showed the maximum % scavenging activity among the tested extracts. It observed from the test results that the phenolic contents in the extracts highly correlate with their antioxidant activity confirming their significant contribution to the antioxidant activities of the plant extracts (Table 3 and Figure 3 & 4). The large variation in the antioxidants activity of the extracts of different parts may result from differences in total phenolic contents in the different parts. The results of the reducing power assessment are depicted in the Figure 1 & 2. The data obtained from the experiment for determining the reducing power of plant extracts in which increasing absorbance values implied increased conversion of Fe^{3+} to Fe^{2+}, hence increasing reducing ability of plant extract, showed that the extract exhibited concentration–dependent ferric reducing ability within the range of plant extract concentrations used for the experiment. Highest reducing power was observed with bark extract in the both Cupric and Ferric ion reducing methods. Bark extract showed stronger Cupric and Ferric reducing capacity than the reference antioxidant ascorbic acid. When substances exhibiting high reducing tendencies donate electrons which can react with free radicals converting them to more stable products in the process, radical chain reactions could be terminated (Pin-Der, 1998). It has been reported earlier that a direct correlation exists between the antioxidant activity and the reducing power of the certain plant extracts (Tanaka et al). The results, obtained from the test of the total phenolic contents, total antioxidant capacity and reducing power of the plant extracts, were observed to relate directly.

IN VIVO ANALGESIC SCREENING OF POLYALTHIA SUBEROSA:

EXPERIMENTAL ANIMAL:

For the experiment Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°c), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

MATERIALS:

Table 5: Materials used in the tests and their sources

Name of the material	Purpose	Source
Nalbuphine	Standard drug in tail immersion test	Incepta pharmaceuticals limited, Bangladesh
Diclofenac-Na	Standard drug in acetic acid induced writhing test	Square pharmaceuticals limited, Bangladesh
Tween 80	Suspending agent	Merck, Germany
Acetic acid	Writhing reflex inducer	Merck, Germany

TAIL IMMERSION TEST:

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Toma et al., 2003). The animals were divided into eight groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 mL/kg body weight), animals of Group II received Nalbuphine at 10 mg/kg body weight while animals of Group III to Group VIII were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of leaves, bark and fruits of *P.suberosa.* From 1-2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined 0, 30, 60 and 90 min after the administration of drugs.

ACETIC ACID-INDUCED WRITHING IN MICE:

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. The animals were divided into eight groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 mL/kg body weight), animals of Group II received Diclofenac-Na at 10 mg/kg body weight while animals of Group III to Group VIII were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of leaves, bark and fruits of *P. suberosa.* Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ahmed et al., 2004).

RESULTS:

ACETIC ACID-INDUCED WRITHING IN MICE:

The results showed that all parts of *P. suberosa* at all doses produced significant (p< 0.05-0.01) inhibition of writhing reaction in a dose dependent manner. Maximum inhibition of writhing (55.15% inhibition) was produced by the bark extract at 400 mg/kg dose whereas fruit and leaf extracts at the same dose displayed a maximum of 53.09% and 38.92% inhibition of writhing response respectively (Table 6).

Table 6: Effect of hydromethanol extract of different parts *on* acetic acid induced writhing in mice.

Groups	Dose (mg/kg)	No. of writhing	% protection
Control	Vehicle	38.8±1.213	-
Diclofenac-Na	10	8.4±1.788	78.35**
Leaves	200	28.4±1.349**	26.80**
Leaves	400	23.7±1.835**	38.92**
Bark	200	28.2±2.248**	27.32**
Bark	400	17.4±2.736**	55.15**
Fruits	200	25.3±2.45*	34.8*
Fruits	400	18.2±2.56**	53.09**

Values are mean ± SEM, (n = 5); ** p < 0.01 and * p < 0.05, Dunnet test as compared to control

TAIL IMMERSION TEST:

The tail withdrawal reflex time following administration of the extracts of *P. suberosa* was found to increase with increasing dose of the sample. In this test, maximum effect was observed after 60 and 90 min of drug administration. The result was statistically significant (p<0.05-0.01) and was comparable to the reference drug Nalbuphine (Table 5). However, hydromethanol extract of the bark of *P. suberosa* was found to be the most potent at all dose levels. The ability of the extracts to increase tail withdrawal reflex time decreased in the following order: Bark> Fruits> Leaves.

Table 7: Effect of hydromethanol extract of different parts of *P. suberosa* on tail withdrawal reflex in mice.

Groups	Dose (mg/kg)	Mean reaction time (sec)				% inhibition		
		0 Min	30 Min	60 min	90 Min	30 min	60 min	90 min
Control	Vehicle	2.18± 0.380	2.44±0.491	2.40± 0.202	2.99±0.321	-	-	-
Nalbuphine	10	2.44±0.391	7.21±1.505	13.29± 1.094	12.88±1.439	66.20	81.92	76.82
Leaves	200	2.85±0.200	4.44±0.975*	4.71± 0.973*	4.86±0.951*	45.06*	49.00*	38.53 *
Leaves	400	2.16±0.127	4.73±1.059**	5.88± 1.436 **	5.82±1.068**	48.43**	59.14**	48.65 **
Bark	200	2.31±0.397	4.67±0.822**	5.52± 1.039**	6.36±1.036**	55.59**	51.84**	63.03 **
Bark	400	2.31±0.242	5.25±0.598***	7.38±1.423** *	7.63±1.575***	60.56 ***	63.99 ***	69.21 ***
Fruits	200	2.62±0.377	4.93±0.871**	5.09±1.020**	5.66±1.021**	50.58**	52.80**	47.24 **

Fruits	400	2.04±0.145	5.20±1.605**	6.48±1.819**	6.38±0.961**	53.07**	62.92**	53.18**

Values are expressed as mean ± SEM, (n = 5);***$p < 0.01$**$p < 0.01$ and* $p < 0.05$, Dunnet test as compared to control

DISCUSSION:

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory responses while tail immersion method is considered to be selective in evaluating central mechanism of pain. The extract inhibited both mechanisms of pain in a dose dependent manner, suggesting that the plant extracts may act as a narcotic analgesic that act via peripheral and central mechanism. The significant reduction in acetic acid-induced writhes by the hydromethanol extract of *P. suberosa* suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs (Koster et al., 1959) and other endogenous substances. The therapeutic benefits of traditional remedies are often attributed to a combination of active constituents (Chindo et al., 2003). For instance, flavonoids are known to target PGs involved in late phase of acute inflammation and pain perception. It is therefore, probable that the biologically active flavonoids components, presence confirmed by phytochemical screening, of the extract might contribute in part to anti-inflammatory and analgesic activities of the extract. The tail immersion test is considered to be selective to examine compounds acting through opoid receptor. The significant increase in mean basal latency produced by the hydromethanol extracts of different parts of the *P. suberosa* suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Bensreti and Sewel, 1983; Headley and Oshaughnessy, 1985; Wigdor and Wilcox, 1987; Pasero et al., 1999). The analgesic effect produced by the extract may be via central mechanisms involving opiate, dopaminergic, descending noradrenergic and serotonergic systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain. Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (Pasero et al., 1999). Antioxidants are now known to play a major role in the resolution of inflammatory conditions (Parke and Sapota, 1996) and several anti-inflammatory agents from plant sources have been found to exhibit antioxidants properties (Sakai et al., 1999; Narendhirakannan et al., 2005). The antioxidant activities of some of these plants have been ascribed to their phenolic constituents (Vijayalakshmi et., 1997). During antioxidant activity screening all the tested extracts (leaves, bark and fruits) has been found to have strong antioxidant activity. So, the antioxidant activity of these extracts might be attributed to the analgesic activity.

IN VIVO ANTIDIARRHEAL SCREENING OF POLYALTHIA SUBEROSA:

EXPERIMENTAL ANIMAL:

For the experiment Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

MATERIALS:

Table 8: Materials used in the tests and their sources

Name of the material	Purpose	Source
Loperamide	Standard drug in both castor oil and magnesium sulphate induced diarrhea	Incepta pharmaceuticals limited, Bangladesh
Tween 80	Suspending agent	Merck, Germany
Castor oil	Diarrhea inducer	Spain
Magnesium sulphate	Diarrhea inducer	Merck, Germany

CASTOR OIL INDUCED DIARRHEA:

The experiment was performed according to the method described by Shoba and Thomas (Shoba and Thomas, 2001).

INITIAL SCREENING:

Briefly, mice fasted for 24 h were randomly allocated to eight groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhea were selected for the final experiment.

TREATMENT PROTOCOL:

Group I received 1% CMC (10 ml/kg, p.o.), groups III to VIII received orally the plant extract (200 and 400 mg/kg), respectively. Group II was given Loperamide (3 mg/ kg, p.o.) in suspension. After 60 min, each animal was given 0.5 ml of castor oil, each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, observed for 4 h and the characteristic diarrheal droppings were recorded.

MAGNESIUM SULFATE INDUCED DIARRHEA:

TREATMENT PROTOCOL:

For the experiment 40 Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g, were taken. The animals were divided in to eight groups of five animals each. Diarrhea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pre-treatment with vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) to the control group (Group-I), loperamide (3 mg/kg) to the positive control group (Group-II), and the hydromethanol extracts of leaves, bark and fruits of *P. suberosa* at the doses of 200 and 400 mg/kg to the test groups (Group-III-VIII) (Doherty, 1981).

RESULTS:

CASTOR OIL-INDUCED DIARRHEA:

In castor oil-induced diarrhea, the hydromethanol extracts of bark, fruits and leaves of *P. suberosa* , at the doses of 200 and 400 mg/kg, reduced the total number of faeces in a dose dependent manner (Table 16). However statistically significant (p <0.05) inhibition of characteristic diarrheal droppings was observed with hydromethanol extracts of bark (69.52% inhibition) and fruits (63.81% inhibition) of the plant at 400 mg/kg dose level. In contrast, the reference antidiarrheal drug Loperamide showed 72.38% inhibition of diarrhea induced by castor oil in the test animals.

Table 9: Effect of hydromethanol extract of different parts *on* castor oil-induced diarrhea in mice.

Groups	Treatment	Dose (p.o.)	No. of faecal droppings in 4 h	% Inhibition of defaecation
Group-I	1% Tween 80 in water	0.4 ml/mouse	21±3.416	-
Group-II	Loperamide	10 mg/kg	*5.8±2.029	*72.38
Group-III	Leaves	200 mg/kg	15±3.342	28.57
Group-IV		400 mg/kg	10±3.149	52.38
Group-V	Fruits	200 mg/kg	13.2±2.849	37.14
Group-VI		400 mg/kg	*7.6±2.229	*63.81
Group-VII	Bark	200 mg/kg	11.6±2.655	44.76
Group-VIII		400 mg/kg	*6.4±2.866	*69.52

Values are expressed as mean ± SEM, (n = 5); * p < 0.05, Dunnet test as compared to control.

MAGNESIUM SULPHATE-INDUCED DIARRHEA:

Similarly in MgSO4-induced diarrheal model, all the extracts of *P. suberosa* at both dose levels reduced the frequency of diarrheal stools in mice in a dose dependent manner (Table 10). But the antidiarrheal actions exhibited by the hydromethanol extracts of the three parts of the plant, at 400 mg/kg dose, were found to be statistically significant (p<0.05) and was comparable to the reference drug Loperamide. The antidiarrheal activity was of the following order: Bark (71.95% inhibition) > Fruits (65.85% inhibition)> Leaves (58.54 inhibition).

Table10: Effect of hydromethanol extract of different parts *P. suberosa* on MgSO4-induced diarrhea in mice.

Groups	Treatment	Dose (p.o.)	No. of faecal droppings in 4 h	% Inhibition of defaecation
Group-I	1% Tween 80 in water	0.4 ml/mouse	16.4±2.527	-
Group-II	Loperamide	10 mg/kg	4±1.979	75.61
Group-III	LEAVES	200 mg/kg	12.2±2.745	25.61
Group-IV		400 mg/kg	*6.8±1.511	58.54
Group-V	FRUITS	200 mg/kg	12±2.708	26.83
Group-VI		400 mg/kg	*5.6±1.862	65.85
Group-VII	BARK	200 mg/kg	8.2±1.765	50.00
Group-VIII		400 mg/kg	*4.6±2.210	71.95

Values are expressed as mean ± SEM, (n = 5); * $p < 0.05$, Dunnet test as compared to control.

DISCUSSION:

Several mechanisms have been previously proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption (Gaginella and Bass, 1978), activation of adenylate cyclase or mucosal cAMP mediated active secretion (Capasso et al., 1994), stimulation of prostaglandin formation (Galvez et al., 1993), platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil(Mascolo et al, 1994). However, it is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (Gaginella et al., 1975). Also ricinoleic acids like other anionic surfactants reduce the net absorption of water and electrolytes (Almeida et al., 1995) causing diarrhea. Loperamide, a drug widely used in the management of diarrhea disorders was reported to be effective in the prevention of diarrhea induced by castor oil, prostaglandins, and cholera toxin (Farack et al., 1981). The pharmacological effect of loperamide is due to its anti-motility and anti-secretory properties (Karim and Adeikan, 1977). Since the hydromethanol extracts of the leaves, bark and fruits of *Polyalthia suberosa* successfully inhibited the castor oil-induced diarrhea, the extract might have exerted its antidiarrheal action via antisecretory mechanism which was also evident from the reduction of total number of wet faces (not shown separately) in the test groups in the experiment. Flavonoids are known to modify the production of cyclooxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) (Moroney et al., 1988) there by inhibiting prostaglandin production. The activation of LOX is induced by fatty meals while COX1and COX-2 is by diarrhea-genic agents. Though several constituents were present in the extracts, it is most likely that flavonoids, present in the plant extracts were responsible to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil (Almeida et al, 1995; Farack et al., 1981; Karim et al., 1977). The antidiarrheal activity of the different extracts may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion. On the other hand, magnesium sulphate had been reported to induce diarrhea by increasing the volume of intestinal content through prevention of reabsorption of water. It had also been reported that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water (Galvez et al., 1993; Mascolo et al., 1994; Veiga et al., 2001; Zavala et al., 1998). The hydromethanol extracts of leaves, bark and fruits of *Polyalthia suberosa* were found to improve the diarrhoeic condition in this model. The extract may have increased the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in mice as compared to the control.

CONCLUSION:

The bark and fruits extracts of the plant *Polyalthia suberosa* possess strong antioxidant potential along with excellent analgesic and antidiarrheal activity while leaves extract possess moderate activity. All these activities may be attributed to the presence of polyphenolic compounds at high concentration in the plants. In recent time natural antioxidants have attracted considerable attention to the

nutritionist, food manufacture rand consumers due to their presumed safety and high therapeutic efficacy. It had been reported that the reactive oxygen species (ROS) were known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, cataracts, chronic inflammation, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and neurodegenerative diseases. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, had been suspected to cause or prompt negative health effects. Hence, strong restrictions had been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. So there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical related diseases. Based on the findings of the study report, this plant can be used as a potential source of natural antioxidants as well as in the treatment of diarrhea and pain. However, further studies are needed to find out the exact mechanism of action and to isolate responsible compounds for those effects displayed by the plant extracts.

REFERENCES:

1. Ahmed F., Selim M. S. T., Das A. K. and Choudhuri M. S. K. (2004) Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. *Pharmazie, 59: 329-333.*
2. Almeida C. E., Karnikowski M. G., Foleto R. and Baldisserotto, B., (1995): Analysis of antidiarrheaic effect of plants used in popular medicine. *Rev. Saude Publica*., 29(6):428-433.
3. Bensreti M.M. and Sewell R. D. E. (1983). Selective effects of dopaminergic modifiers on antinociception produced by different opioid receptor agonists. *Pro. Br. Pharmacol. Soc. pp. 70.*
4. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. (2001). Antioxidant principles from *Bauhinia terapotensis*. *J. Nat. Prod*. ; 64: 892–895.
5. Capasso F., Mascolo N., Izzo A. A. and Gaginella T. S. (1994). Dissociation of castor oil-induced diarrhea and intestinal mucosal injury in rat: effect of NG-nitro-l-arginine methyl ester. *British J. Pharmacol. 113:1127–1130.*
6. Chindo B. A., Amos S., Odutola A. A., Vongtau H. O., Abah J., Wambebe C. and Gamaniel K. S. (2003). Central nervous system activity of the methanolic extract of *Ficus platyphylla* stems bark. *J. Ethnopharmacol. 85: 131–137.*
7. Doherty S. S. (1981) Inhibition of arachidonic acid release, mechanism by which glucocorticoids inhibit endotoxin-induced diarrhoea. *British Journal of Pharmacology. 73: 549–554.*
8. Farack U. M., Kantz, U. and Loeseke, K. (1981): Loperamide reduces the intestinal secretion but not the mucosa C-AMP accumulation induced by cholera toxin. *Naungn Schmiedebergs Archive of Pharmacol*., *317:178.179.*
9. Gaginella T. S. and Bass P. (1978). Laxatives: an update on mechanism of action. *Life Sci. 23: 1001-1010.*
10. Gaginella T. S., Stewart J. J., Olsen W. A. and Bass P. (1975). Action of recinoleic acid and structurally related fatty acid on the gastrointestinal tract. *J. Pharmacol. and Exp. Ther. 195: 355–356.*
11. Galvez A., Zarzuelo M. E., Crespo M. D., Lorente M., Ocete A. and Jimenez J. (1993). Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of active flavonoid constituent. *Planta Med. 59:333–336.*
12. Galvez A., Zarzuelo M. E., Crespo M. D., Lorente M., Ocete A. and Jimenez J. (1993). Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of active flavonoid constituent. *Planta Med. 59:333–336.*
13. Ghani A. (2003) Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh. Dhaka, Bangladesh, *pp.362-363, 502-504.*
14. Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AKS, Mehrotra S, Palpu P. (2003). Studies on the Antioxidant Activities of *Desmodium gangeticum*. *Biol. Pharm. Bull*. 26: 1424-1427.
15. Headley P. M. and O'Shaughnessy C. T. (1985) Evidence for opiate and dopamine interaction in striatum. *Br. J. Pharmacol. 86, pp. 700.*
16. Karim, S. M. M., Adeikan, P. G., (1977) the effects of loperamide on prostaglandin-induced diarrheal in rats and man. *Prostaglandins, 13: 321.331.*
17. Kumaran A, Karunakaran RJ. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *L.W.T*. 40: 344-352.
18. Mascolo N., Izzo A. A., Autore G., Barbato F. and Capasso F. (1994) Nitric oxide and castor oil-induced diarrhea. *J. Pharmacol. Exp. Ther. 268:291–295.*
19. Morone M. A., Alcaraz M. J., Forder R. A., Carey F., Hoult and J. R. S. (1988) Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J Pharm Pharmacol*, *40: 787.792.*
20. Narendhirakannan R. T., Subramanian S. and Kandaswamy M. (2005) Free radical scavenging activity of Cleome gynandra L. leaves on adjuvant induced arthritis in rats. Mol. *Cell Biochem. 276: 71-80.*

21. Oyaizu M. (1986) Studies on Products on Browning reaction – Antioxidative activities of products of Browning reaction prepared from Glucosamine. *Japanese Journal of Nutration*, *44, 307 – 315.*

22. Parke D. V. and Sapota A. (1996) Chemical toxicity and reactive species. *Int. J. Occup. Med. Environ. Health*. *9: 119-123.*

23. Pasero C., Paice J. A. and McCaffery M. (1999) Basic Mechanisms underlying the causes and effects of pain. In: *Pain. (McCaffery M., Pasero C., eds), Mosby, St. Louis pp. 15 – 34.*

24. Pasero, Chris, McCaffery and Margo (1999). *Pain: clinical manual*. St. Louis: *Mosby.* ISBN 0-8151-5609-X.

25. Pin-Der D. (1998). Antioxidant activity of burdock (*Arctium lappa* Linne): It's scavenging effect on free-radical and active oxygen. *J. Amer. Oil Chemists' Soc. 75(4): 455-461.*

26. Prieto P. Pineda M. and Aguilar M. (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269*, 337- 341.*

27. Resat A., Kubilay G., Mustafa O. and Saliha E. K. (2004) Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: *CUPRAC Method, J. Agric. Food Chem., 52, 7970-7981.*

28. Sakai A., Hirano T., Okazaki R., Okimoto N., Tanaka K. and Nakamura T (1999). Large-dose ascorbic acid administration suppresses the development of arthritis in adjuvant-infected rats. *Arch Orthop. Trauma Surg. 119: 121-126.*

29. Shoba F. G. and Thomas M. (2001) Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhoea. *J. Ethnopharmacol.76: 73-76.*

30. Tanaka M, Kuie C. W., Nagashima Y. and Taguchi T., (1998) Application of antioxidative Malliard reaction products from histidine and glucose to sardine products. *Nippon suisan Gakkaaishi 54: 1409-1414.*

31. Toma W., J. S. Graciosa, C. A. Hiruma-Lima F. D. P. Andrade W., Vilegas and Souza Brita A. R. M. (2003). Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. *J. Ethnopharmacol., 85: 19-23.*

32. Veiga V. F., Zunino L., Calixto J. B., Pititucci M. L. and Pinato A. C. (2001) Phytochemical and antioedematogenic studies of commercial copaiba oils available in Brazil. *Phytother. Res. 15 (6): 476-480.*

33. Vijayalakshmi T., Muthulakshmi V. and Sachdanandam P. (1997). Salubrious effect of Semecarpus anacardium against lipid peroxidative changes in adjuvant arthritis studied in rats. *Mol. Cell Biochem. 175:65-69.*

34. Wigdor S. and Wilcox G. L. (1987). Central and systemic morphine-induced antinociception in mice: Contribution of descending serotonergic and noradrenergic pathways. *J. Pharmacol. Exp. Ther. 242: 90 - 95.*

35. Zavala M. A., Perez S., Perez C., Vargas R. and Perez R. M. (1998). Antidiarrhoeal activity of Waltheria americana, *Commelina coelestis* and *Alternanthera repens*. *J. Ethnopharmacol.* 61: 41–47.

