



**PHYTOCHEMICAL SCREENING AND ANALGESIC ACTIVITIES OF
TWO BANGLADESHI MEDICINAL PLANTS:
Diospyros peregrina and *Alocasia fornicata***

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Abstract: This study deals with crude methanolic extract of the stem bark of *Diospyros peregrina* and the rhizome of *Alocasia fornicata* were investigated for phytochemical screening and possible analgesic activity. Preliminary phytochemical screening showed that both extracts possessed the active principles - alkaloids, steroids, flavonoids and tannins. The analgesic activity was evaluated for its central and peripheral pharmacological actions using tail immersion method and acetic acid-induced writhing test in mice respectively. Both extracts produced a significant increase in pain threshold in tail immersion methods in a dose dependent manner. The crude extracts displayed significant reduction in acetic acid induced writhing in mice with a maximum effect of 72.1 % reduction at 500 mg/kg and 88.05 % reduction at 400 mg/kg body weight which are comparable to the standard analgesic drug, diclofenac sodium (78.09 %).

Keywords: *Diospyros peregrina*, *Alocasia fornicata*, analgesic, tail immersion

Introduction

Diospyros peregrina (Gaertn.) Gurke. (Fam. Ebenaceae) locally known as Deshi Gab is a medium to large sized tree planted for its edible fruits in different areas of Bangladesh. Bark contains myricyl alcohol, triterpenes and saponin (Ghani, 1998). Ethanolic extract of stem is hypoglycemic, diuretic and anti-cancer. The fruits of *Diospyros peregrina* are useful in hyperglycemia, hyperlipidemia and augmented oxidative stress (Dewanjee *et al.*, 2009).

Alocasia fornicata (Roxb.) belongs to the family Araceae (Bengali name-Bish kachu) is a giant arum plant with a big rootstock and large leaves. Leaves and roots have a high content of soluble oxalates. Leaf is used against tumors. It also acts as a mild laxative and diuretic (Ghani, 1998). As part of our ongoing phytochemical and pharmacological screening in the present study, we evaluated the analgesic activities of methanol extract of *D. peregrina* bark and methanol extract of *A. fornicata* rhizome.

Materials and Methods

Chemicals and drugs: Methanol, Ethanol (80%) and Acetic acid were purchased from Merck, Germany. Tween 80 and Diclofenac-Na were obtained from India. All other reagents were of analytical grade.

Plant material: The plant *Diospyros peregrina* and *Alocasia fornicata* were collected from a village of Nilphamari in the month of May 2008. A voucher specimen for this collection has been

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maintained in Bangladesh National Herbarium, Dhaka, Bangladesh. The collected plants were shade-dried for four week. The plants were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Extraction: The powdered plant samples (500 g) were soaked in 1.5 L of methanol for 16 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extracts were concentrated with a rotary evaporator and it afforded 15 g of the methanolic extract.

Animal: For the experiment male Swiss albino mice, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: $24 \pm 1^\circ\text{C}$, relative humidity: 55-65% and 12hrs light/12 hrs 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. The institutional animal ethical committee approved all protocols for animal experiment.

Phytochemical Screening: The freshly prepared crude extract was qualitatively tested for the presence of various phytochemical constituents (Ghani, 2003). Following tests were performed for the identification of different chemical groups present in the extract (Evans, 1989).

Test for Carbohydrates: Molisch's test: Two ml solution of the extract of the plant material was taken in a test tube. Two drops of freshly prepared 10% alcoholic solution of α -naphthol was taken in test tube and thoroughly mixed. 2ml of conc. sulphuric acid was given to flow down the side of the inclined test tube so that the acid forms a layer beneath the aqueous solution. A red or reddish violet ring was formed at the junction of the two layers if a carbohydrate was present. A dark purple solution was formed on standing or shaking. The test tube was shaken and allowed to stand for 2 minutes, and then it was diluted with 5ml of water. A dull violet precipitate was formed immediately which confirmed the presence of carbohydrates.

Tests for Tannins: Ferric chloride test: Five ml solution of the extract was taken in a test tube. Then 1ml of 5% ferric chloride solution was added. Greenish black precipitate was formed which confirmed the presence of tannins.

Potassium dichromate test: 5ml solution of the extract was taken in a test tube. Then 1ml of 10% potassium dichromate solution was added. A yellow precipitate was formed in the presence of tannins.

Test for Flavonoids: A few drops of conc. hydrochloric acid were added to a small amount of methanolic extract of the plant material. Immediate development of a red color indicated the presence of flavonoids.

Test for Saponins: One ml solution of the extract was diluted to 20ml with distilled water and shaken in a graduated cylinder for 15 minutes. 1cm layer of foam indicated the presence of saponins.

Test for Gums: Five ml solution of the extract was taken and then Molisch's reagent and sulphuric acid were added. Presence of red violet ring at the junction of two liquids indicated the presence of gums.

Test for Steroids: Sulphuric acid test: One ml solution of chloroform extract was taken and then 1ml sulphuric acid was added. Presence of red color indicated the presence of steroids.

Tests for Alkaloids: Mayer's test: Two ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a test tube. Then 1ml of Mayer's reagent was added. Yellow color precipitate was formed and that indicated the presence of alkaloids.

Dragendorff's test: 2ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a test tube. Then 1ml of Dragendorff's reagent was added. Orange brown precipitate was formed which indicated the presence of alkaloids.

Analgesic Activity: Acetic acid-induced writhing test: The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but diclofenac-Na was administered intraperitoneally 15 min before injection of acetic acid. After an

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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).

Tail immersion (tail flick) 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). 1 to 2 cm of the tail of mice was immersed in warm water 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 sec. 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 before and 0, 30, 60 and 90 min after the administration of drugs.

Results and Discussion

Phytochemical screening of the stem bark of *Diospyros peregrina* revealed that the plant contains alkaloids, saponins flavonoids and tannins whereas the rhizome of *Alocasia formicata* gave positive reactions for flavonoids, tannins, alkaloids, and reducing sugar (Table 1).

Table 1. Results of chemical group test of the methanolic fruit extract of *Diospyros peregrina* and *Alocasia formicata*

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
ME of <i>D. peregrina</i>	+	+	-	+	-	+	+
ME of <i>A. formicata</i>	+	+	+	+	-	+	-

ME: Methanolic extract; (+): Present; (-): Absent

The oral administration of both *D. peregrina* and *A. formicata* extract significantly inhibited writhing response induced by acetic acid in a dose dependent manner. Crude methanolic extract of the barks of *D. peregrina* and the rhizome of *A. formicata* are administered in the two doses, 250mg/kg and 500mg/kg and 200 mg/kg and 400mg/kg (higher dose) respectively. In both extracts, the higher dose decreased the frequency of acetic acid induced writhing in mice more effectively than the lower dose. The inhibition of the writhing found 72.1% for 500 mg/kg and 88.05% for 400 mg/kg body weight of mice in *D. peregrina* and *A. formicata* respectively that was very much similar to that with the diclofenac-Na. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid (Ahmed et al. 2006). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ronaldo et al. 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Vogel and Vogel, 1997). Table 2 and Table 3 showed the effects of the extract on acetic acid-induced writhing in mice.

Table 2. Effect of the methanolic extract of *D. peregrina* on acetic acid induced writhing in mice

Group	Treatment & Dose	Writhings [†]	% of writhing	% of protection
Group I	1% Tween 80 in water (10 mL/Kg)	25.8± 5.46	100	0.00
Group II	Diclofenac sodium (25 mg/kg)	11 ±1.87	21.91	78.09
Group III	Extract of <i>D. peregrina</i> (250 mg/kg) [‡]	9.6 ± 1.8	37.20	62.8
Group IV	Extract of <i>D. peregrina</i> (500 mg/kg) [‡]	7.2 ± 1.35	27.90	72.1

[‡]Administered 45 min before 0.7% acetic acid administration (10 ml/kg)

[†]Counted for 10 min, starting 5 min after acetic acid administration;

Values are mean ± SEM (n = 5)

Table 3. Effect of the methanolic extract of *A. fornicata* on acetic acid induced writhing in mice

Group	Treatment & Dose	Writhings [†]	% of writhing	% of protection
Group I	1% Tween 80 in water (10 mL/Kg)	50.2 ± 1.53	100	0.00
Group II	Diclofenac sodium (25 mg/kg)	11 ± 1.87	21.91	78.09
Group III	Extract of <i>A. fornicata</i> (200 mg/kg) [‡]	25 ± 5.41	37.20	50.02
Group IV	Extract of <i>A. fornicata</i> (400 mg/kg) [‡]	6 ± 1.87	27.90	88.05

[‡]Administered 45 min before 0.7% acetic acid administration (10 ml/kg)

[†]Counted for 10 min, starting 5 min after acetic acid administration;

Values are mean ± SEM (n = 5)

The tail withdrawal reflex time following administration of the extract of *D. peregrina* was found to increase with increasing dose of the sample. The result was statistically significant and was comparable to the reference drug diclofenac sodium. The result is summarized in the Table 4 and Table 5.

Table 4. Effects of the methanolic extract of *D. peregrina* on tail withdrawal reflex of mice induced by tail immersion method.

Groups	Dose (mg/kg)	Mean reaction time (s) before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Group-I	10	2.2±0.75	3.20 ± 1.16	2.60 ± 0.49	3.4 ± 1.020	-	-	-
Group-II	25	4.2 ± 0.75	8.06 ± 0.54	8.12 ± 0.45	7.54 ± 0.81	60.297	67.98	54.90
Group-III	250	4.47 ± 1.88	3.24 ± 1.15	3.62 ± 2.11	4.01 ± 1.77	1.234	28.176	15.211
Group-IV	500	6.49 ± 1.37	3.78 ± 1.18	4.02 ± 2.31	4.01 ± 1.78	15.343	35.323	15.211

Group I animals received vehicle (normal saline)

Group II received Diclofenac Sodium 25 mg/kg body weight,

Group III and Group IV were treated with 250 and 500 mg/kg body weight (p.o.) of the crude extract of *D. peregrina*.

Values are mean ± SEM, (n = 5)

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Table 5. Effects of the methanolic extract of *A. fornicata* on tail withdrawal reflex of mice induced by tail immersion method

Groups	Dose (mg/kg)	Mean reaction time (s) before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
		Group-I	10 ml	2.2±0.84	3.20 ± 1.31	2.60 ± 0.55	3.4 ± 1.02	-
Group-II	25	4.2 ± 0.84	8.06 ± 0.60	8.12 ± 0.51	7.54 ± 0.81	59.852	67.98	54.907
Group-III	200	2.99 ±0.78	3.45 ±0.83	6.15 ± 1.11	6.03 ± 1.10	7.246	57.723	43.615
Group-IV	400	5.63 ±0.37	4.27±0.93	5.35± 1.04	7.41± 1.22	25.058	51.401	54.116

Group I animals received vehicle (Normal Saline)

Group II received Diclofenac Sodium 25 mg/kg body weight,

Group III and Group IV were treated with 250 and 500 mg/kg body weight (p.o.) of the crude extract of *A. fornicata*

Values are mean ±SEM, (n = 5)

Preliminary phytochemical screening showed the presence of flavonoids, alkaloid, steroid and tannin in the plant extracts. Recent studies suggested that the inflammatory tissue damage was due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (Parke and Sapota, 1996). There were also reports on the role of flavonoid, a powerful antioxidant (Brown and Rice-Evans, 1998; Vinson et al. 1995), in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al. 2001; Rao et al. 1998). So it can be assumed that cyclooxygenase (COX) inhibitory activity together with antioxidant activity may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system responsible for the synthesis of prostaglandins and eventually relieve pain-sensation. Tail immersion tests were considered to be selective to examine compounds acting through opioid receptor (Hasan et al., 2010; Ramabadrhan et al., 1989). Therefore, the extract increased mean basal latency attributed to the fact that it might act via centrally mediated analgesic mechanism.

Conclusion

Based on the results of the present study, it can be concluded that the plant extract possesses remarkable analgesic potential. However, further studies are needed to understand the exact mechanisms of antioxidant and analgesic action and to isolate the compound(s) responsible for such activity.

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