



**PHYTOCHEMICAL SCREENING AND THE EVALUATION OF THE ANTIOXIDANT, TOTAL PHENOLIC CONTENT AND ANALGESIC PROPERTIES OF THE PLANT**

***PANDANUS FOETIDUS* (FAMILY: PANDANACEAE)**

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**ABSTRACT**

*Pandanus foetidus* is an important medicinal plant having widespread application in a variety of disorders. The aim of this study was the phytochemical investigation and evaluation of the anti-oxidant, total phenolic content and analgesic activities of the whole plant *Pandanus foetidus*. Phytochemical screening of the ethanolic extract of *Pandanus foetidus* ensured the presence of carbohydrates, glycosides, alkaloids, tannins, flavonoids and steroids. The anti-oxidant activity was measured by DPPH free radical scavenging activity (IC<sub>50</sub>=48 µg/ml). The plant *Pandanus foetidus* shows total phenolic content test due to the presence of reducing sugar, tannins and flavonoids.

The crude ethanolic extract of the tubers of *Pandanus foetidus* exhibited significant analgesic activity at a dose of 250 mg/kg and 500 mg/kg with 71.15% and 80.77 % inhibition of writhing respectively.

**KEYWORDS:** *Pandanus foetidus*, phytochemical screening, DPPH, writhing inhibition.

**INTRODUCTION**

Plants represent a rich source of antimicrobial agent<sup>1</sup> and natural antioxidants<sup>2</sup>. Many plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines<sup>3</sup>. Plants generally produce many secondary metabolites which constitute an important source of microbicides, anti-oxidants. Many natural substances having anti-oxidant and anti-microbial properties have been used in health foods for medicinal and preservative purposes<sup>4</sup>. Again drugs which are presently used for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone<sup>5</sup>. All of these drugs present well known side and toxic effects. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop safer drugs. *Pandanus foetidus* is a common plant of sundarban. *Pandanus foetidus* is a dense bushy shrub. It grows in a prolific manner over host plants (or other support) with inter-twined stems. The plant has extensive medicinal uses. Leaves are alexiteric, tonic and aphrodisiac. It is used in leprosy, smallpox, syphilis, scabies and leucoderma. *Pandanus foetidus* has neuropharmacological activity. Leaves and spadix are used in diabetes. The oil and otto obtained from the bracts are considered stimulant and antispasmodic and are administered in headache and rheumatism. The root is considered diuretic, depurative and tonic<sup>6</sup>.

**MATERIALS AND METHODS**

**Collection of the Plant Sample**

The whole plant of *Pandanus foetidus* was collected from the mangrove forest sundorban. The time of collection was October, 2011 at the daytime. The fresh whole plants were collected from the healthy host plants. During collection, any type of adulteration was strictly prohibited. The plants were mounted on paper and the sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 35572).

**Preparation of Plant Extract**

The collected plants were separated from undesirable materials and dried in shade for 18 days. Shade drying ensured that the chemical components in the plant were not degraded. The plant was grounded into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place. The plant was extracted by cold extraction method. 400 gm of grinded tubers powder was soaked in 600 ml of ethanol in a glass container for eight days accompanying regular shaking and stirring. The extract was separated from the plant debris by filtration by a piece of clean, white cotton material and it was repeated twice. The filtrate (ethanol extract) was taken into a rotary evaporator and the remaining ethanol was completely evaporated. Then this filtrate was taken into a beaker. The opening of beaker was wrapped by a sheet of aluminum foil. The aluminum foil was perforated for the complete evaporation of any remaining ethanol. The beaker was kept in dry and cool place for several days. It rendered the extract a deep purple color.

**Phyto-chemical Screening**

**Composition of Reagents Used for the Different Chemical Group Tests**

The following reagents were used for the different chemical group test<sup>7</sup>.

**Mayer's Reagent:** 1.36 gm mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm of potassium iodide in 20 ml of water.

**Dragendroff's Reagent:** 1.7 gm basic bismuth nitrate and 20 gm tartaric acid were dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm potassium iodide and 40 ml water.

**Fehling's Solution A:** 34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

**Fehling's Solution B:** 17.6 gm of sodium potassium tartarate and 7.7 gm of sodium hydroxide were dissolved in sufficient water to produce 100 ml. Equal volume of above solution were mixed at the time of use.

**Benedict's Reagent:** 1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

**Molish Reagent:** 5 gm of pure  $\alpha$ -naphthol was dissolved in 50 ml of ethanol.

#### Tests Procedure for Identifying Different Chemical Groups

The following tests were performed for identifying different chemical groups present in the plant extract.

#### Tests for Tannins:

**Ferric Chloride Test:** 5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added. Greenish black precipitate and indicated the presence of tannins.

**Potassium Dichromate Test:** 5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added. The formation of a yellow precipitate indicated the presence of tannins.

#### Carbohydrate Tests

##### Benedict's tests (Test for reducing sugar)

0.5 ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. A red color precipitate of cuprous oxide was formed which indicate the presence of a reducing sugar.

##### Fehling's test (Standard test for reducing sugar)

2ml of an aqueous extract of the plant material was added 1ml of a mixture of equal volumes of Fehling's solutions A and B. Boiled for few minutes. A red or brick red color precipitate was formed in the presence of a reducing sugar.

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

##### Test for Steroids:

**Sulphuric Acid Test:** 1 ml solution of chloroform extract was taken and added to 1ml Sulphuric acid. Presence of red color indicated the presence of steroid.

##### Test for Alkaloids:

**Mayer's Test:** 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1 ml of Mayer's reagent was added. Formation of yellow color precipitate indicated the presence of alkaloids.

**Dragendroff's Test:** 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1 ml of Dragendroff's reagent was added. Formation of orange brown precipitate indicated the presence of alkaloids.

##### Flavonoids Test

Added a few drops of concentrated hydrochloric acid to a small amount of an alcoholic extract of the plant material. Immediate development of a red color was observed which indicated the presence of flavonoids.

##### Tests for Glycosides:

**General Test:** A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1ml of water and a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.

**Fehling's solution test:** A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. Brick-red precipitate was considered as an indication for the presence of glycosides.

#### Evaluation of anti-oxidant activity

Brand-Williams method or DPPH assay<sup>8</sup> was used to estimate free radical scavenging activities of the ethanolic extract of *Pandanus foetidus*. 50 mg of the extracts was dissolved in 50 ml of alcohol (98 % ethanol) to prepare 1000  $\mu$ g/ml solution of extract. Solution of different concentrations such as 512  $\mu$ g/ml, 256  $\mu$ g/ml, 128  $\mu$ g/ml, 64  $\mu$ g/ml, 32  $\mu$ g/ml, 16  $\mu$ g/ml, 8  $\mu$ g/ml, 2  $\mu$ g/ml and 1  $\mu$ g/ml were obtained by serial dilution technique. In the same way, various concentration of ascorbic acid solution was prepared. First, 20 mg DPPH powder was measured by electronic balance and mixed with 500 ml of ethanol to prepare 0.004 % DPPH solution. It should be kept in cool, dry and dark place. At first, we took ten test tubes and labeled for were 1 $\mu$ g/ml, 2 $\mu$ g/ml, 4 $\mu$ g/ml, 8 $\mu$ g/ml, 16 $\mu$ g/ml, 32 $\mu$ g/ml, 64 $\mu$ g/ml, 128 $\mu$ g/ml, 256 $\mu$ g/ml, and 512 $\mu$ g/ml. Then we took 2 ml of solution of each concentration into a test tube designed for each concentration. We added 6 ml of 0.004 % DPPH solution into every test tube and kept it in dark place for 30 minutes. In the same manner solution of ascorbic acid took in seven test tube and DPPH solution was applied and kept in dark place for 30 minutes. DPPH was also applied on the blank test tube at the same time where only ethanol was taken as blank. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm by spectrophotometric method and corresponding percentage of inhibitions were calculated by using the following equation

$$\% \text{ inhibition} = [1 - (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}})] \times 100 \%$$

Where  $\text{Abs}_{\text{sample}}$  is the absorbance of the sample material and  $\text{Abs}_{\text{control}}$  is the absorbance of the control reaction (containing all reagents except the test material). Then percent inhibitions were plotted against respective concentrations.  $\text{IC}_{50}$  values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph. Ascorbic acid was used as positive control.

#### Quantitative analysis of total phenolic content

The anti-oxidant potential of the ethanolic extract of *Pandanus foetidus* was determined using Folin-Ciocalteu reagents with analytical grade gallic acid as the standard<sup>9</sup>. Folin's method measures -OH groups in a sample based on the fact that light absorption increases as OH groups in a sample increase. First, 2 gm powder of *P. foetidus* leaf was measured by electronic balance and mixed with 100 ml of alcohol (98 % ethanol) homogenized for 15 min. at 25000 rpm prepare ethanolic solution of extract. Then ethanolic extract of *P. foetidus* leaf was filtered with laboratory grade filter paper. In case of standard solution 6 concentration of the gallic acid was prepared by proper dilution method. These concentrations were 400 mg/L, 200 mg/L, 100 mg/L, 50 mg/L, 25 mg/L, and 12.5 mg/L. Then 1ml solution was taken from each concentration of given above. And dilute each concentrated solution dilute with 10 ml distill water and 1 ml FC reagent, kept for 5 min at 22°C. After 5 min. 2ml of  $\text{Na}_2\text{CO}_3$  was added to the solution, and kept 1 hour in dark. Then it was ready for taking absorbance. At first, we took six volumetric flasks and labeled for 400 mg/L, 200 mg/L, 100 mg/L, 50 mg/L, 25 mg/L, and 12.5 mg/L. Then we took 1 ml of solution of each concentration into a test tube designed for each concentration. We added 10 ml of distill water and 1 ml of FC reagent (Dilute 10 fold) solution into every test tube and kept it in dark place for 5 minutes at 22° c. Then we added 2 ml solution of  $\text{Na}_2\text{CO}_3$  and kept in dark place for 60 minutes. After 60 minutes we took the reading of every test tube at 570 nm. Each concentration's reading was taken for

two times for accuracy. The reading was noted down carefully.

**Evaluation of analgesic activity**

The peripheral analgesic activity of tubers of *Pandanus foetidus* was measured by the acetic acid induced writhing test<sup>10</sup>. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, i.e. the sample having analgesic activity will

inhibit writhing<sup>11</sup>. Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Diclofenac at oral dose of 100 mg/kg was used as standard analgesic agent. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhing was calculated for 10 min, 5 min after the application of acetic acid.

**Table 1: Results of the Different Chemical Tests Performed**

Chemical Group test	Specific test	Observation	Interference
Test for Alkaloids	a)Mayer's test	positive	Presence of Alkaloids
	b)Dragendroff's test	positive	
Test for Steroid	a) Sulphuric acid test	Positive	Presence of steroid
Test for Flavonoids	-	Positive	Presence of Flavonoids
Test for Saponins	-	Negative	Absence of Saponins
Test for Tannins	a)Ferric Chloride Test	Positive	Presence of Tannins
	b)Potassium dichromate test	Positive	
Test for Gums	-	Negative	Absence of gums
Test for Reducing Sugars	a) Benedict's Test	Positive	Presence of reducing sugar
	b) Fehling's Test	Positive	
Test for glycosides	a) General Test	Positive	Presence of glycosides
	b) Fehling's solution test	Positive	

**Table 2: % Inhibition of Writhing Of the Different Test Samples**

Group	Number of Writhing (Mean ± SEM)	% of Inhibition of Writhing
1) Control	39.00±4.74	-
2)Standard	6 ± 4.58*	86.64
3) Ethanol extract of tuber (500mg/kg)	7.50 ±1.94	80.77
4) Ethanol extract of tuber (250mg/kg)	12± 4.42*	71.15

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnett's Test): \*P<0.001, All values are means of individual data obtained from five mice (n = 5).

**RESULTS**

**Phytochemical Screening**

The chemical group tests were performed and the results are mentioned in the table 1. Results indicated that carbohydrates, glycosides, alkaloids, tannins, flavonoids and steroids were detected in the crude ethanol extract.

**In Vitro Antioxidant Activity**

The antioxidant activity of the crude ethanolic extract was measured on the basis of its DPPH scavenging activity. The concentration of the crude ethanolic extract needed for 50% scavenging (IC<sub>50</sub>) of DPPH was found to be 48 µg/ml which is mild comparable to that of ascorbic acid (IC<sub>50</sub> = 3.90 µg/ml), a well-known standard antioxidant.

**Total phenolic content**

The anti-oxidant potential of the ethanolic extract of *Pandanus foetidus* was determined using Folin-Ciocalteu reagents with analytical grade gallic acid as the standard. The plant *pandanus foetidus* shows total phenolic content test due to the presence of reducing sugar, tannins and flavonoids. Total phenol contents that is measure by Folin Ciocalteu reagent in terms of gallic acid equivalent (GAE) is about 0.451of GAE/gm powder.

**Analgesic activity**

The ethanolic extract of *Pandanus foetidus* exhibited significant analgesic effect in acetic acid induced writhing of white albino mice (Swiss-Webster strain). The extract produced 71.15% and 80.77% writhing inhibition (p < 0.001) at doses of 250 and 500 mg/kg-body weight respectively. The results are shown in table 2.

**DISCUSSION**

The present study confirms the use of the tubers of *Pandanus foetidus* as an analgesic agent. NSAIDS offer relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and

bradykinin were suggested to play an important role in the pain process. Therefore, it is likely that the extract might suppress the formation of these substances or antagonize the action of these substances and thus exert its analgesic activity.

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