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Phytochemical screening and evaluation of cytotoxic activity of *Pandanus fascicularis L.* (Fruits)

K.M. Rahat Maruf Jitu,[1] Dipti Debnath,[2*] Sharmeen Asad,[3] Rajib Chandra Das,[4] Abida Sultana[4]

ABSTRACT

The present study was conducted to evaluate possible phytochemicals present, and cytoctoxic activity of extract of fruits of *Pandanus fascicularis L*. Phytochemical screening was carried out using the standard test methods of different chemical group. Evaluation of cytotoxic activity was done using the brine shrimp lethality bioassay. The aqueous, methanolic, ethanolic, ethyl acetate, pet ether and chloroform extracts show presence of maximum phytochemicals such as alkaloids, tannins, flavonoids, steroids, saponins, proteins, terpens, phenols, glycosides, carbohydrate in different fractions etc. During cytotoxicity test, the positive control groups showed nonlinear mortality rates at lower concentrations and linear rates at higher concentrations. The LC50 values of Chloroform extract was found 1.0636 µg/ml where the positive control vincristine sulphate showed LC_{50} at a concentration of 0.200µg/ml. Therefore, the plant extract possess potent cytotoxic effect.

Key words: phytochemicals, cytotoxicity, alkaloids, flavonoids, *Pandanus fascicularis L.*

*Corresponding Author:
Dipti Debnath, Department of Pharmacy, Atish Dipankar University of Science and Technology, Bangladesh
diptidebnath@rocketmail.com

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INTRODUCTION

Focus on medicinal plant research has increased throughout the world.[1] According to the recent report of World Health Organization (WHO), still 80% of the world's population uses medicinal plants for different medical purposes.[2] *Pandanus fascicularis (P. fascicularis)* L. commonly known as screw pines are palm-like evergreen trees or shrubs belong to the genus *Pandanus*. *Pandanus* is mainly distributed in tropical and subtropical regions of the world. *P. fascicularis* has a significant presence in South Asia.[3] The flower oil of this plant is used in headache, earache, disorders of the blood, and as stimulant and antispasmodic. Roots are extensively applied in the treatment of skin diseases and osteoarthritis. Moreover, different species of *Pandanus* are used traditionally to treat diabetes.[4] Our present study focuses on determination of phytochemicals and cytotoxic effect of crude extract of fruits of *P. fascicularis* L.

Phytochemicals obtained from medicinal plants show a variety of pharmacological effects in human body. Fruits and herbs containing phytochemicals protect our body from different diseases.[5] Cancer or tumor is the most common cause of death in both developed and developing countries. There are many methods to describe how cancer spread throughout the body. Actually cancer occurs on a specific part of our body and then invade to the other parts of our body very quickly and ultimately causes the death of the patient.[6] So it is very necessary to identify cancer at early stage. However, there are several approaches of cancer treatments including surgery, radiation therapy and chemotherapy. All of these approaches are aimed to destroy cancerous cell from the body. Each approach possesses several side effects.[7] That is why it is now demand of the present era to discover drug with fewer side effect.

METHODS AND MATERIALS

Chemicals and reference drugs

In this study, all the chemicals and reagents of analytical grade were used. These were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

Collection and identification of the plant

For this present investigation the fruits of *P. fascicularis L.* were collected by the authors from the St. Martin Island of Bangladesh, in July 7, 2013. The plant was identified and authenticated by the expert botanist of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 39684). During collection any type of adulteration was strictly prohibited.

Plant extracts preparation

The fruits of *P. fascicularis L.* were thoroughly washed with water after collection. The fruits were

[1]Department of Pharmacy, Noakhali Science and Technology University, Bangladesh
[2]Department of Pharmacy, Atish Dipankar University of Science and Technology, Bangladesh
[3]Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh.
[4]Department of Applied Chemistry and Chemical Engeering, Noakhali Science and Technology University, Bangladesh.

shade-dried and crushed into coarse powder using a mechanical grinder.[8] About 500g of the powdered materials of the plant were soaked in 1.5 liters of chloroform at room temperature for two weeks. The sample mixture were shacked and stirred at regular interval during this time. Then the solution was filtered using filter cloth and Whatman's filter paper and concentrated with a rotary evaporator. It rendered a brown granulars which was then designated as crude chloroform extracts for further study.

Phytochemical Screening

The Phytochemical analysis is done to see the presence of various phytoconstituents.[1] Phytoconstituents are the raw ingredients for pharmacological activities. In this work phytochemical screening was conducted on fruit extract of *P. fascicularis* by aqueous, ethanol, methanol, chloroform, ethyl acetate, and pet ether solvents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorff's reagent and Mayer's reagent; flavonoids with the use of Mg and HCI; tannins with ferric chloride and potassium dichromate solutions; saponins with ability to produce stable foam; staroids with Libermann-Burchard reagent; reducing sugars with Benedict's reagent and Fehling's reagents.[1] Thus all phytochemicals were screened through standard procedures. These were carefully identified by characteristic color changes using standard procedures.

Cytotoxic activity

Brine shrimp lethality bioassay

The measurement of toxicity plays a vital role in drug discovery and is a useful tool in biological, especially ecological investigations. It also serves as a tool for screening plant extracts of possible medicinal value. In this study, we used simple brine shrimp bioassay test of Meyer with slight modification by using *Artimia salina* as test organism, which was collected from a pet shop.[9,10]

Brine shrimp hatching

Sea water was prepared by dissolving 38 g sea salt (pure NaCl) in one liter of distilled water, which is then filtered to get clear solution of 3.8% concentration.[10] In a suitable plastic or glass vessel sea water was taken and shrimp eggs were added to one side of the vessel and allowed to hatch for 24 h till the mature nauplii were found. Continuous oxygen and light supply were provided to support the hatching process.

Sample preparation

All the test samples (crude extracts) were taken in vials and dissolved in 100 µl of pure dimethyl sulfoxide (DMSO) to get stock solutions. Then 50 µl of solution was taken in the first test tube containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In every case, 50 µl samples were added to test tube and fresh 50 µl DMSO was added to vial.

Negative control group test

100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as negative control groups.

Positive control group test

Here we used vincristine sulphate (VINCRIRST®, Techno Drugs Ltd., Bangladesh) as a positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 40 µg/ml from which serial dilutions were made using DMSO to get 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 and 0.078125 µg/ml respectively. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

Counting of nouplii

After 24 h, the number of survived nauplii in each vial was counted by using magniflying glass. From this data the percent (%) of mortality of brine shrimp nauplii was calculated for each concentration.

Statistical Analysis

All the above assays were conducted in triplicate and repeated threes for consistency of results and statistical purpose. The data were expressed as Mean ± SD and analyzed by using SPSS software of 10 version.

RESULTS

Phytochemical Screening

The phytochemical screening conducted on fruit extract of *Pandanus fascicularis* revealed the presence of alkaloids, tannins, flavonoids, steroids, saponins, proteins, terpens, phenols, glycosides, carbohydrate in different fractions (Table 1).

Table 1 Result of phytochemical screening of different fractions of fruits of *Pandanus fascicularis*

Bioactive constituents	Aqueous extract	Ethanolic extract	Methanolic extract	Chloroform extract	Ethyl acetate extract	Pet ether extract
Alkaloids	-	+	+	+	-	-
Steroids	-	+	+	+	-	+
Terpenoids	-	+	+	+	-	-
Phenols	-	+	+	-	+	-
Glycosides	+	+	+	-	-	-
Carbohydrates	+	+	+	-	+	-
Proteins	+	+	+	+	-	-
Flavanoids	+	+	+	+	+	-
Saponins	-	+	+	+	-	-
Tannins	-	+	+	+	-	+

Table 2 Effect of chloroform extract (fruits) of *P. fascicularis* on brine shrimp nauplii

Conc. (C) (µg/ml)	Log C	No. of alive nauplii added	No. of dead nauplii	% Mortality	LD_{50} (µg/ml)
1	0	10	4	40	1.0636
5	0.6989	10	7.5	75	
10	1	10	8.5	85	
20	1.301	10	10	100	
50	1.6989	10	10	100	
100	2	10	10	100	

Table 3 Effect of vincristine sulphate on brine shrimp nauplii

Conc (C) (µg/ml)	Log C	No. of alive nauplii added	No. of dead nauplii	% Mortality	LD_{50} (µg/ml)
1	0	10	6	60	0.2
5	0.6989	10	8	80	
10	1	10	9	90	
20	1.301	10	9	90	
50	1.6989	10	10	100	
100	2	10	10	100	

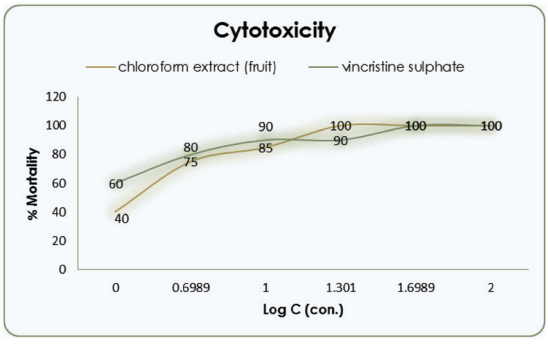

Figure 1 Comparative presentation of cytotoxic activity of chloroform soluble extracts of *P. fascicularis* with standard vincristine sulphate

Cytotoxic activity

In this study we used chloroform soluble extracts of *P. fascicularis*. Plotting of log of concentration versus percent mortality for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) was determined for the samples. The positive control groups showed nonlinear mortality rates at lower concentrations and linear rates at higher concentrations. There was no mortality in the negative control groups indicating the test as a valid one and the results obtained are only due to the activity of the test agents. Following the procedure of *Meyer et al.*,

1982[10] the lethality of the Dimethyl-Sulphoxide (DMSO) soluble Chloroform extract was determined and the summary of the results is expressed in Table-2. The LC50 values of Chloroform extract for fruit of the plant was found 1.0636 µg/ml. The positive control vincristine sulphate showed LC_{50} at a concentration of 0.200µg/ml (Table-3). When this result is compared with data obtained for Chloroform extract of *P. fascicularis* was found almost identical and the value is represented at Figure-1.

DISCUSSION AND CONCLUSION

In different parts of the world, people still use medicinal plants in the treatment of various disorders. Medicinal plants possess different types of phytochemicals which exert a variety of pharmacological effects in human body.[1] In the present study, phytochemical screening revealed the presence of several phytochemicals like tannins, flavonoids, steroids, saponins, proteins, terpens, phenols, glycosides, carbohydrate and alkaloids. This fruit also showed significant cytotoxic effect (Figure 04). Bioactive compounds in a plant are responsible for exhibiting cytotoxic activity. According to few previous studies, flavonoids and tannins may possess remarkable cytotoxic and anti-tumour properties.[2]

Brine shrimp lethality bioassay (BSLB) method was chosen to determine cytotoxic potency because this is easiest to conduct than any other methods and also said that the cytotoxic compounds generally exhibit significant activity in the BSLB; for all of this aspect this method become one of the recommended guideline for the detection of antitumor compounds and pesticides due to its low cost.[10,11] This bioassay also found to exhibit positive relationship with the human solid tumour cell lines. Hence, cytotoxic effect of the plant extracts will enunciate for further cell line assay.[11,12] We want to conclude here with a massage that significant lethality (as LC50 value less than 100 ppm or µg/ml) of the plant extract to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigations.

REFERENCES

1. Uddin SMN, Amin MN, Shahid-Ud-Daula AFM, Hossain H, Haque MM, Rahman MS, et al. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of *Stephania japonica* Linn. J. Med. Plants Res. 2014; 8(37): 1127-1133.
2. Amin MN, Banik S, Ibrahim M, Moghal MMR, Majumder MS, Siddika R, et al. A Study on *Ardisia solanacea* for Evaluation of Phytochemical and Pharmacological Properties. International Journal of Pharmacognosy and Phytochemical Research. 2015; 7(1); 8-15.
3. Rajeswari J, Kesavan K, Jayakar B. Phytochemical and pharmacological evaluation of prop roots of Pandanus fascicularis Lam. Asian Pac J Trop Med. 2011; 4(5): 649-653.
4. Kumari S, Wanjari M, Kumar P, Palani S. Antidiabetic activity of *Pandanus fascicularis* Lamk - aerial roots in alloxan-induced hyperglycemic rats. Int J Nutr Pharmacol Neurol Dis. 2012; 2: 105-10.
5. Tanna MTH, Nath AK, Amin MN, Ibrahim M, Chowdhury MI, Mukul MEH, et al. Evaluation of antioxidants, membrane stabilizing, cytotoxic and anthelmintic activity with phytochemical screening of *Chromolaena odorata*: A medicinal shrub. Int J Pharm. 2016; 6(1): 53-61
6. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanumaethiopicum*). DARU J Pharm Sci. 2012; 20: 76.
7. Ueda JY, Tezuka Y, Banskota AH, Le Tran Q, Tran QK, Harimaya Y, et al. Antiproliferative activity of Vietnamese medicinal plants. Biol Pharm Bull. 2002; 25(6): 753-60.
8. Apu AS, Chowdhury FA, Khatun F, Jamaluddin ATM, Pathan AH, Pal A. Phytochemical Screening and In vitro Evaluation of Pharmacological Activities of Aphanamixis polystachya (Wall) Parker Fruit Extracts. Tropical Journal of Pharmacological research, 2013, 12(1): 111-116.
9. Majumder MS, Amin MN, Moghal MMR, Banik S, Kar A, Hossain MM. Anthelmintic and Cytotoxic Activities of Two Medicinal Plants: *Polygonum viscosum* and *Aphanamixis polystachya* Growing in Bangladesh. J. Sci. Res. 2014; 6(2): 339-345.
10. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982 ; 45(1): 31-4.
11. Manilal A, Sujith S, Seghal KG, Selvin J, Shakir C. Cytotoxic potentials of red alga, *laurenciabrandenii* collected from the Indian coast. Global J Pharmacol. 2009; 3: 90–94.
12. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor pre-screens. Phytochem Ana. 1991; 2: 107–111.