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Pritesh Ranjan Dash
Teaching Assistant,
Department of Pharmacy,
BRAC University, 41, Pacific
Tower, Mohakhali, Dhaka-1212,
Bangladesh.
Email: priteshranjan@bracu.ac.bd

Zara Sheikh
Senior Lecturer,
Department of Pharmacy,
BRAC University, 41, Pacific
Tower, Mohakhali, Dhaka-1212,
Bangladesh.
Email: zarasheikh@bracu.ac.bd

Preliminary studies on phytochemicals and cytotoxic activity of methanolic rhizome extract of *Hedychium coronarium*

Pritesh Ranjan Dash, Zara Sheikh

Abstract

The methanolic extract of the rhizome of *Hedychium coronarium* (Family- Zingiberaceae) was screened for the presence of various phytochemicals by standard procedures and the cytotoxic activity was evaluated by brine shrimp lethality bioassay. The present study indicates that the rhizome contain carbohydrates, flavonoids, saponins, steroids and alkaloids. In brine shrimp lethality bioassay, the extract showed potent cytotoxic action. The degree of lethality was found to be directly proportional to the concentration of the extract ranging from the lowest concentration (1.25 µg/ml) to the highest concentration (320 µg/ml). The LC₅₀ value of the methanolic rhizome extract was 0.39 µg/ml, whereas the LC₅₀ of the reference anticancer drug vincristine sulphate was 0.52 µg/ml.

Keywords: *Hedychium coronarium*, cytotoxicity, brine shrimp, *Artemia salina*.

1. Introduction

Many medicinal plants are used in modern medicine where they occupy a very significance place as raw material for important drugs and plants used in traditional system of medicine in pharmaceutical houses are collected from wild sources [1]. Medicinal plants are great important to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are: alkaloids, tannins, flavonoids and phenolics compounds. Many of these indigenous medicinal plants are also used for medicinal purposes [2]. *Hedychium coronarium* (Bengali name: Dolon Champa) is an erect herb belonging to the family Zingiberaceae. The plant is widely available in tropical and subtropical regions, such as Japan, India, Brazil, South China, Southeast Asian countries including Bangladesh [3]. This plant has tremendous medicinal properties and its various parts are used in traditional as well as modern medicine. The rhizome of the plant is used in the treatment of diabetes, cold, body aches, headache, lancinating pain, contusion, inflammation and rheumatic pain. The rhizome has anti-cancerous, antioxidant, anti-hypertensive, diuretic, leishmanicidal, anti-malarial activities and also used in irregular menstruation, piles bleeding and stone in urinary tract. Recently, antifungal activity of *Hedychium coronarium* crude extracts has been reported. Cancer chemoprevention activity is also reported recently of labdane diterpenes from rhizomes of *Hedychium coronarium*. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita [4]. Hence the present paper reports the phytochemical and cytotoxic activity of methanolic rhizome extract of *Hedychium coronarium*.

2. Materials and methods

2.1 Plant material

The rhizome of *Hedychium coronarium* was collected from the local area of Mirpur, Dhaka, during the first week of January, 2010 and was identified (Accession No. 34,484) by Bangladesh National Herbarium, Mirpur, Dhaka. Collected plants, after cutting into small pieces, were dried and pulverized into a coarse powder and stored into an air-tight container.

2.2 Extraction and sample preparation

The pulverized coarse powder of the rhizome of *Hedychium coronarium* (150 gm) was extracted with methanol by successive cold extraction. The extract obtained, were filtered off and evaporated to dryness in an oven at low temperature. The extract rendered concentrate of reddish color.

Correspondence:

Pritesh Ranjan Dash
Teaching Assistant,
Department of Pharmacy,
BRAC University, 41, Pacific
Tower, Mohakhali, Dhaka-1212,
Bangladesh.
Email: priteshranjan@bracu.ac.bd

2.3. Phytochemical screening

The methanolic rhizome extract obtained was subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Ghani ^[5, 6].

2.3.1. Test for carbohydrates

2.3.1.1. Molisch's test: 2ml solution of the extract of the plant material was taken in a test tube. 2 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 reddish violet ring was formed at the junction of the two layers which confirmed the presence of carbohydrates. 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.

2.3.1.2. Fehling's test: 2ml of an aqueous extract of the plant material add 1ml of a mixture of equal volumes of Fehling's solutions A and B. Boiled for a few minutes. A brick-red precipitate was formed immediately which confirmed the presence of carbohydrates.

2.3.2. Test for flavonoids: 0.5 ml of an alcoholic extract of the sample was taken in a test tube. Then a small piece of zinc ribbon and 5-10 drops of conc. hydrochloric acid was added. Boiled the solution for a few minutes. Development of orange to red (flavones) color which indicated the presences of flavonoids.

2.3.3. Test for saponins

2.3.3.1 Frothing test: 0.5ml of alcoholic extract was diluted to 10ml with distilled water and shaken in a graduated cylinder for 3-5 minutes. Production of persistent frothing indicated the presence of saponins.

2.3.4. Test for steroids

2.3.4.1. Salkowski test: 2ml solution of chloroform extract was taken and then 1ml of conc. sulphuric acid was added. Presence of red color indicated the presence of steroids.

2.3.5. Test for proteins

2.3.5.1. Million's test: Small amount of an extract of the plant material was taken in a test tube. Then 5-6 drops of Millions reagent was added. Formation of a white precipitate turning red on heating indicated the presences of proteins in the sample.

2.3.5.2. Biuret's test: Small amount of an extract of the plant material was taken in a test tube. Then 5-6 drops of 10% sodium hydroxide solution and 1-2 drops of 3% copper sulphate solution were added. A red color indicated the presence of proteins.

2.3.6. Test for tannins

2.3.6.1. Ferric chloride test: 2ml 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.

2.3.6.2. Potassium dichromate test: 2ml 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.

2.3.6.3. Lead acetate test: 2ml of an aqueous extract of the plant material was taken in a test tube and added a few drops of a 1% solution of lead acetate. A yellow precipitate was formed which confirmed the presence of tannins.

2.3.7. Test for glycosides: A small amount of alcoholic extract was dissolved in 1 ml of water and adding a few drops of aqueous sodium hydroxide solution. A yellow color developed in the presence of glycosides.

2.3.8. Test for glucosides: A small amount of an alcoholic extract was dissolved in water and alcohol and boiled with Fehling's solutions A and B. Production of yellow color indicated the presence of glucosides. Another portion of the extract was dissolved in water and alcohol, boiled with a few drops of dilute sulphuric acid, neutralize with sodium hydroxide solution and boil with Fehling's solution A and B. A brick-red precipitate which indicated the presence of glucosides.

2.3.9. Test for alkaloids

2.3.9.1. Mayer's test: 2ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a watch glass. Then 1ml of Mayer's reagent was added. Creamy white precipitate indicated the presence of alkaloids.

2.3.9.2. Dragendroff's test: 2ml solution of the extract and 0.2ml of dilute Hydrochloric acid were taken in a watch glass. Then 1ml of Dragendroff's reagent was added. Orange red precipitate indicated the presence of alkaloids.

2.3.9.3. Hager's test: 2ml solution of the extract and 0.2ml of dilute Hydrochloric acid were taken in a watch glass. Then 1ml of Hager's reagent was added. Yellow crystalline precipitate indicated the presence of alkaloids.

2.4. Cytotoxicity studies

2.4.1. Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was carried out according to Meyer *et al.* ^[7] to investigate the cytotoxicity of the extract. 5 mg of each of the extract was measured and dissolved in DMSO. Serial dilution was then carried out in order to obtain the concentration of 1.25 μ g/ml to 320 μ g/ml. 5 ml of artificial sea water was added into all the test tubes. *Artemia salina* was used as a convenient monitor for cytotoxic screening. The eggs of the brine shrimps were hatched in artificial seawater (prepared by using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 24 hr under the light. The hatched shrimps were allowed to grow by 48 h to get shrimp larvae called nauplii. After 48 hr, active nauplii were attached to one side in a glass Petri dish by using a micropipette. The nauplii were then separated from the eggs by aliquoting them in another glass Petri dish containing artificial sea water and used for the assay. Suspension containing 10 nauplii was added into each test tube and was incubated at room temperature of 25 ± 1 °C for 12 hr under the light. The tubes were then examined after 24 hr and the number of surviving larvae in each test tube was counted with the aid of a 3 \times magnifying glass. The percentage of mortality

was plotted against the logarithm of concentration. The concentration that would kill 50% of the nauplii (LC_{50}) was determined from probit analysis [8] as well as linear regression equation using the software "Microsoft Excel-2003".

Vincristine sulfate was used as standard in this bioassay.

3. Results

3.1. Photochemical screening

Table 1: Results of phytochemical screening

S. No	Test	Result
1	Carbohydrates	+
2	Flavonoids	+
3	Saponins	+
4	Steroids	+
5	Proteins	-
6	Tannins	-
7	Glycosides	-
8	Glycosides	-
9	Alkaloids	+

(+) = Presence, (-) = Absence

3.2. Cytotoxic activity

3.2.1. Brine shrimp lethality bioassay

The degree of lethality shown by the extract was found to be directly proportional to the concentration of the extract ranging

from the lowest concentration (1.25 $\mu\text{g/ml}$) to the highest concentration (320 $\mu\text{g/ml}$). The LC_{50} value of methanolic rhizome extract was 0.39 $\mu\text{g/ml}$ while the LC_{50} of the reference anticancer drug vincristine sulphate was 0.52 $\mu\text{g/ml}$ (Table 3).

Table 2: Effect of *Hedychium coronarium* on brine shrimp lethality test in *Artemia salina*.

conc. ($\mu\text{g/ml}$)	Log conc.	No. of nauplii taken	no. of nauplii dead				Average no. of nauplii dead	% mortality	Vincristine Sulfate				
			Trial-1	Trial-2	Trial-3	Trial-4			Std. Conc ($\mu\text{g/ml}$)	Log conc.	Nauplii taken	Nauplii dead	% mortality
1.25	0.090	10	5	3	4	4	4	40	0.156	-0.806	10	3	30
2.5	0.39	10	6	5	5	4	5	50	0.312	-0.505	10	4	40
5	0.69	10	6	6	6	5	5.75	57.5	0.625	-0.204	10	5	50
10	1	10	7	7	6	5	6.25	62.5	1.25	0.096	10	6	60
20	1.30	10	7	7	6	6	6.5	65	2.5	0.397	10	8	80
40	1.60	10	8	7	7	6	7	70	5	0.698	10	9	90
80	1.90	10	8	7	7	6	7	70	10	1	10	10	100
160	2.20	10	8	8	7	7	7.5	75	20	1.310	10	10	100
320	2.50	10	9	9	7	7	8	80	40	1.602	10	10	100

Mortality (%) = Number of dead brine shrimps \times 100/ Total number of brine shrimps.

Table 3: Result of *Hedychium coronarium* against on *Artemia salina*.

Sample	LC_{50} ($\mu\text{g/ml}$)	Regression equation	R^2
Vincristine Sulphate	0.52	$y = 32.61x + 59.22$	0.942
Methanolic extract	0.39	$y = 14.81x + 44.065$	0.944

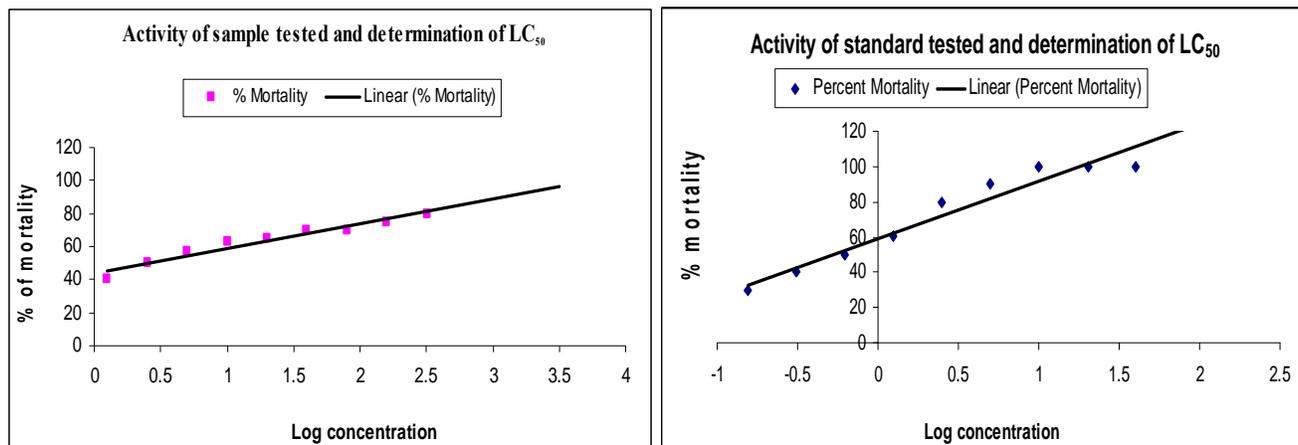


Fig 1: Effect of *Hedychium coronarium* and Vincristine sulphate on brine shrimp lethality bioassay.

4. Discussion

The results of phytochemical screening showed that the methanolic extract of the rhizome of *Hedychium coronarium* contained carbohydrates, flavonoids, saponins, steroids, alkaloids (**Table 1**). Alkaloids has addictive or pain killing or poisonous effect and sometimes help in important cure. Saponins may help to prevent colon cancer. Flavonoids possess antiallergic, anti-inflammatory, antiviral and antioxidant activities. Steroids are used to suppress various allergic, inflammatory and autoimmune disorders. The cytotoxicity bioassay against *Artemia salina* is a simple and inexpensive method to test cytotoxicity, to biodirect fractionation of natural products and as a predictor of antitumor and pesticidal activity^[9]. It also indicates antiviral, antiplasmodial, antifilarial, antimalarial activities^[10]. In this cytotoxic activity study, mortality of the nauplii was observed in all the experimental groups. Control group nauplii remained unchanged (no lethality/mortality), is indicative of the cytotoxicity of the extract. The rate of mortality of the nauplii found to be increased with increased concentration of the sample (**Table 2**). A plot of log concentration of the test sample versus percentage of mortality on a graph paper showed an approximately linear correlation between them. From this graphical plotting (**Figure 1**), the LC₅₀ value was found to be 0.39 µg/ml in methanol extracts of rhizome of *Hedychium coronarium*, whereas the LC₅₀ of the reference anticancer drug vincristine sulphate was 0.52 µg/ml (**Table 3**). From the phytochemical screening, the presence of alkaloids and steroids was observed. So the observed cytotoxic action may be due to the presence of such compounds. Again, reports exist on the role of alkaloids and steroids in cytotoxic activity of plant extracts^[11-13]. However, phenolics and flavonoids are also known to show cytotoxicity in Hoechst 33258 fluorescence assay by inhibiting cellular DNA in a concentration-dependent manner^[14]. The positive response obtained in this assay suggests that the extract may have bioactive compounds.

5. Conclusion

Results revealed that rhizome of *Hedychium coronarium* have quite a number of chemical constituents, which may be responsible for many pharmacological activities. In the brine shrimp lethality bioassay the extract showed potent cytotoxic activity. Further work is required to investigate the extract of rhizome of *Hedychium coronarium* for various pharmacological activities for the benefit of human beings.

6. Acknowledgements

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7. Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. All listed authors read and approved the final manuscript.

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