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Phytochemical screening and evaluation of cytotoxic and hypoglycemic properties of *Mangifera indica* peels

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ABSTRACT

Objective: To investigate the presence of different phytoconstituents in *Mangifera indica* (*M. indica*) peel and evaluate its cytotoxicity to *Artemia salina* and hypoglycemic potential in Swiss albino mice.

Methods: The methanolic extract of *M. indica* peel was used to determine the presence of phytoconstituents. Brine shrimp lethality bioassay method was followed to determine the cytotoxic potential of plant extract. In the case of hypoglycemic activity, oral administration of extract at 200 and 400 mg/kg and standard glibenclamide at 10 mg/kg was done, followed by determining the percentage of reduction of plasma glucose from the initial level.

Results: The methanolic extract of *M. indica* peel showed the presence of flavonoid, saponin, steroid, tannins, terpenoids, glycosides and alkaloids. In brine shrimp lethality bioassay, the LC_{50} of the extract and standard vincristine sulfate was found to be 2.04 and 0.41 μg/mL, respectively. After 90 and 150 min, the methanolic extract at 200 and 400 mg/kg showed prominent plasma glucose reduction of 13.95%, 22.48% and 14.16%, 26.18% respectively compared to standard glibenclamide showing 14.90% and 20.67% plasma glucose reduction.

Conclusions: This current research affirms prominent cytotoxic and moderate hypoglycemic potential of *M. indica* peel. Further bioactivity guided isolation of phytoconstituents and investigation on higher animals can lead to development of new drug molecules.

1. Introduction

As traditional medicine, the use of plant for the treatment of different diseases has been going on since the dawn of human history, owing to the presence of different phytoconstituents. These phytoconstituents, also known as secondary metabolites, are a huge source of molecules with outstanding diversity which helps to continue the drug discovery process [1].

Medicinal plants can exhibit different pharmacological activities such as hypoglycemic activity and cytotoxicity. Brine shrimp lethality bioassay is a technique to investigate the cytotoxic potential. This is based on cellular mechanism that may occur via necrosis which is characterized by loss of membrane integrity, death of cell or apoptosis, a genetic program of controlled cell death [2]. Like other pharmacological activities, the hypoglycemic activity is also exhibited by medicinal plants, and glucose tolerance test is usually done for the hypoglycemic activity. It is a therapeutic test where glucose is given followed by testing the blood to determine the time it takes to be cleared from the blood. The test is typically used to test for diabetes, insulin resistance, reactive hypoglycemia and rarer issue of starch digestion system.

Mangifera indica (*M. indica*) is a large spreading evergreen tree which belongs to the family Anacardiaceae [3]. This plant is

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indigenous in India and Myanmar. Apart from its application as food, the different parts of this plant possess different activities. For example, the bark is well known for its use in the treatment of diarrhea; ripe fruit is used to treat habitual constipation; the seeds are used as astringent to the bowels and leaves are used to treat piles [3]. There have also been many reported bioactivities like antioxidant, immunomodulatory, anti-inflammatory, anti-allergic, antiviral, antibacterial, antifungal and monoamine oxidase inhibitory activities of this plant [4]. In this current research, the objectives were to prepare methanolic extract of the peels of *M. indica* and analyze its cytotoxic and hypoglycemic effects.

2. Materials and methods

2.1. Collection and identification of M. indica

The entire plant of *M. indica* along with its fruit was collected from Dhaka, Bangladesh in March, 2015 and identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. The plant had been given an accession number of 41226 by the herbarium which can be used for further references.

2.2. Preparation of extract

After collecting the fruit of *M. indica*, the peel was separated and sundried for about a week to convert it into powder by using a grinder. For preparing methanolic extracts, the powder was soaked in methanol in two separate jars. The jars were kept closed with foil paper for seven days with occasional stirring. Then the soaked peel was filtered by cloth, cotton and Whatman No. 1 filter paper. The filtrate was dried in rotary evaporator at 50 °C for 40 min. Then it was poured in a beaker and kept in the fume hood for further evaporation of the solvent. After a week, sticky extract was obtained, which was kept in a dry place at normal temperature. The crude extract was used for phytochemical and pharmacological evaluation.

2.3. Drugs and chemicals

The drugs glibenclamide (standard grade) and vincristine sulfate were obtained from university laboratory. Tween 80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Pharmaceuticals Ltd., Bangladesh. Analytical reagent grade chemicals were used in all cases. Dimethyl sulphoxide (DMSO) were obtained from university laboratory.

2.4. Experimental animals

Swiss albino mice of either sex, weighed 25 g each, were collected from the animal house of State University of Bangladesh situated in Dhanmondi, Dhaka, Bangladesh. They were housed in standard polypropylene cages and kept under controlled conditions [room temperature of (24 ± 2) °C, relative humidity of 60%–70%]. Twenty mice were utilized to assess hypoglycemic activity of *M. indica*. All experimental procedures involving animals were conducted in accordance to ethical guidelines of the Faculty of Biological Science, Dhaka University (FBS/16/2016) and approved by the Institutional Ethical Committee of Faculty of Biological Science, University of Dhaka.

2.5. Phytochemical screening

The methanolic extract of the peel of *M. indica* was taken as the stock solution. This stock solution was then subjected to successive standard tests for phytochemical identification to find out the presence of different constituents including flavonoids, saponins, tannins, steroids, terpenoids, glycosides, alkaloids and carbohydrates [5–11].

2.6. Brine shrimp lethality bioassay

The brine shrimp lethality bioasssay was performed according to Mayer technique [12]. Shrimp nauplii were hatched in saline solution which mimicked ocean water. Then 4 mg of each test sample was taken and dissolved in 100 μL of pure DMSO in a glass vial. Then 50 μL of this solution was taken in a test tube containing 5 mL of simulated seawater. Thus the final concentration of the prepared solution in the first test tube was 400 μg/mL. Then a series of solutions having concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.781 μg/mL respectively were prepared by serial dilution method. In the case of negative control, the same protocol of dilution was followed without adding the test sample.

A total of 2 mg vincristine sulfate was dissolved in DMSO to prepare the positive control. Ten nauplii were added in each of the test tubes. These test tubes were kept for 24 h after which the number of survived nauplii was counted and percentage of mortality was calculated. Then a graph of percentage of mortality *v.s.* log values of concentration was drawn to figure out the LC_{50} values.

2.7. Evaluation of hypoglycemic activity

In this current study, the hypoglycemic effect of methanolic extract of the peel of *M. indica* at 200 and 400 mg/kg was analyzed. Twenty mice were randomly taken and divided into four groups, each containing five mice. At first, 10 g glucose, dissolved in 100 mL water, was administered orally by means of a long needle with a ball-shaped end to raise the blood glucose level of the mice. The tail of each mouse was pricked and blood was taken into the strip of diabetes measuring machine. Current blood glucose level of every mouse was recorded which will be used as baseline data. Then 0.2 mL extract at 200 and 400 mg/kg, control (1% Tween 80 solution in saline) and the standard glibenclamide at 10 mg/kg were given orally. Blood glucose level was checked after 0, 30, 90 and 150 min. The percentage reduction of plasma glucose level was determined by the following formula:

$$\text{Percentage reduction of plasma glucose level} = \frac{a - b}{a} \times 100$$

where, a indicates initial plasma glucose level and b indicates plasma glucose level at time 't'. The data was then recorded in a chart to measure and compare the percentage reduction of plasma glucose level between the standard and the sample.

3. Results

3.1. Phytochemical screening

The preliminary phytochemical screening of methanolic extract of the peel of *M. indica* revealed the presence of

flavonoids, saponins, tannins, steroids, terpenoids, glycosides and alkaloids.

3.2. Brine shrimp lethality bioassay

In the case of brine shrimp lethality bioassay, the methanolic extract of peel of *M. indica* showed prominent activity (Table 1). After plotting the values of percentage of mortality against log concentration, the LC_{50} value of this extract was found out to be 2.04 μg/mL. The standard vincristine sulfate showed LC_{50} value of 0.41 μg/mL (Figure 1).

Table 1
Cytotoxic effects of methanolic extract of *M. indica* peel.

Sample concentration (μg/mL)	Log concentration	Percentage of mortality
400	2.602 060	90
200	2.301 030	60
100	2.000 000	30
50	1.698 970	40
25	1.397 940	20
12.5	1.096 910	20
6.25	0.795 880	0
3.125	0.494 850	0
1.56	0.193 125	0
0.781	–0.107 350	0

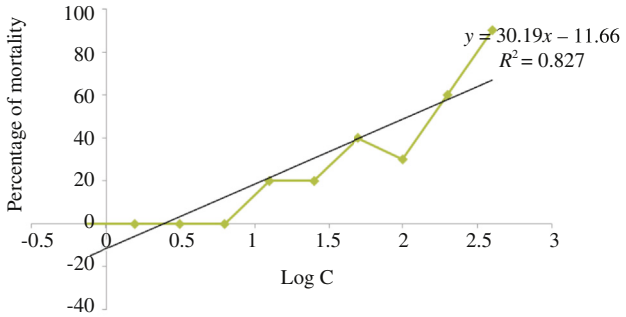

Figure 1. Percentage of mortality *v.s.* log concentration for methanolic extract of *M. indica* peel.

3.3. Hypoglycemic activity

The effects of methanolic extract of *M. indica* peel at 200 and 400 mg/kg on plasma glucose level were observed (Table 2) to evaluate their hypoglycemic activity. The extract at 200 and 400 mg/kg showed significant reduction of plasma glucose comparable to standard glibenclamide after 90 and 150 min of oral administration of sample and standard (Figure 2).

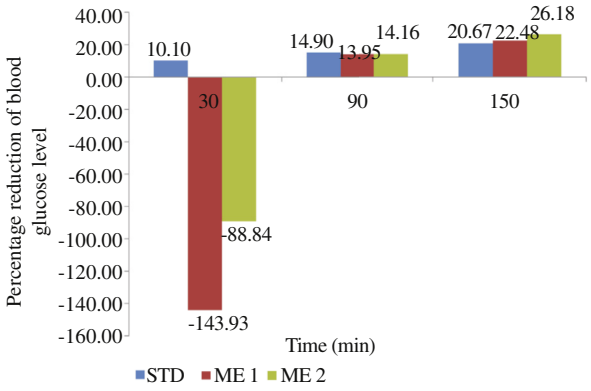

Figure 2. Percentage reduction of plasma glucose level by test materials and standard.
STD: Glibenclamide; ME 1: Methanolic extract of peel of *M. indica* at 200 mg/kg; ME 2: Methanolic extract of peel of *M. indica* at 400 mg/kg.

4. Discussion

4.1. Brine shrimp lethality bioassay

We can see that vincristine sulfate which is an anticancer drug has cytotoxic effect and the LC_{50} value was 0.41 μg/mL, whereas the LC_{50} value of *M. indica* peel extract was 2.04 μg/mL.

Due to the presence of different compounds in the extract, the LC_{50} value is very prominent which represents its high cytotoxic effect. There are some flavonoids which were reported for their strong anticancer properties. Furthermore, cholesterol lowering and cytotoxic properties are attributed to the presence of saponins [13]. Steroids and alkaloids also have cytotoxic properties [14]. Tannins possess anticancer property which is evident from its inhibitory activity towards growth of MCF-7 breast cancer cells [15]. Various types of terpenoids have the capability to suppress the growth of cancer cells, and they are also found efficacious as anticancer agents in preclinical studies [16]. The LC_{50} value of 2.04 μg/mL of the methanolic extract of *M. indica* peel may be due to the presence of these secondary metabolites. In order to confirm whether this cytotoxic activity is attributed to the presence of a single compound or compounds, we have to further investigate the extract for isolation of bioactive compounds and assess their cytotoxic activity. Based upon this study, we can also identify lead anticancer molecules having specific cytotoxicity toward various cell lines.

4.2. Hypoglycemic effect

The methanolic extract showed dose dependent reduction of plasma glucose level with time. This reduction is more

Table 2
Plasma level of glucose of mice at different time (mmol/L).

Code No.	0 min	30 min	90 min	150 min
CTL	5.700 0 ± 0.141 4	10.660 0 ± 0.350 7	7.340 0 ± 0.167 3	5.660 0 ± 0.207 4
STD	4.160 0 ± 0.054 8***	3.740 0 ± 0.114 0***	3.540 0 ± 0.151 6***	3.300 0 ± 0.200 0***
ME 1	3.870 0 ± 0.941 8	9.440 0 ± 2.173 2	3.330 0 ± 0.277 5***	3.000 0 ± 0.538 5**
ME 2	4.660 0 ± 0.568 3	8.800 0 ± 2.941 1	4.000 0 ± 1.081 7*	3.440 0 ± 1.031 0

Values are presented as mean ± SD (n = 5). ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$ compared to control. CTL: Control (1% Tween 80 solution in normal saline); STD: Glibenclamide; ME 1: Methanolic extract of peel of *M. indica* at 200 mg/kg; ME 2: Methanolic extract of peel of *M. indica* at 400 mg/kg.

prominent after 150 min of oral administration of sample (22.48% and 26.18% reduction for methanolic extract of *M. indica* peel at 200 and 400 mg/kg respectively after 150 min), which is comparable to standard glibenclamide (20.67% after 150 min). So, it can be said that the extract was moderately effective as hypoglycemic agent.

Phytoconstituents have got inherent capability to reduce the plasma glucose at various phases. Flavonoids have been reported to stimulate peripheral glucose uptake and express the enzymes responsible for metabolism of carbohydrate [17]. Alkaloids are also hypoglycemic in nature [18]. Among many enzymes, α-amylase is one which helps human body to breakdown complex polysaccharides into oligosaccharides and disaccharides. α-Glucosidase then hydrolyzes these into simple absorbable monosaccharides which is responsible for increase in postprandial glucose levels [19,20]. Tannins have α-amylase and α-glucosidase inhibition capability [21]. Again, lupeol type terpenoid is also reported for its α-amylase inhibition activity [22]. Saponins can stimulate the beta cells and pancreatic islets of the pancreas which can lead to the decrease in blood glucose [23]. Stimulation of 5-adenosine monophosphate activated protein kinase and insulin receptor/insulin receptor substrate 1/phosphatidylinositol 3-kinase/Akt signaling pathways leading to decrease in blood glucose is also demonstrated by saponin. So, saponins can also be another potential hypoglycemic compound [24]. Isolation of the active phytoconstituents and further evaluation of hypoglycemic activities can assert the reason for showing hypoglycemic property. Besides, further studies can be carried out to strengthen the glucose lowering activity of peels of *M. indica* using streptozotocin- and aloxan-induced diabetic rat models for chronic studies.

From the above discussion, it can be concluded that the methanolic extract of the *M. indica* peel possesses prominent cytotoxic and moderate hypoglycemic activity. The preliminary screening of the extract and its pharmacological activity paves the way towards discovering new drug molecules. Therefore, further extensive studies on isolation of the phytoconstituents and investigation on clinical level can lead to the development of new therapeutic formulations.

Conflict of interest statement

We declare that we have no conflict of interest.

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