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Phytochemical and Pharmacological Evaluation of Ethanol Leaves Extract of *Hydnocarpus kurzii* (King) Warb

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ABSTRACT

Hydnocarpus kurzii, is a plant found in the Southeast Asian region. Various parts of the plant such as barks, fruits, leaves have many therapeutic uses in traditional medicinal system. After reviewing various journals and on the basis of traditional uses, our work was designed to conduct various phytochemical and pharmacological tests (antioxidant, antidiabetic, analgesic, laxative, diuretic, antihelminthic, blood anticoagulation) on the leaves of the plant. The extract showed DPPH free radical and Hydrogen peroxide scavenging activities (IC_{50} values were 58 µg/mL and 67 µg/mL, respectively). Reducing power assay was also performed (RC_{50} = 79 µg/mL). Total phenolic, flavonoid, tannin content and total antioxidant capacities of the plant were found to be 242 mg GAE/g, 365 mg QE/g, 289 mg GAE/g and 274 mg AAE/g, respectively. The plant caused a significant reduction in blood glucose level in oral glucose tolerance test (OGTT) and alloxan-induced diabetic test, though it did not show any response in *α*-glucosidase enzyme inhibitory test. The extract exhibited significant analgesic, laxative, diuretic, anticoagulant and antihelminthic activities in a dose-dependent manner and these were compared with the respective standard drugs. All of these results signified the plants' utilization in traditional medicinal system. We hope that these preliminary studies will be helpful for conducting further research on this plant in order to isolate promising drug molecule.

Keywords: *Hydnocarpus kurzii*, antioxidant, antidiabetic, analgesic, diuretic, anticoagulant.

Introduction

Mankind has always depended on medicinal plants for eradication and management of diseases. *Hydnocarpus kurzii*- a medicinal plant from Achariaceae family, is cultivated in areas of Southeast Asia, tropical Africa and tropical South America. In Bangladesh, it is commonly found in the Chittagong Hill Tracts, Cox's Bazar, Moulavi Bazar, Mymensingh and locally known as Chaulmoogra. *H. kurzii* is a 12-15 meter tall evergreen, dioecious tree. The lanceolate leaves are 18-25 cm long. The reddish brown fruits are orange shaped.[1,2] The main medicinal ingredient- chaulmoogra is extracted from the seeds which are used to treat different types of diseases such as scabies, eczema, psoriasis, scrofula, ringworm, and intestinal parasitic worms.[2] Besides the oil, its leaves, fruits and barks are also used in the traditional medicinal system for their analgesic, purgative, antioxidant, antidiabetic, haematinic and cytotoxic properties.[2] Different cyclopentenyl fatty acid derivatives such as hydnocarpic acid, chaulmoogric acid, gorlic acid along with oleic acid, palmitic acid, myristic acid, stearic acid, palmitoleic acid, linoleic acid, linolenic acids, gallic acid, hydnocarpin were isolated from various parts of this plant.[1,3] Following the review of its traditional uses, we selected its leaves to quantify the antioxidative components, radical scavenging properties and evaluate its different pharmacological properties like antioxidant, antidiabetic, analgesic, laxative, diuretic, antihelminthic and blood anticoagulation properties. Our work is aimed at justifying the plant's usage in the traditional medicinal system so that further investigations to obtain specific and quantified results and to separate pure bioactive compound(s) can be conducted on the plant for the development of new drug leads.

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Materials and Methods

Chemicals

Analytical grade reagents such as 2,2-diphenyl-1-picryl hydrazyl (DPPH) (Sigma, USA), FC reagent (Merck, India) along with Na_2CO_3 (Loba, India), $NaNO_2$ (Loba, India), $AlCl_3$ (Loba, India), NaOH (Loba, India), H_2O_2 (Merck, Germany), Na_2HPO_4. $2H_2O$ (Loba, India), $NaH_2PO_4.2H_2O$ (Loba, India), $FeCl_3$ (Merck, Germany), ascorbic acid (Merck, Germany), powdered alloxan (Merck, Germany), and α-glucosidase enzyme from *Saccharomyces cerevisiae* (Sigma, USA) were used. All of the medicines used for pharmacological tests were purchased from the Square Pharmaceuticals Ltd. and Eskayef Pharmaceuticals Ltd., Bangladesh.

Collection and extraction of plant materials

H. kurzii leaves were collected from the botanical garden of Bangladesh Agricultural University, Mymensingh in June, 2019. The dried sample was identified by Sarder Nasir Uddin, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. Then an authentication number was provided (DACB-64653). After shade-drying properly for 45 days, cold extraction was carried out with 96% ethanol on 280 gm of dried powdered leaves to get 15.876 gm crude extract (yield = 5.67%) which was used to conduct the experiments mentioned in this study.

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Animals
For conducting some pharmacological experiments, young Swiss-albino mice (*Mus musculus*), possessing weights of 20–25 gm, were collected from the animal house of Jahangirnagar University, Bangladesh. For adaptation, the mice were kept in optimum environmental condition for 2-3 weeks in the animal house of Pharmacology Laboratory of Pharmacy Discipline, Khulna University, Bangladesh. All of the experiments were conducted in an attenuated, separated and noiseless condition. All of the experimental procedures performed in this study involving animals were in accordance with the ethical standards of Animal Ethics Committee (AEC), Khulna University, Khulna-9208, Bangladesh [Ref: KUAEC-2020/10/20].

Phytochemical screening
The extract was tested for the presence of different types of secondary metabolites such as, reducing sugars, polyphenols, flavonoids, tannins, glycoside, steroids, terpenoids, alkaloids using the method of Golder *et al*.[4]

Evaluation of in vitro antioxidant activity
Qualitative antioxidant activity test
This test was performed by using Silica gel pre-coated Thin Layer Chromatography (TLC) plates developed in polar, medium polar and nonpolar solvent systems. A suitable diluted plant stock solution was spotted on TLC plates and then 0.02% DPPH solution of ethanol was sprayed on the plates. Any hydrogen donating molecule from the extract will react with DPPH and bleach it.[4]

DPPH free radical scavenging assay
DPPH free radical scavenging property of *H. kurzii* was evaluated according to method of Golder *et al*.[4] Plant extract solutions of different concentrations (4096-1 µg/mL) were prepared and then DPPH solution (methanol was used as solvent) were added to each. Then absorbance was taken at 517 nm by the help of Thermo Scientific Multiskan Ex microplate photometer. Then antioxidant activity was expressed in terms of IC_{50} (µg/mL concentration of plant sample required to scavenge 50% of DPPH free radicals formation). The IC_{50} was calculated from the curve of log concentration versus percent inhibition.

Hydrogen peroxide scavenging assay
The ability of the *H. kurzii* extract to scavenge Hydrogen peroxide was determined according to the method of Golder *et al*.[4] In this assay different concentrations (800-6.25 µg/mL) of plant extract were prepared and then Hydrogen peroxide (40 mM) was added in each. Then absorbance was measured at 230 nm. Percentage of Hydrogen peroxide scavenging by the extract and standard compounds was calculated from log concentration-inhibition curve and was expressed as IC_{50}.

Reducing power assay
Antioxidants show their reducing power by converting ferric iron to ferrous iron.[5] Substances possess reduction capacity can react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}). Reducing power of the *H. kurzii* extract was measured according to the method of Debnath *et al*.[6] In this assay, plant extract of different concentrations (800-6.25 µg/mL) were prepared and was mixed with 0.2 M phosphate buffer, 1% potassium ferricyanide, 10% trichloroacetic acid (TCA), distilled water and 0.5 mL of ferric chloride (0.1%). Finally, absorbance was taken at 700 nm and percentage of reduction was calculated from the calibration curve and was expressed as RC_{50} (concentration of the sample in µg/mL required to reduce 50% of potassium ferricyanide).

Evaluation of total content of secondary metabolites
Total phenolic content assay
Total phenolic content of the extract was determined by using Folin-Ciocalteu reagent with analytical grade gallic acid as the standard.[7] Then it was expressed as mg of gallic acid equivalent per gram of dry extract using the calibration curve.

Total flavonoid content assay
Total flavonoid content of the extract was determined using aluminum chloride colorimetric assay.[7] Then it was expressed as mg of quercetin equivalent per gram of dry extract using the calibration curve.

Total tannin content assay
Total tannin content of the extract was determined using the Folin-Ciocalteu reagents.[7] Then it was expressed as mg of gallic acid equivalent per gram of dry extract using the calibration curve.

Total antioxidant capacity assay
Total antioxidant capacity of sample extracts was determined by phosphomolybdate method.[8] Then it was expressed as mg of Ascorbic acid equivalent per gram of dry extract using the calibration curve.

Evaluation of antidiabetic activity
Oral glucose tolerance test
Oral glucose tolerance test (OGTT) is a worldwide famous method used to assess how quickly exogenous glucose can be cleared from blood. In this test, oral glucose tolerance and antihyperglycaemic activity test of plant extract were screened according to the method of Mazumder *et al.* where glibenclamide at 5 mg/kg was administered as standard drug.[9] After administering glucose solution orally at 2 gm/kg body weight, blood glucose level (mmol/l) of each mouse were measured at the 0, 30, 60, 90, 120 and 150 minutes with a glucometer.

Alpha-glucosidase enzyme inhibitory activity test
Alpha-glucosidase enzyme inhibitory activity of *H. kurzii* extract was evaluated using method described by Ivan and Muhammad with slight modifications.[10,11] Voglibose was used as the positive control. Different concentrations of *H. kurzii* and voglibose were prepared. Then potassium phosphate buffer, alpha-glucosidase enzyme and p-nitrophenyl glucopyranoside were mixed with the extract and standard. Then the reaction was terminated by adding Sodium Carbonate solution. Finally, absorbance was taken at 405 nm using a Thermo Scientific Multiskan Go spectrophotometer. The α-glucosidase inhibitory activity was calculated using the curve of log concentration versus percent inhibition and was expressed as was expressed as IC_{50} (conc. of sample required to inhibit 50% of enzyme).

Alloxan-induced diabetic test
Antidiabetic test of *H. kurzii* extract was performed using alloxan-induced diabetes model in Swiss albino mice.[12] Alloxan causes diabetes by a mechanism which basically involves partial degradation of the beta (β) cells of pancreatic islets [13].The mice were kept fasting up to 12 hours before the starting of the experiment. Freshly prepared 5% alloxan monohydrate solution was prepared and it was administered intraperitoneally at 125 mg/kg doses to induce diabetes. After one week, mice having a fasting blood glucose level of 200–300 mg/dL were treated as diabetic mice. Mice having blood glucose level higher than 300 mg/dL were excluded from this test because it was assumed that their pancreatic β cells were considered almost completely damaged.[13] Then, mice were divided into five groups (I: nondiabetic control, II: diabetic control, III: *H. kurzii* extract- 50 mg/kg, IV: *H. kurzii* extract- 100 mg/kg, V: Glibenclamide, standard drug- 5 mg/kg). Group I and II were administered with normal saline solution. Mice were orally administered with the sample extracts and standard drug once daily for 28 days. Blood glucose level (mg/dL) was measured at 0^{th}, 1^{st}, 5^{th}, 7^{th}, 14^{th} and 28^{th} days with the help of a glucometer.

Evaluation of analgesic activity by acetic acid-induced writhing method
Peripheral analgesic activity of *H. kurzii* extract was tested according to the acetic acid induced writhing method in mice as described by Debnath *et al*.[6] *H. kurzii* extract was given at doses of 250 mg/kg and 500 mg/kg body weight. Diclofenac sodium at the dose of 25 mg/kg body weight was used as standard.[6] After 30 minutes interval of oral administration of *H. kurzii* and Diclofenac sodium, 0.7% acetic acid was administered intraperitoneally for induction of writhing in mice

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(constriction of abdomen, turning of trunk and extension of hind legs). Then after 15 minutes, no. of writhing was counted up to 5 minutes for each mouse. Percent inhibition of writhing in comparison to the control group was taken as an index of analgesia and was calculated by the following formula:

Inhibition of writhing (%) = $[(W_c - W_t) \times 100] / W_c$

Where W_c is the average number of writhing reflexes in the control group, W_t is the average number of writhing reflexes in the test group

Evaluation of laxative activity
Evaluation of laxative activity of *H. kurzii* extract was carried out following the method described by Capasso *et al*. with minor modifications.[14] Bisacodyl at the dose of 10 mg/kg was used as standard drug. *H. kurzii* extract was given at doses of 250 mg/kg and 500 mg/kg body weight. After 12 hours fasting, mice were orally administered with the *H. kurzii* extract and bisacodyl. Then after keeping those in metabolic cages for next 16 hours, the stool was collected & weighed. This experiment was performed in duplicate.

Evaluation of diuretic activity
This diuretic test of *H. kurzii* extract was carried out by the method described by Lahlou *et al.* with minor modification.[15] Furosemide at 5 mg/kg was used as standard drug. *H. kurzii* extract was given at doses of 250 mg/kg and 500 mg/kg body weight. The concentrations of reference diuretic (furosemide) as well as the *H. kurzii* extract were adjusted in such a way that each animal was administered with 2 mL of solution every time. The diuretic activity was calculated using the following equations.[16]

Urinary excretion = [Total urinary output (v_o) / Total liquid administered (v_i)] x 100

The diuretic action was calculated by dividing the urinary excretion of test group by the urinary excretion of control group and the diuretic activity was then measured by dividing the diuretic action of test group by the diuretic action of control group.

Evaluation of antihelminthic activity
Antihelminthic activity of *H. kurzii* extract was evaluated by the method described by Mahalder *et al.*[17] Live *P. cervi.* was collected from intestine of freshly slaughtered cattle at local abattoirs of Gallamari, Khulna. Albendazole at 15 mg/mL was used as standard drug. Plant samples were prepared at 50, 25, 12.2 and 6.25 mg/mL concentrations. Six of *P. cervi* were placed in each petri dish containing 10 mL of the above prepared solutions of *H. kurzii* extract and albendazole. After waiting for a while, the paralysis time was considered when no movement was observed even after vigorously shaking. When the parasites could not move even when shaken strongly, dipped in hot water (50ºC) or subjected to outer stimuli, then the time for death was recorded.

Evaluation of blood anticoagulant activity
In vitro blood anticoagulation activity of *H. kurzii* extract was performed according to the prothrombin time (PT) test described by Salzman *et al.*[18] Fresh human blood was collected from healthy individuals by using a sterile syringe. Then pure platelet plasma (PPP) was separated from blood after centrifuging at 3000 rpm for 15 minutes. Warfarin at 5 mg/mL was used as standard drug. *H. kurzii* solution was prepared at 350, 175 and 87.5 mg/mL concentrations by serial dilution. Then 0.2 mL plasma, 0.1 mL of 0.9% saline 0.3 mL $CaCl_2$ (25 mM) were added separately in the above concentrations of *H. kurzii* extract and warfarin in different test tubes. After incubating the test tubes at 37°C in a water bath, the clotting time was recorded with a stop watch by tilting the test tubes every 5 seconds. Every test was carried out three times and the average clotting time was noted.

Statistical analysis
All of the statistical evaluations of test results were carried out by using Dunnet's test for one-way ANOVA analysis ($p < 0.05$, versus control). Pairwise comparisons were carried out with Post-hoc Tukey test ($p < 0.05$, versus standard/extract) within the mean values. Data analysis was done with SPSS software of IBM Corporation, New York, USA (version 16.0).[19]

Results and Discussion

Qualitative phytochemical screening conducted on *H. kurzii* extract demonstrated the presence of many phytochemical groups such as carbohydrates, tannins, phenolics, flavonoids, steroids, alkaloids, glycosides and terpenoids. These phytochemical groups may be responsible for different pharmacological activities.
In our qualitative antioxidant assay, the extract spotted TLC plates were kept under UV light at 254 and 366 nm. Then several UV positive and fluorescent components were found. Then, the plates were sprayed with previously prepared 0.2% DPPH solution that resulted in the development of yellow colors which indicated the presence of antioxidant compounds. Then in quantitative measurements of antioxidant tests, total content of phenolic, flavonoid and tannin compounds of *H. kurzii* were found to be 242 mg gallic acid equivalent (mg GAE)/g, 365 mg quercetin equivalent (mg QE/g) and 289 mg GAE/g, respectively (Table 1). The *H. kurzii* extract possessed a good number of antioxidant compounds and from the total antioxidant capacity test, the obtained antioxidant capacity was 274 mg AAE/g (Table 1).
Free radicals (molecules containing one or more unpaired electrons) are continuously produced in our body by different oxidase enzymes and they cause lipid peroxidation, DNA bases fragmentation which lead to different diseases such as inflammation, cancer, cardiovascular and respiratory disorders, atherosclerosis, muscular dystrophy, diabetes and neurological disorders.[7,20] Due to the presence of phytoconstituents like polyphenols, flavonoids, tannins, alkaloids, terpenoids, glycosides, plants are a great source of antioxidants.[7,21] Our results indicate that this plant possess good antioxidative phytochemicals which will be helpful to combat different oxidative reactions and protect our body from oxidative damages.
After determining the qualitative antioxidative potential, we put our focus on different radical scavenging assays. DPPH, a well-known stable radical can quickly accept an electron or hydrogen from the antioxidant molecules to develop into a stable molecule DPPH-H and get converted into pale yellow color from violet color.[7] In the DPPH free radical scavenging assay, the calculated IC_{50} (50% inhibitory concentration) values were 58 µg/mL and 13 µg/mL for *H. kurzii* and ascorbic acid, respectively. Hydrogen peroxide, a strong oxidative agent gets rapidly decomposed into oxygen and water and thus Hydroxyl radicals (OH) are produced. In the Hydrogen peroxide scavenging assay, the calculated IC_{50} values were 67 µg/mL and 25 µg/mL for *H. kurzii* extract and ascorbic acid, respectively (Table 1). Reducing power of antioxidants convert ferric iron to ferrous iron which is beneficial for human health.[5] In reducing power assay, maximum absorbance was found for the concentration of 800 µg/mL and that was 1.284 whereas that for ascorbic acid was 1.624. After calculating the results, the RC_{50} (50% reducing concentration) values of *H. kurzii* extract and ascorbic acid were 79 µg/mL and 40 µg/mL, respectively (Table 1).
Diabetes, a chronic metabolic disorder (marked with high blood sugar) is further associated with severe complications including renal failure, cardiac disorder, neurological complications, cerebro-vascular disease, blindness, long term dysfunctions and failure of organs like liver, kidney, heart, lungs, muscles, pancreas, thereby promoting premature death.[9,22] Plants possessing different types of secondary metabolites, especially the antioxidant compounds are potent therapeutic agents to combat diabetes and associated diseases. α-glucosidase and α-amylase inhibitory activities of tannins, flavonoids, polyphenolic compounds performed in different *in vivo* and *in vitro* models revealed that they have a high potential to serve as a remedy against type 1 and type 2 diabetes.[9] In our OGTT experiment, the *H. kurzii* extract reduced the

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blood glucose level significantly with the period of time (Figure 1). In another experiment percentage inhibition of *α*-glucosidase by the voglibose was calculated at respective concentrations (IC_{50} = 0.3 mg/mL) as shown in. In this test, *H. kurzii* extract did not show any inhibitory effect towards α-glucosidase. So, it can be suggested that, *H. kurzii* extract may reduce blood glucose level without having any inhibitory effect on α-glucosidase enzyme. Alloxan-induced diabetes is one of the commonly used models to induce diabetes mellitus in the experimental animals as it selectively destroys the insulin-producing β-cells of the pancreas, leaving the non-beta cells and other endocrine and non-endocrine islet cell types intact.[23] After administering alloxan once, the measured blood glucose level were found to be decreased significantly over the period of time (average values are plotted in the Figure 3). Thus, from the above tests we can say that, the *H. kurzii* extract showed antidiabetic activity on the mice model. Compounds having antidiabetic properties of this plant like palmitoleic acid, gallic acid, linoleic acid, oleic acid, hydnocarpin may be responsible for these effects. Besides these, presence of other phytochemical groups such as tannins, flavonoids, polyphenolics, alkaloids, glycosides, flavonoids, steroids are also responsible for antidiabetic properties as their antidiabetic property is well reported.[9]

Algesia (pain) is an obnoxious sensation which is usually originated by an external or internal unpleasant stimulus. Intraperitoneal administration of 0.7% acetic acid leads to the biosynthesis of leukotrienes & prostaglandins. These released prostaglandins, mainly prostacyclin (PGI_2), PGE_2 and $PGF_{2\alpha}$ have been responsible for pain sensation or writhing.[6] In our analgesic test, the *H. kurzii* extract showed significant inhibition of writhing impulse by 32.26% and 64.52% at 250 mg/kg and 500 mg/kg doses, respectively. Diclofenac sodium showed 80.65% inhibition of writhing at the dose of 25 mg/kg (Table 2). These significant inhibitions of writhing impulse of *H. kurzii* extract might be due to the presence of compounds like oleic acid, linoleic acid, linolenic acid, palmitoleic acid, gallic acid, hydnocarpin and other phytochemical groups such as alkaloids, glycosides, terpenoids, polyphenolic compounds, tannins as their analgesic property already reported.

Constipation is a very common bowel problem that is a predisposing factor to various colorectal disorders (enlarged hemorrhoids, colorectal cancer etc). Laxatives are used frequently to treat constipation by adding bulk to intestinal contents by retaining water within the bowel lumen and increasing the frequency and ease of defecation.[24] In our laxative test, we observed that *H. kurzii* extract significantly increase weight of stool in dose dependent manner. The weight increases were found 64.45% and 95.56% at the doses of 250 mg/kg and 500 mg/kg whereas bisacodyl increased the stool weight 115.56% at the dose of 10 mg/kg (Table 3). Thus, we can say that *H. kurzii* extract showed good laxative activity. The possible reason for showing the laxative activity of *H. kurzii* extract may be the presence of stearic acid and other phytochemical groups like terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids. These compounds have been previously found to be responsible for laxative activity in plants.[24]

At present, hypertension is one of the major causes of death of people worldwide. About 25% of the world's adult population has hypertension and this is likely to increase to 30% by 2025.[25] In treating hypertension, diuretics are the first-line choice of drugs as they increase urinary output by inhibiting the reabsorption of water and other electrolytes. This leads to the reduction of blood volume and consequently, reduction of blood pressure.[26] In medicine, diuretics are frequently used to treat heart failure, liver cirrhosis, hypertension, chronic renal failure, liver cirrhosis, water poisoning, and certain kidney diseases.[25] In our diuretic test, we observed that *H. kurzii* extract significantly increased the volume of urine in the dose-dependent manner. Thus, it showed both good diuretic action and diuretic activity over the period of time. The urinary excretion and diuretic action of *H. kurzii* extract are shown in Figure 4 and 5, respectively. Previous studies have demonstrated that several compounds such as gallic acid, palmitic acid, myristic acid, linoleic acid and other phytochemical groups like flavonoids, saponins, volatile oils, sterols, glycosides or organic acids are responsible for diuretic effect.[27,28]

Parasitic worms, also known as helminths, can be found in the intestine of human and other vertebrates where they cause diseases by secreting toxins and stealing vital nutrients from host bodies. Medicinal plants have been of great interest in recent years for the management of helminthiasis due to contain wide range of antihelminthic compounds.[17] In antihelminthic test, both paralysis and death time were monitored and recorded for the *H. kurzii* extract on *P. cervi*. The values were found in dose-dependent manner. *H. kurzii* extract showed paralysis time at 56.42 min, 50.23 min, 42.48 min and 36.52 min for the doses of 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL and 50 mg/mL respectively where 8.5 min for Albendazole at the 15 mg/mL dose. The death time for the extract were 72 min, 56.82 min, 49.83 min and 41.72 min for the respective doses for the *H. kurzii* extract and for Albendazole it was 18.08 min (Table 4). From these results we can assume that *H. kurzii* extract did not show very promising antihelminthic activity. Compounds like chaulmoogric acid, hydnocarpic acid, linolenic acid and phytochemicals such as tannins, alkaloids, flavonoids may be responsible for this antihelminthic activity as such property was previously reported.[29]

Blood is the most important biological fluid that carries different nutrients to the cell from food. Hemophilia is one type of bleeding disorder that impairs body's ability to form blood clots. This problem may be life threatening to patients who suffer deep wound, serious injury due to severe blood loss.[30] In our blood anticoagulant assay, the clotting time was monitored for the warfarin and *H. kurzii* extract at different doses. These values are given in table 5. Considering the results, we can say that *H. kurzii* extract showed promising anticoagulant properties. Different phytoconstituents like linoleic acid, linolenic acid as well as alkaloids, tannins, flavonoids and steroids have previously been reported to be responsible for blood anticoagulant property.[31,32]

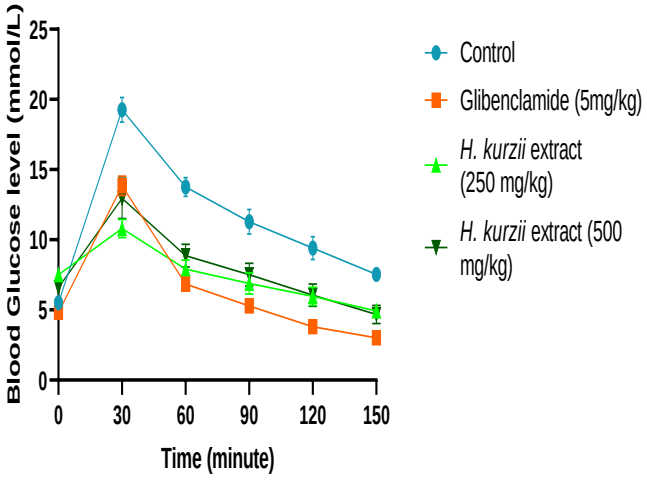

Figure 1: Comparison of blood glucose levels (mmol/L) at different time points for control, standard and *H. kurzii* extract in the oral glucose tolerance test.

Table 1: Approximate IC_{50} values of different radicals scavenging assays and total content of secondary metabolites (phenolics, flavonoids and tannins) of *H. kurzii* extract

Sample extract	TPC (mg GAE/g)	TFC (mg QE/g)	TFC (mg GAE/g)	TAC (mg/AAE)	DRSA (IC_{50} µg/mL)	HPSA (IC_{50} µg/mL)	RPA (RC_{50} µg/mL)
H. kurzii	242	365	289	274	58	67	79

TPC (Total Phenolic Content), TFC (Total Flavonoid Content), Total Tannin Content (TTC), TAC (Total Antioxidant Capacity), DRSA (DPPH Radical Scavenging Activity), HPSA (Hydrogen Peroxide Scavenging Activity), RPA (Reducing Power Assay)

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Table 2: Effects of *H. kurzii* extract on acetic acid induced writhing in mice

Treatment group	Dose (mg/kg)	Mean writhing	% inhibition of writhing
Negative control	-----	$12.4 \pm 4.16^{\theta\blacktriangle\Delta}$	------
Standard (Diclofenac Na)	25	$2.4 \pm 1.14^{*\blacktriangle}$	$80.64 \pm 9.21^{*\blacktriangle}$
H. kurzii extract	250	$8.4 \pm 1.14^{*\theta\Delta}$	$32.26 \pm 9.2^{*\theta\Delta}$
H. kurzii extract	500	$4.4 \pm 0.89^{*\blacktriangle}$	$64.52 \pm 7.21^{*\blacktriangle}$

Data are means of five replicates ± SD (standard deviation); *P < 0.05 vs. Control (Dunnett's t test); $^{\theta}$P < 0.05 vs. Diclofenac Na 25 mg/kg; $^{\blacktriangle}$P < 0.05 vs *H. kurzii* extract 250 mg/kg; $^{\Delta}$P < 0.05 vs. *H. kurzii* extract 500 mg/kg (pair-wise comparison by Post Hoc Tukey test)

Table 3: Effects of *H. kurzii* extract on laxative test

Treatment group	Dose (mg/kg)	Mean stool weight (gm)	% increase of stool weight
Negative control	------	$0.445 \pm 0.045^{\theta\blacktriangle\Delta}$	------
Standard (Bisacodyl)	10	$0.97 \pm 0.01^{*\blacktriangle}$	$115.56 \pm 2.22^{*\blacktriangle}$
H. kurzii extract	250	$0.745 \pm 0.495^{*\theta}$	$65.55 \pm 7.77^{*\theta}$
H. kurzii extract	500	$0.885 \pm 0.035^{*}$	$96.65 \pm 5.55^{*}$

Data are means of two replicates ± SD (standard deviation); *P < 0.05 vs. Control (Dunnett's t test); $^{\theta}$P < 0.05 vs. Bisacodyl 10 mg/kg; $^{\blacktriangle}$P < 0.05 vs *H. kurzii* extract 250 mg/kg; $^{\Delta}$P < 0.05 vs. *H. kurzii* extract 500 mg/kg (pair-wise comparison by Post Hoc Tukey test).

Table 4: Effects of *H. kurzii* extract on antihelminthic test

Treatment group	Dose (mg/mL)	Mean paralysis time (min)	Mean death time (min)
Negative control	------	------	------
Standard (Albendazole)	15	$8.51 \pm 0.75^{\blacktriangle\Delta*\Psi}$	$18.08 \pm 0.62^{\blacktriangle\Delta*\Psi}$
H. kurzii extract	6.25	$36.52 \pm 2.02^{\theta\Delta*\Psi}$	$41.72 \pm 1.42^{\theta\Delta*\Psi}$
H. kurzii extract	12.5	$42.42 \pm 0.78^{\theta\blacktriangle*\Psi}$	$96.65 \pm 5.55^{\theta\blacktriangle*\Psi}$
H. kurzii extract	25	$50.23 \pm 1.35^{\theta\blacktriangle\Delta\Psi}$	$56.82 \pm 0.98^{\theta\blacktriangle\Delta\Psi}$
H. kurzii extract	50	$56.42 \pm 1.88^{\theta\blacktriangle\Delta*}$	$71.00 \pm 2.64^{\theta\blacktriangle\Delta*}$

Data are means of six replicates ± SD (standard deviation); $^{\theta}$P < 0.05 vs. Albendazole 15 mg/mL; $^{\blacktriangle}$P < 0.05 vs *H. kurzii* extract 6.25 mg/mL; $^{\Delta}$P < 0.05 vs. *H. kurzii* extract 12.5 mg/mL, *P < 0.05 vs. *H. kurzii* extract 25 mg/mL, $^{\Psi}$P < 0.05 vs. *H. kurzii* extract 50 mg/mL (pair-wise comparison by Post Hoc Tukey test)

Table 5: Effects of *H. kurzii* extract on anticoagulant test

Treatment group	Dose (mg/mL)	Coagulation time (min)
Negative control	0.9% Saline	$8.70 \pm 0.28^{\theta\blacktriangle\Delta\psi}$
Standard (Warfarin)	5	$58.9 \pm 0.14^{*\blacktriangle\Delta\psi}$
H. kurzii extract	87.5	$27.40 \pm 0.14^{*\theta\Delta\psi}$
H. kurzii extract	175	$37.00 \pm 2.40^{*\theta\blacktriangle\psi}$
H. kurzii extract	350	$48.95 \pm 1.77^{*\theta\blacktriangle\Delta}$

Data are means of three replicates ± SD (standard deviation); *P < 0.05 vs. Control (Dunnett's t test) $^{\theta}$P < 0.05 vs. Warfarin 5 mg/mL; $^{\blacktriangle}$P < 0.05 vs *H. kurzii* extract 87.5 mg/mL; $^{\Delta}$P < 0.05 vs. *H. kurzii* extract 175 mg/mL, $^{\psi}$P < 0.05 vs. *H. kurzii* extract 350 mg/mL, (pair-wise comparison by Post Hoc Tukey test)

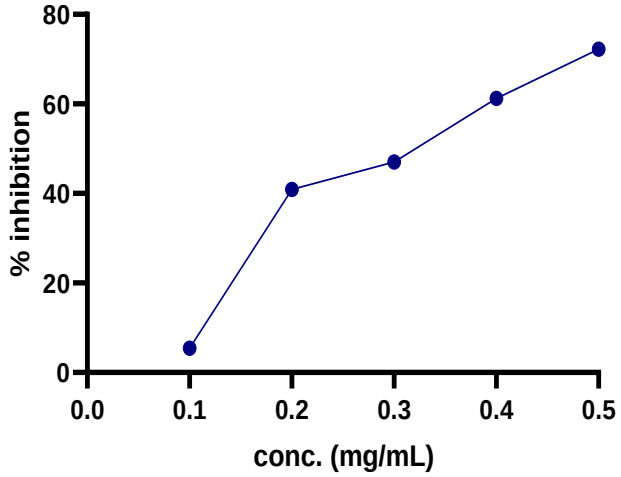

Figure 2: Graphical presentation of α-glucosidase enzyme inhibitory activity of voglibose

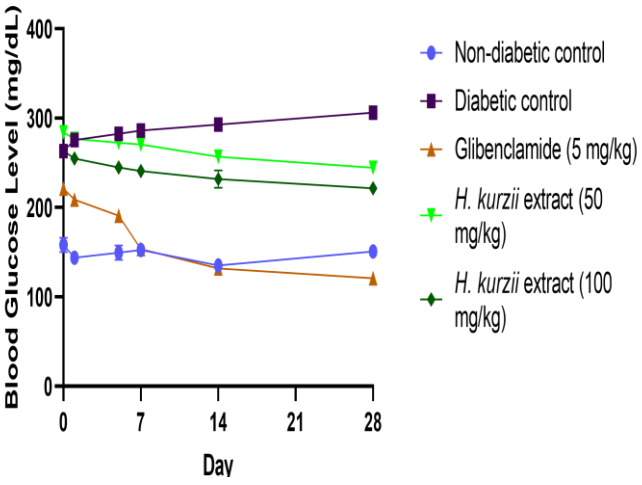

Figure 3: Comparison of blood glucose levels (mg/dL) at different time points for control, standard and *H. kurzii* extract in the alloxan-induced diabetic test.

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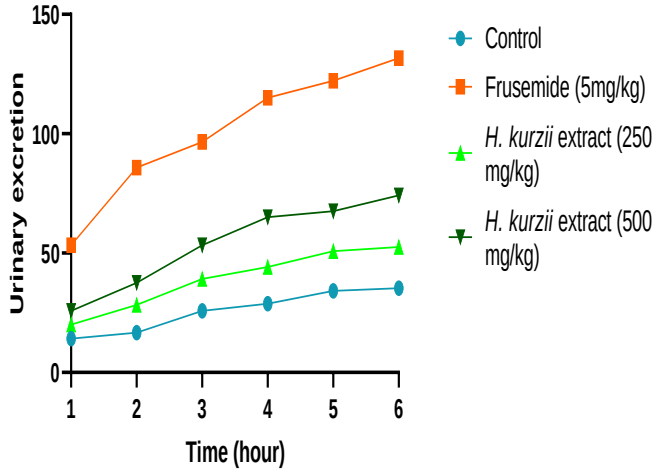

Figure 4: Urinary excretion of *H. kurzii* extract and frusemide

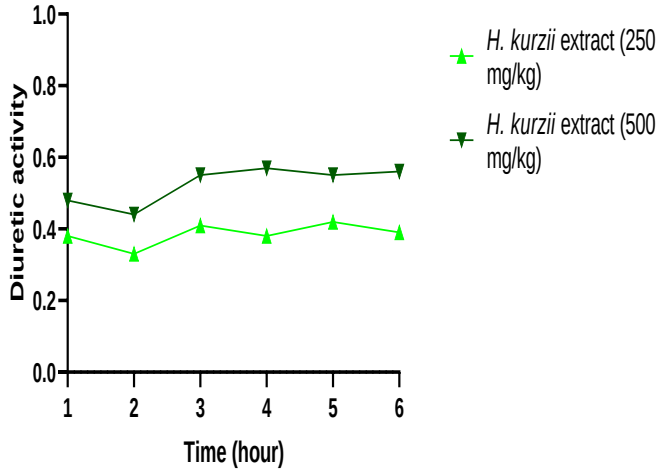

Figure 5: Diuretic activity of *H. kurzii* extract comparing with frusemide

Conclusion

Our present study concludes that the leaves of *H. kurzii* extract showed promising antidiabetic activities in both OGTT and alloxan-induced diabetic test in mice model. This extract also demonstrated good antioxidative capacity and possess substantial amount of secondary metabolites. The extract further displayed significant analgesic, laxative, diuretic activities in specific model. This plant only showed mild anticoagulant property. These pharmacological activities of the plant may be attributed to the presence of multiple phytoconstituents. The results justify the plant's usage in the traditional medicinal system. Further researches and mechanistic investigations are suggested to obtain specific and quantified results. Isolation of pure compound(s) is also suggested for the development of new drug leads.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Ghani A. Medicinal plants of Bangladesh: Chemical constituents and uses. (1st ed.). Dhaka: Asiatic society of Bangladesh; 1998; 1:467p.
2. Rameshkumar K, Hishan A, Latha P. Chemistry and therapeutic potential of chaulmoogra oil. In: Govil JN (Ed.) Recent Progress in Medicinal Plants. Studium Press, LLC, USA. 2011; 33:339-356p.
3. Rahmatullah M. Oral glucose tolerance and preliminary phytochemical and toxicity studies on *Hydnocarpus kurzii* bark methanolic extract. J Chem Pharm Res. 2015; 7:640-643.
4. Golder M, Sadhu SK, Biswas B, Islam T. Comparative pharmacologic profiles of leaves and hypocotyls of a mangrove plant: *Bruguiera gymnorrhiza*. Adv Tradit Med. 2020; 20(3):395-403.
5. Biswas M, Haldar PK, Ghosh AK. Antioxidant and free radical scavenging effects of fruits of *Dregea volubilis*. J Nat Sc Biol Med. 2010; 1:29-34.
6. Debnath SL, Kundu P, Golder M, Biswas B, Sadhu SK. Phytochemical characterization and evaluation of pharmacological activities of leaves of a mangrove plant species-*Aegiceras corniculatum*(L.) Trop J Nat Prod Res. 2020; 4(9):516-522.
7. Biswas B, Golder M, Islam T, Sadhu SK. Comparative antioxidative and antihyperglycemic profiles of pneumatophores of two mangrove species *Avicennia alba* and *Sonneratia apetala*. Dhaka Univ J Pharm Sci. 2018; 17(2):205-211.
8. Mahfuz A, Salam FBA, Deepa KN, Hasan AN. Characterization of *in-vitro* antioxidant, cytotoxic, thrombolytic and membrane stabilizing potential of different extracts of *Cheilanthes tenuifolia* and Stigmasterol isolation from *n*-hexane extract. Clin Phytosci. 2019; 5:1-10.
9. Mazumder K, Sumi TS, Golder M, Biswas B, Kerr PG. Antidiabetic profiling, cytotoxicity and acute toxicity evaluation of aerial parts of *Phragmites karka* (Retz.). J Ethnopharmacol. 2020; 113781.
10. Lawag IL, Aguinaldo AM, Naheed S, Mosihuzzaman M. α-Glucosidase inhibitory activity of selected Philippine plants. J Ethnopharmacol. 2012; 144:217-219.
11. Qaisar MN, Chaudhary BA, Sajid MU, Hussain N. Evaluation of α-glucosidase inhibitory activity of dichloromethane and methanol extracts of *Croton bonplandianum* Baill. Trop J Pharm Res. 2014; 13:1833-1836.
12. Kujur R, Singh V, Ram M, Yadava HN, Singh K, Kumari S. Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetis rats. Pharmacogn J. 2010; 2(14):27-32.
13. Dunn JS and McLetchie N. Experimental alloxan diabetes in the rat. The Lancet. 1943; 242:384-387.
14. Capasso F, Mascolo N, Autore G, Romano V. Laxatives and the production of autacoids by rat colon. J Pharm Pharmacol. 1986; 38:627-629.
15. Lahlou S, Tahraoui A, Israili Z, Lyoussi B. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. J Ethnopharmacol. 2007; 110:458-463.
16. Van Arman C. The diuretic activity of isocytosines. J Pharmacol Exp Ther. 1954; 111:285-292.
17. Mahaldar K, Saifuzzaman M, Irin T, Barman AK, Islam MK. Rahman MM, Islam AI. Analgesic, anthelmintic and toxicity

studies of *Solanum violaceum* Linn. leaves. Orient Pharm Exp Med. 2016; 16:147-152.
18. Salzman E. The epidemiology, pathogenesis, and natural history of venous thrombosis. In: Hemostasis and thrombosis: Basic principles and clinical practice. Lippincott Williams & Wilkins (LWW). Philadelphia, Pennsylvania, United States; 1994; 18(7):867 p.
19. Sarkar KK, Rahman MM, Shahriar AAE, Mitra T, Golder M, Zilani MNHZ, Biswas B. Comparative neuropharmacological and cytotoxic profiles of *Alstonia scholaris* (L.) and *Mimusops elengi* (L.) leaves. Adv Tradit Med. 2020.
20. Barman AK, Murshid GMM, Saifuzzaman M, Mahaldar K, Bojic M, Biswas NN. Study of antioxidant potential, and quantification of major polyphenols in *Leonurus sibiricus* L. leaves using HPLC. Pharmacol. 2017; 17:55-67.
21. Ramproshad S, Das R, Mondal B, Saha L. Phytochemical evaluation and antioxidant activities of *Spilanthes calva* DC. Pharmacol. 2019; 1:289-300.
22. Chakrabarty B, Acharyya RN, Kundu N, Mahmud I, Hossain H, Biswas NN, Uddin SJ, Dev S, Das AK. Evaluation of anti-diabetic and anti-allergic activities of *Brownlowia tersa* (L.) Kosterm leaves extract and determination of its phenolic compounds by HPLC-DAD. Trop J Nat Prod Res. 2020; 4(8):326-333.
23. Hammarström L, Hellman B, Ullberg S. On the accumulation of alloxan in the pancreatic β-cells. Diabetologia. 1967; 3:340-345.
24. Tessema MY, Wubneh ZB, Asrie AB. Laxative activities of 80% methanolic extract of the leaves of *Grewia ferruginea* Hochst Ex A Rich in mice. J Evid Based Integr Med. 2020; 25:1-8.
25. Landazuri P, Loango N, Restrepo B. Medicinal plants used in the management hypertension. J Anal Pharm Res. 2017; 5(2):00134.
26. Shah S, Anjum S, Littler W. Use of diuretics in cardiovascular disease: Hypertension. Postgrad Med J. 2004; 80:271-276.
27. Affan I, Azizah Z , Rivai H. Overview of the Phytochemicals of medicinal plants to lower blood pressure. Sch Acad J Pharm. 2020; 9(11):318-329.
28. Rodríguez-Garcíav CM, Ruiz-Ruiz JC, Peraza-Echeverria L, Peraza-Sa´nchez SR, Torres-Tapia LW, Pe´rez-Brito D, Tapia-Tussell R, Herrera-Chale´ FG, ´ Segura-Campos MR, Quijano-Ramayo A, Ramo´n-Sierra JM, OrtizVa´zquez E. Antioxidant, antihypertensive, anti-hyperglycemic, and antimicrobial activity of aqueous extracts from twelve native plants of the Yucatan coast. PloS One 2019; 14(3):1-17.
29. Zahir AA, Rahuman AA, Bagavan A, Geetha K, Kamaraj C, Elango G. Evaluation of medicinal plant extracts and isolated compound epicatechin from *Ricinus communis* against *Paramphistomum cervi*. Parasitol Res. 2012; 111:1629-1635.
30. Colledge NR, Walker BR, Ralston S , Davidson S. Davidson's principles and practice of medicine.(22nd ed.). China: Elsevier; 2014; 22:1050-1053p.
31. Tanelli F, Belardo TG, Peiro CHF, Gama RMd, Fonseca FLA, Armando-Junior J. Preliminary studies on anticoagulation and antiplatelet activities of *Plinia cauliflora* (Mart.) Kausel Leaves Extract. J Appl Pharm Sci. 2017; 7:115-118.
32. Tyagi CK, Jhade D, Shah SK. Isolation, evaluation and characterization of isolated compounds from aqueous extract of *Cestrum nocturnum*. Int J Phytomed. 2017; 8:491-499.