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Phytochemical investigation and assessment of pharmacological properties of leaves of *Duabanga grandiflora*

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Abstract
Duabanga grandiflora- a medicinal plant from Lythraceae family, is native to some countries of South Asian region. This plant has many uses in folk medicine. But there are lack of scientific reports comparing to its therapeutic importance. Our study was designed to assess phytochemical and biological effects of *D. grandiflora* leaves. From the phytochemical investigation we came to know that this extract possesses many important phytochemical groups as secondary metabolites. This extract showed the presence of many antioxidant compounds in both qualitative and quantitative antioxidant assays. In DPPH radical and hydrogen peroxide scavenging assay, the SC_{50} were 58 and 50 µg/ml, respectively. In $FeCl_3$ reducing power assay, the RC_{50} was found to be 57 µg/ml. Total content of phenolic, flavonoid and tannin were measured as 127 mg GAE/g, 285 mg QE/g and 132 mg GAE/g, respectively. This plant showed significant blood glucose level reduction over the short period of time in oral glucose tolerance test and for long term in alloxan-induced diabetic tests, although it did not express any significant response to α-glucosidase enzyme inhibition. In acetic acid-induced writhing test, this plant showed significant pain inhibition which was followed by significant reduction in paw edema induced by formalin. *D. grandiflora* increased the latency of defecation and decreased the stool count in castor oil-induced diarrheal test. Lastly, we also observed prominent CNS depression activity of this extract in open field model in mice. All of these results were compared with standard drugs and the responses were found in dose-dependent manner. These observations signify the utilization of this plant in folk medicinal system and we hope that these initial results will be beneficial to conduct higher research on this plant to isolate newer bioactive molecules.

Keywords: *Duabanga grandiflora*, antioxidant, antihyperglycemic, analgesic, antidiarrheal, CNS depressive

Introduction
Duabanga grandiflora is a large, evergreen tree from the family Lythraceae. This tree is instinctive to the Southeast Asian countries like Nepal, India, Bangladesh, Laos, Cambodia, Malaysia, Myanmar, Thailand and Vietnam etc. In Bangladesh, it is locally known as 'Bandorhula' and found in in the forest and hill tracts of the district of Mymensingh, Sherpur, Jamalpur, Netrokona. This deciduous plant grows very rapidly. This tree is tall up to 40-80 feet. Leaves are oblong in shape, 25 cm long and 10 cm broad and are oppositely arranged. The young red leaves turn into green in mature stage. Flowers are clustered from leaf axils. The white flowers are large about 5-6 cm and bears ill smell. The sepal structure is bell shaped and very wide. There are many stamens. The fruits are round in shape and resembles the shape of a small orange. This plant is propagated by seed or cutting [1, 2]. Several types of compounds are reported to be present from this plant like 5-formylfurfuryl esters, duabanganals, 3-hydroxy-4-methoxycinnamaldehyde, 5-formylfurfurol eight pentacyclic triterpenes, a benzofuran derivative, an ellagic acid derivative, 5- formylfurfuryl ester, latifolinal, vanillin, β-sitosterol, β-sitosterol glucoside etc. [2, 3]. This plant has some therapeutic applications in folk medicine. This plant is useful to treat skin diseases such as scabies, eczema, atopic dermatitis, headache, epileptic disorders [4]. People of northern Thailand use its leaves poultices to treat stomach pains. Leaves are used for skin whitening, treating pain and swelling [5]. Its anticancer property is already reported [6].

Depending on the traditional usage of this plant, we aimed our focus to investigate the unexplored biological properties of leaves of this plant.

Materials and Methods

Collection and identification of plant samples

Leaves of *D. grandiflora* were collected from the botanical garden of Bangladesh Agricultural University, Mymensingh, Bangladesh in January, 2019. During collection, fresh leaves were carefully collected and precautions were taken to avoid any type of admixture. Dried plant samples were sent to Bangladesh National Herbarium, Dhaka for identification and an accession no. was provided from there for future reference (DACB-64654).

Preparation of the crude extract

After collection, the plant samples were shade dried for about 50 days. The dried leaves were ground to obtain coarse powder by a suitable grinder. With 300 gm of powdered sample, cold extraction was performed with ethanol (96%). The whole mixture was then sealed in a tight flat-bottomed glass container. After 14 days, it was filtered and 18.7 gm gummy crude extract (yield: 6.2% w/w) was obtained.

Chemicals and drugs

To conduct the analytical experiments laboratory grade reagents were used such as acetic acid (Merck, Germany), formalin (Merck, Germany), 2,2-diphenyl-1-picryl hydrazyl (DPPH) (Sigma, USA), Na_2CO_3 (Loba, India), $NaNO_2$ (Loba, India), $AlCl_3$ (Loba, India), NaOH (Loba, India), H_2O_2 (Merck, Germany), gallic acid (Sigma, USA), Folin-Ciocalteu (FC) reagent (Merck, India), quercetin (Sigma, USA), ascorbic acid (Merck, Germany) and powdered alloxan (Merck, Germany). Standard drugs such as loperamide, diclofenac sodium and glibenclamide were purchased from Square Pharmaceuticals Limited (Bangladesh), ibuprofen and glucose powder were purchased from ACI Laboratories (Bangladesh) and GSK Bangladesh Limited, respectively.

Animals

To conduct the pharmacological tests, 4-5 weeks aged Swiss albino mice (*Mus musculus*) having 20–25 gm weights were procured from the Pharmacology laboratory animal house from Pharmacy Department, Jahangirnagar University, Bangladesh. Mice were placed in proper environmental to be adapted for 3 weeks in the Animal house of Khulna University, Bangladesh so that they could adopt with the environmental condition. The mice were feed with rodent foods and distilled water. Metabolic waste and others debris were cleaned regularly to maintain hygienic environment. The experiments mentioned in this manuscript were performed in noiseless condition in accordance with the ethical permission of Animal Ethics Committee (AEC), Khulna University, Khulna-9208, Bangladesh [Ref: KUAEC-2021/03/04].

Phytochemical assessment

In order to investigate the presence or absence of different phytochemicals, a qualitative screening of *D. grandiflora* leaves extract was conducted by the method of Ayoola *et al*., 2008 [7].

In vitro qualitative antioxidant tests

Qualitative antioxidant test of *D. grandiflora* extract was carried out using thin layer chromatographic (TLC) technique by the method of Sadhu *et al*., 2003 [8]. A small amount of extract was mixed and then the diluted plant solution was dotted by a capillary tube on pre-coated silica gel TLC plates. Those were kept horizontally in different types of solvent systems, non-polar, medium polar and polar and chromatogram was allowed to be developed. After developing, those plates were dried and observed under 254 nm and 366 nm wavelength to observe the existence of UV positive and fluorescent compounds. DPPH solution (0.02% w/v) was then sprayed on those plates for the formation of yellow spots to confirm the presence of antioxidant compounds.

In vitro quantitative antioxidant assays

a) Measurement of total content of secondary metabolites

i. Determination of total phenolic content (TPC)

TPC of *D. grandiflora* extract was determined according to Folin-Ciocalteu method described by Debnath *et al*., 2020 where gallic acid was used as standard [9]. 0.5 ml of 1 mg/ml extract and 0.04-0.15 mg/ml gallic acid were mixed with 5 ml diluted (1:10) FC reagent and 4 ml 7% w/v Na_2CO_3 solution separately. This mixture was vortexed for 15 seconds. Thirty minutes later, UV absorbance was taken at 765 nm wavelength. TPC was denoted as mg of gallic acid equivalent per gram of dry extract by using the gallic acid calibration curve.

ii. Determination of total flavonoid content (TFC)

TFC of *D. grandiflora* extract was determined according to aluminum chloride colorimetric method by described by Debnath *et al*., 2020 where quercetin was used as standard [9]. 1 ml of 1 mg/ml extract and 0.25-1 mg/ml quercetin were mixed with 5 ml distilled water, 0.3 ml 5% w/v $NaNO_2$, 0.3 ml 10% w/v $AlCl_3$ and 2 ml of 1M NaOH. Then, distilled water was added with this mixture to make the volume 10 ml. Fifteen minutes later, UV absorbance was taken at 510 nm wavelength. TFC was denoted as mg of quercetin equivalent per gram of dry extract using the quercetin calibration curve.

iii. Determination of total tannin content (TTC)

TTC of *D. grandiflora* extract was determined according to Folin-Ciocalteu method by Debnath *et al*., 2020 where gallic acid was used as standard [9]. 0.5 ml of 1 mg/ml extract and 0.04-0.15 mg/ml gallic acid were mixed with 5 ml diluted (1:10) FC reagent and 4 ml 7% w/v Na_2CO_3 solution separately This mixture weas vortexed for 15 seconds. Thirty minutes later, UV absorbance was taken at 725 nm wavelength. TTC was denoted as mg of gallic acid equivalent per gram of dry extract using the gallic acid calibration curve.

b) Free radical scavenging assay

i. DPPH free radical scavenging assay

DPPH free radical scavenging assay was performed according to the method of Biswas *et al*., 2018 with slight modifications [10]. By serial dilution, different concentrations (1-1024 µg/ml) of *D. grandiflora* extract and ascorbic acid (1-1024 µg/ml) were prepared and 10 µl of each concentration was added with 190 µl 0.008% w/v DPPH solution (in methanol). After keeping those in dark environment for 30 minutes, the absorbance was taken at 517 nm wavelength by a Thermo Scientific Multiskan Ex microplate photometer. From those reading, calibration curve of log concentration vs percent inhibition was derived and by the help of those, the scavenging ability of DPPH free radicals was expressed as SC_{50} (µg/ml, concentration of plant extract required to scavenge 50% of free radical).

ii. Hydrogen peroxide radical scavenging assay

Hydrogen peroxide radical scavenging activity of *D. grandiflora* extract was determined by the method of Golder *et al*., 2020 [11]. By serial dilution, different concentrations (12.5-800 µg/ml) of the plant extract and ascorbic acid (6.25-800 µg/ml) were prepared and 1 ml of each concentration was added with 3 ml of phosphate buffer and 6 ml of prepared hydrogen peroxide solution were mixed together. After 10 minutes incubation, absorbance was taken at 230 nm wavelength using UV spectrophotometer. From the curve of log concentration versus percent inhibition, free radical scavenging activity was calculated at expressed in SC_{50} (µg/ml, concentration of plant extract required to scavenge 50% of hydrogen peroxide free radicals).

c) Reducing power assay

Most of the antioxidant compounds also possess reduction capability. The ability of *D. grandiflora* extract to reduce $FeCl_3$ to $FeCl_2$ was performed by the method described by Debnath *et al*., 2021 [12]. By serial dilution, different concentrations (12.5-800 µg/ml) of the extract and ascorbic acid (6.25-800 µg/ml) were prepared and 1 ml of each concentration was added with 2.5 ml 0.2 M phosphate buffer and 2.5 ml 1% w/v potassium ferricyanide. These mixtures were incubated at 50 ºC for 20 minutes. After cooling, 2.5 ml 10% trichloroacetic acid (TCA) was added with each previous mixture and centrifuged at 3000 rpm for 15 minutes. After this, 2.5 ml of upper layer was separated and further mixed with 2.5 ml distilled water and 0.5 ml ferric chloride (0.1%). Absorbance was taken at 700 nm wavelength after 5 minutes and percentage of reduction was calculated from the calibration curve and finally it was expressed as RC_{50} (µg/ml, concentration of the plant extract required to reduce 50% Fe^{3+}).

Assessment of antihyperglycemic activity

i. Oral glucose tolerance test (OGTT)

OGTT is a very common technique which is used to assess the body's own glucose metabolism capacity. OGTT of *D. grandiflora* extract was performed by the method described by Saha *et al*., 2021 [13]. The extract was orally given at 250 mg/kg and 500 mg/kg body weight to the test groups of mice. Mice of positive control group and control group were administered with glibenclamide at 5 mg/kg and normal saline solution, respectively. After 30 minutes, 2 gm/kg glucose solution was orally given to each mouse and blood glucose level (mmol/l) of the mice was determined instantly with the help of a glucometer. Then the blood glucose level was again determined at 30, 60, 90, 120 and 150 minutes.

ii. Alloxan-induced diabetic test

Alloxan-induced diabetic test for the *D. grandiflora* extract was performed by the method of Kujur *et al*., 2010 [14]. 5% w/v alloxan monohydrate solution was intraperitoneally administered to the mice (kept fasting for 12 hours) at 125 mg/kg doses for inducing diabetes. Seven days later, mice that showed fasting blood glucose level at 200–300 mg/dl were considered as diabetes affected mice. But the mice possessed fasting blood glucose level more than 300 mg/dl were discarded from this experiment as it was supposed that their beta cells of pancreas were almost fully destructed [15]. Then the selected mice were divided into five groups to conduct the study (I: nondiabetic control, II: diabetic control, III: *D. grandiflora* extract- 50 mg/kg, IV: *D. grandiflora* extract- 100 mg/kg, V: glibenclamide (standard)- 5 mg/kg). Mice of group I and II were treated with normal saline solution orally whereas mice of group III, IV and V were orally administered daily with two doses of *D. grandiflora* extract (at 50 mg/kg and 100 mg/kg) and glibenclamide, respectively for the next 28 days. In these periods, blood glucose level (mg/dl) of the experimented mice was measured at 0^{th}, 1^{st}, 5^{th}, 7^{th}, 14^{th} and 28^{th} days by a glucometer.

iii. Alpha-glucosidase inhibitory test

The ability of *D. grandiflora* extract to show inhibition to α-glucosidase enzyme was evaluated by the method of Lawag *et al*., 2012 with some modifications [16]. 0.1M potassium dihydrogen phosphate buffer solution (pH 6.8), 0.1M α-glucosidase enzyme, 5 mM p-nitrophenyl glucopyranoside (pNPG) and 0.1M Na_2CO_3 solution was prepared. *D. grandiflora* extract and voglibose (as standard) solution were prepared at 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml concentrations. 20 µl of varying concentrations of the extract and voglibose were separately mixed with 50 µl potassium phosphate buffer and 10 µl enzyme solution and these were incubated in a 96-well plate at 37 °C for 15 minutes. Then 20 µl of pNPG was added to each well and further incubated at 37 °C for 15 min. The reaction was terminated by adding 50 µl of Na_2CO_3 solution. Finally, absorbance was taken at a wavelength of 405 nm by a Thermo Scientific Multiskan Go spectrophotometer. Alpha-glucosidase inhibitory activity was denoted as the IC_{50} (conc. of plant extract required to inhibit 50% of enzyme) and calculated by the following equation-

Inhibitory activity (%) = $[(A_s-A_c)/ A_s] \times 100$
where, A_c = absorbance in the presence of test substance
A_s = absorbance of control

Assessment of peripheral analgesic activity

Acetic acid-induced writhing method in mice was performed for *D. grandiflora* extract for determining the peripheral analgesic activity by the method described by Debnath *et al*., 2021 [12]. The plant extract was orally given at 250 mg/kg and 500 mg/kg body weight to the test groups of mice. Mice of positive control group and control group were administered with diclofenac Na at 25 mg/kg and 1% v/v tween 80 at 10 ml/kg body weight doses, respectively. After 30 minutes, 0.7% v/v acetic acid solution was intraperitoneally injected to each mouse at 0.01x ml doses (where, x= body weight of each mouse in gm). After 15 minutes, no. of writhing (constriction of abdomen and extension of hind legs) was counted.
The inhibition of writhing in comparison to the mice of control group was taken as an index of analgesia and it was calculated by the following formula:

Inhibition in writhing (%) = $[(W_c - W_t) \times 100]/W_c$
where, W_c = average number of writhing reflexes in the control group and
W_t = average number of writhing reflexes in the test group

Assessment of anti-inflammatory activity

In order to assess the anti-inflammatory activity of *D. grandiflora* leaves extract, formalin-induced paw edema test was performed in mice by the method of Sadeghi *et al*., 2014 [17]. The extract was orally given at 250 mg/kg and 500 mg/kg body weight to the test groups of mice. Mice of positive control group and control group were administered with Ibuprofen at 100 mg/kg and 1% v/v tween 80 at 10 ml/kg body weight doses, respectively. Then, linear circumference of the right hind paw was measured by a slide caliper. Thirty

minutes later, 0.1 ml formalin (2% v/v) was injected into the subplantar area of right hind paw of the mice to induce swelling. Linear circumference of the treated paw was measured at 1st, 2nd, 3rd and 4th hour after formalin injection. Then change in paw size was calculated as = (paw size after formalin injection at pre-determined times -paw size before formalin injection)

Percent inflammation of paw edema = (I_s/I_c) x100
where, I_s = inflammation of the test group
I_c = inflammation of the control group
Percent inhibition of inflammation = 100 - % inflammation

Assessment of antidiarrheal activity

Castor oil-induced diarrheal method in mice was performed for *D. grandiflora* extract for the assessment of antidiarrheal activity by the method of Niemegeers *et al*., 1984 [18]. The extract was orally given at 250 mg/kg and 500 mg/kg body weight to the test groups of mice. Mice of positive control group and control group were administered with loperamide at 3 mg/kg and 1% v/v tween 80 at 10 ml/kg body weight doses, respectively. 0.5 ml castor oil was orally given to each mouse after 30 minutes for the induction of diarrhea. Each mouse was kept separated from one another and blotting paper was placed under them. For next 4 hours, first defecation period and no. of stool were counted. The defecation reduction was calculated by the following equation:

Defecation reduction (%) = $[(S_c - S_t)/ S_c] \times 100$
where, S_c = average number of stools in the control group,
S_t = average number of stools in the test group

Assessment of neuropharmacological behavior

Neuropharmacological behavior (CNS excitatory or depressant) of mice for the effect of *D. grandiflora* extract was evaluated according to the open field model described by Gupta *et al*., 1971 [19]. This experiment was performed in a sound attenuated room. The extract was administered orally at 250 mg/kg and 500 mg/kg body weight to the test groups of mice. Mice of positive control group and control group were administered with diazepam at 1 mg/kg and 1% v/v tween 80 at 10 ml/kg body weight doses, respectively. Afterwards, each mouse was placed in one of the corner squares and the number of squares crossed by the animals was counted for next 3 minutes on 0, 30, 60, 90 and 120 minutes during the study period.

Statistical analysis

SPSS (version 25) was used to conduct the statistical analysis. The obtained values are expressed as mean ± standard deviation. An analysis of variance (ANOVA), and then Tukey's post hoc test were used to determine significant differences considering the significance value was considered < 0.05 [13].

Results and Discussion

Each plant in nature is a storehouse of numerous phytochemicals. Depending on the chemical structure and biosynthetic derivation, these are divided as primary and secondary metabolites. Among them, secondary metabolites are responsible for expressing different biological activities. These phytochemicals exhibit diverse pharmacological activity which are beneficial to both plant itself and human. In order to get cure from diseases, human have been relying on these medicinal plants from the very ancient period [20]. In our phytochemical investigation, different phytochemicals like reducing sugars, tannins, flavonoids, gums, steroids, alkaloids, glycosides, terpenoids and acidic compounds were found to be present in *D. grandiflora* leaves extract (Table 1). Expression of different therapeutic activities of this plant are mainly responsible for the presence of these compounds.

Free radicals are the atoms or group of atoms having an odd (unpaired) number of electrons and these can be formed when oxygen get interacted with certain molecules. These are produced under certain environmental conditions and during normal cellular function in the body. Free radicals are formed naturally in the body by different oxidase enzymes and play harmful role in affecting normal physiological processes. These cause lipid peroxidation, stimulating oxidation of sulfhydryl groups, modification in genetic arrangement. These accelerate chain reactions and in the long run patients suffer from many life-threatening diseases like cancer, stroke, atherosclerosis, diabetes, neurological, cardiac and renal impairment. Antioxidants are molecules that neutralize or scavenge different types of free radicals and thus prevent us from those associated diseases [13]. Most of the plants are enriched in numerous antioxidant molecules. Polyphenols, flavonoids and tannins are mostly antioxidant compounds and these have significant medicinal properties. Phenolic compounds reduce the possibilities of developing severe chronic diseases like gout, cancer, stroke, diabetes and cardiovascular disease. Tannins also reduce the lipid peroxidation and DNA mutation [21]. On the other hand, flavonoids reveal anti-allergic and anti-inflammatory properties as well as protective effects against vascular, renal and hepatic disorders [22]. In the qualitative antioxidant test, *D. grandiflora* extract revealed the presence of yellowish spots at both 254 nm and 360 nm. These indicated the existence of antioxidant compounds in this plant. From the quantitative measurements of antioxidant tests, total phenolic, flavonoid and tannin content of *D. grandiflora* extract were found to be 127 mg gallic acid equivalent (mg GAE)/g, 285 mg quercetin equivalent (mg QE/g) and 132 mg GAE/g, respectively (Table 2).

DPPH is a common stable radical which can easily accept an electron or proton from antioxidant compounds and develops a stable diamagnetic molecule DPPH-H and thus it gets transformed violet color to pale yellow color [23]. Due to being unstable in nature, DPPH quickly attacks to the nearby cells in our body. Thus, it causes lipid peroxidation, stimulation of sulfhydryl groups and fragments the DNA bases. In the long run, it generates many life-threatening diseases. The antioxidants compounds from different plants serve us to combat these types of free radicals associated damages. In the DPPH radical scavenging assay, both the *D. grandiflora* extract and ascorbic acid scavenged the DPPH at various degrees depending on their concentration. With these results, percent inhibition of DPPH free radical was calculated and the resulting SC_{50} values were 58 and 13 µg/ml, respectively (Table 2).

Hydrogen peroxide itself does not act as a free radical, rather if forms free radicals like Hydroxyls, peroxide and superoxide in aqueous solutions. Like other free radicals, these free radicals are also responsible for causing different types of free radicals associated diseases. In the Hydrogen peroxide radical scavenging assay, both the *D. grandiflora* extract and ascorbic acid scavenged the free radicals from hydrogen peroxide at various degrees depending on their concentration. With these results, percent inhibition of free radicals was calculated and

the resulting SC_{50} values were 50 and 40 µg/ml, respectively (Table 2).

Antioxidants not only serve as free radical scavengers but also show reducing properties. These reducing property is also beneficial to human. In the ferric chloride reducing assay, both the extract and ascorbic acid reduced the $FeCl_3$ at various degrees in different concentrations. From the readings, the calculated RC_{50} of the extract and ascorbic acid are 57 and 41 µg/ml, respectively (Table 2). From the above results we can assume that *D. grandiflora* leaves are rich in antioxidant contents and so that they can act as free radical scavengers.

Diabetes mellitus (type II diabetes) is the most common metabolic disorder that affects many people worldwide. People with diabetes have a multiple time risk of all-cause mortality and presence of diabetes is also associated with increased hepatic and cardiovascular disease, stroke, cancer, renal disease [24]. Abnormal rise in blood glucose level (hyperglycemia) is associated to onset of type II diabetes in human [25]. It is such a serious disease that can`t be cured completely. Strict regulations of discipline, diet and drug is only the way to control the blood glucose at normal level. In our OGTT and alloxan-induced diabetic test, we observed that *D. grandiflora* extract significantly reduced the elevated blood glucose level over the period of time (Figure 2 and 3). Alpha-glucosidase and alpha-amylase are the mediatory enzymes responsible for absorption of glucose from food sources. Medicinal plants rich in phenolic, flavonoid and tannin contents show inhibitory effect on alpha-amylase and alpha-glucosidase [26]. These enzyme inhibitors act by directly blocking the active centers of the enzyme at several sites [27]. In our experiment, although voglibose showed inhibition of alpha-glucosidase enzyme (IC_{50} = 0.3 mg/ml) as shown in figure 4, but no inhibitory effect of *D. grandiflora* extract was observed. So, it is assumed that the antihyperglycemic effect of the extract might be mediated by some other mechanism(s). However, different polyphenolic compounds, tannins and flavonoids and compounds like vanillin, β-sitosterol and 3-hydroxy-4-methoxycinnamaldehyde might be responsible for antihyperglycemic effect of this extract [13, 28-30].

Pain is always a galling perception which is provoked by different external or internal obnoxious stimulus. These stimuli tend to release intracellular arachidonic acid from the phospholipids of the affected tissues. These cause the secretion of many intracellular components like prostaglandins, mainly prostacyclin (PGI_2) and some other PGE_2 and $PGF_{2\alpha}$, cytokines, leukotrienes. These are responsible for pain sensation [31]. Diclofenac Na is a very common NSAID (nonsteroidal anti-inflammatory drug) used in the pain treatment. Although causing many gastrointestinal and some other adverse effects, it is still the most prescribed analgesic all over the world [32]. Worldwide, acetic acid-induced writhing model in rodent animals is used to evaluate the peripheral analgesic activity of any administered compound. Intraperitoneal administration of 0.7% v/v acetic acid induces many endogenous pain mediators, such as prostaglandins, histamine, serotonin (5-HT), bradykinin and substance that sensitize pain nerve endings [33]. In the acetic acid-induced writhing test, the *D. grandiflora* extract revealed significant inhibition of writhing impulse by 50% and 70% at 250 mg/kg and 500 mg/kg doses, respectively whereas diclofenac Na inhibited the writhing impulse 81% in 25 mg/kg dose (Table 3).

Inflammation is another type of cellular response to injured tissues like pain. At the inflamed sites, the affected cells release many inflammatory mediators that start the initialization of inflammatory response. Inflammation, pain and pyrexia are different pathological conditions associated by different types of mediators. But these mediators are produced by similar mechanisms [34]. Formalin-induced paw edema method in mice is a very common widely accepted test for evaluating the anti-inflammatory response for any compound. Intraplantar injection of formalin produces the release of different inflammatory mediators like prostaglandins, bradykinin, histamine, serotonin etc. [35]. Ibuprofen, as an NSAID can reduce the swelling or edema by reducing the synthesis of pain and inflammatory mediators [36]. In the formalin-induced paw edema method, the *D. grandiflora* extract revealed potent reduction of the paw edema in mice within the observing period at both the doses of 250 mg/kg and 500 mg/kg doses (Figure 5). So, we may conclude that the extract contains different compounds to exhibit these analgesic and anti-inflammatory activities. Compounds like vanillin, β-sitosterol, 3-hydroxy-4-methoxycinnamaldehyde and derivatives of benzofuran and ellagic acid might be responsible for exerting these effects [28-30, 37, 38].

Diarrhea is one of the major gastrointestinal disorders that is responsible for both morbidity and mortality of many of children and adults all over the world. Increased gastrointestinal motility, intake of unhygienic food and gastrointestinal infections deteriorate the normal state of stomach and these cause diarrheal disorders [39]. Castor oil-induced diarrheal method in mice is a very common laboratory process for the assessment of antidiarrheal activity of any sample or plant extract. The main component of castor oil, ricinoleic acid can irritate and cause inflammation of gastrointestinal mucosa and it leads increased motility and secretion [40]. In the castor oil-induced diarrheal test, *D. grandiflora* extract both increased the latent time of defecation (83 and 172 minutes at 250 mg/kg and 500 mg/kg doses, respectively) and reduction in defecation (59% and 72% at 250 mg/kg and 500 mg/kg doses, respectively) (Table 4). Thus, we can assume that *D. grandiflora* extract showed very good antidiarrheal effect. Phytochemicals in this plant like alkaloids, tannins, flavonoids and terpenes may be responsible for this antidiarrheal action [41].

The central nervous system (CNS) is the most complex and most important part of human body. Eighty-six billion neurons are the main regulatory part of CNS. Excitement or depressive effect of these neurons exhibit different types of neuropharmacological behavior. The open field test is used to assess the general locomotor activity levels, anxiety, and willingness to explore in animals (usually rodents) in scientific research. The number of squares crossed by mice at regular interval express both excitement or depressive effect of CNS. The more the number of squares crossing, the more the excitement effect of CNS and *vice versa* [42]. In our open field model, *D. grandiflora* extract showed us CNS depression effect over the period of time. With the advancement of time, less no. of squares was crossed by mice at both 250 mg/kg and 500 mg/kg doses (Figure 6). So, compounds like benzofuran derivatives and other phytochemicals like glycosides, alkaloids, tannins, saponins, flavonoids might be responsible for this CNS depressant activities [37, 43]. These CNS depressive agents are helpful in treating different neurological disorders like Alzheimer disease, Parkinson disease, epilepsy, trauma, anxiety, convulsion etc. [44].

Conclusion

Our study was conducted on leaves of *D. grandiflora* to investigate its different pharmacological properties relating to its local uses in folk medicine. From our observation, it might be concluded that *D. grandiflora* leaves extract possessing numerous phytochemicals is rich in antioxidant content and it has very good radical scavenging capacity. This plant showed potent antihyperglycemic, analgesic, anti-inflammatory, antidiarrheal and CNS depressive effects. These results also justify the traditional usage of this plant in a scientific manner. Our observations will be helpful to use this plant to mitigate related cases and for isolation of bioactive molecules.

Fig 1: Photo of *Duabanga grandiflora* leaves

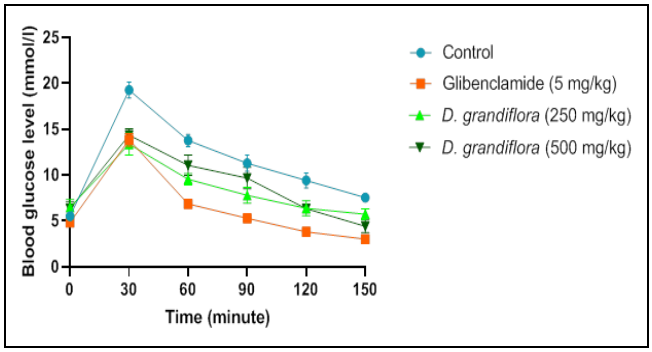

Fig 2: Comparison of blood glucose level (mmol/l) of *D. grandiflora* extract with negative control and positive control group over period of time

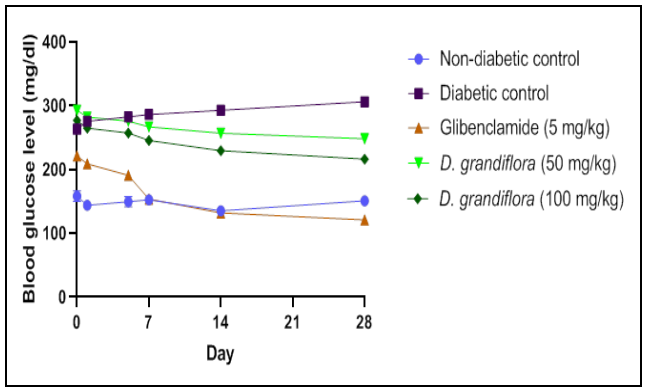

Fig 3: Comparison of blood glucose level (mg/dl) of *D. grandiflora* extract with negative control and positive control group over period of time in alloxan-induced diabetic test

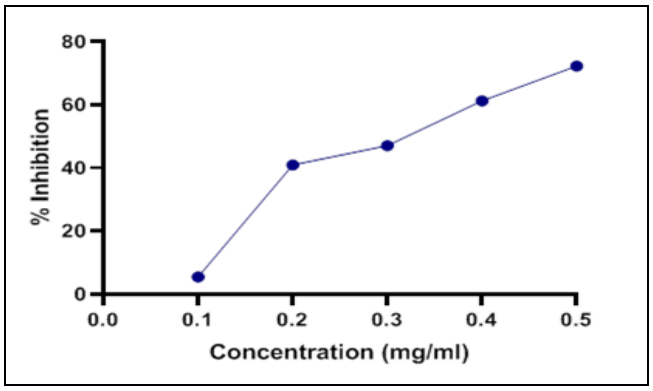

Fig 4: Alpha-glucosidase inhibitory activity of voglibose

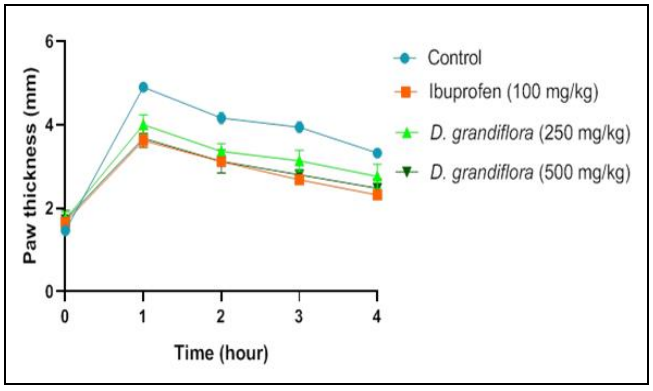

Fig 5: Comparison of paw thickness (mm) of *D. grandiflora* extract with negative control and positive control group over period of time

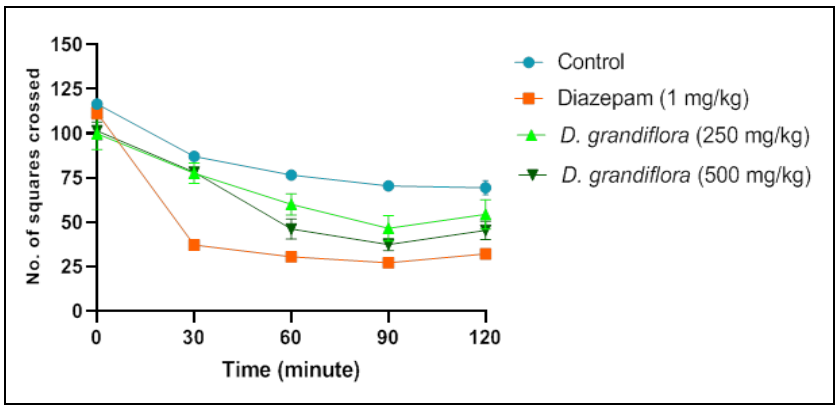

Fig 6: No. of squares crossed by mice at different times (for *D. grandiflora* extract with negative and positive control)

Table 1: Presence or absence of different phytochemical groups in *D. grandiflora* extract

Phytochemical groups	Reducing sugars	Tannins	Flavonoids	Saponins	Gums	Steroids	Alkaloids	Glycosides	Xanthoproteins	Terpenoids	Acidic compounds
D. grandiflora extract	+	+	+	-	+	+	+	+	-	+	+

'+' indicates presence and '-' indicates absence

Table 2: SC_{50} and RC_{50} values of different antioxidative assays and total content of secondary metabolites (phenolics, flavonoids and tannins) of *D. grandiflora* extract

Sample	DRSA (SC_{50} µg/ml)	HPSA (SC_{50} µg/ml)	RPA (RC_{50} µg/ml)	TPC (mg GAE/g)	TFC (mg QE/g)	TFC (mg GAE/g)
D. grandiflora extract	58	50	57	127	285	132
Ascorbic acid	13	40	41			

DRSA (DPPH radical scavenging activity), HPSA (hydrogen peroxide scavenging activity), RPA (reducing power assay), TPC (total phenolic content), TFC (total flavonoid content), TTC (total tannin content)

Table 3: Representation of effects of *D. grandiflora* extract on acetic acid-induced writhing in mice

Treatment group	Dose (mg/kg)	Mean writhing	% Inhibition of writhing
Negative control	------	$12.4\pm2.302^{\theta\blacktriangle\Delta}$	------
Standard (diclofenac Na)	25	$2.4\pm1.14^{*\blacktriangle}$	$80.645\pm9.194^{*\blacktriangle}$
D. grandiflora extract	250	$6.2\pm0.837^{*\theta}$	$50\pm6.747^{*\theta}$
D. grandiflora extract	500	$3.8\pm0.837^{*}$	$69.355\pm747^{*\blacktriangle}$

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 *D. grandiflora* 250 mg/kg; $^{\Delta}P<0.05$ vs. *D. grandiflora* 500 mg/kg (pair-wise comparison by Post Hoc Tukey test)

Table 4: Representation of effects of *D. grandiflora* extract on castor oil-induced diarrhea in mice

Treatment group	Dose (mg/kg)	Average latent period of defecation	Average no. of stool	% Inhibition of defecation
Negative control	------	$34.75\pm4.349^{\theta\blacktriangle\Delta}$	$17\pm1.826^{\theta\blacktriangle\Delta}$	------
Standard (loperamide)	3	$176.75\pm13.099^{*\Delta\blacktriangle}$	$3.5\pm0.577^{*\Delta\blacktriangle}$	$79.412\pm3.396^{*\Delta}$
D. grandiflora extract	250	$82.75\pm5.123^{*\theta\Delta}$	$7\pm0.816^{*\theta}$	$58.823\pm4.802^{*\theta}$
D. grandiflora extract	500	$171.5\pm8.021^{*\Delta}$	$4.75\pm0.957^{*}$	$72.058\pm5.631^{*}$

Data are means of five replicates ± SD (standard deviation); * $P<0.05$ vs. control (Dunnett's t test); $^{\theta}$ $P<0.05$ vs. loperamide 3 mg/kg; $^{\blacktriangle}$ $P<0.05$ vs *D. grandiflora* 250 mg/kg; $^{\Delta}P<0.05$ vs. *D. grandiflora* 500 mg/kg (pair-wise comparison by Post Hoc Tukey test)

Conflict of interest
There is no point of confliction of interest among the authors.

Authors' declaration
It is declared by the authors that the presented works in this manuscript are completely original. All types of liability for claims regarding to any content of this manuscript will be totally borne by them.

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References
1. Hooker JD, Cathcart JF, Fitch WH. Illustrations of Himalayan Plants: Chiefly Selected from Drawings Made for the Late JF Cathcart, Esq. of the Bengal Civil Service: L. Reeve 1855.
2. Akhter F, Islam QS, Adib M, Al Faruq A, Ibrahim M. Phytochemical screenings and bioactivities of *Duabanga grandiflora* (Roxb. ex DC.) Walp. Bangladesh Pharm. J. 2019;22(1):68-72.
3. Kaweetripob W, Mahidol C, Prachyawarakorn V, Prawat H, Ruchirawat S. 5-Formylfurfuryl esters from *Duabanga grandiflora.* Phytochemistry 2012;76:78-82.
4. Santiago C, Lim KH, Loh HS, Ting KN. Inhibitory effect of *Duabanga grandiflora* on MRSA biofilm formation via prevention of cell-surface attachment and PBP2a production. Molecules 2015;20(3):4473-82.
5. Tsukiyama M, Sugita T, Kikuchi H, Yasuda Y, Arashima M, Okumura H, *et al*. Effect of *Duabanga grandiflora* for human skin cells. Am. J Chinese Med 2010;38(02):387-99.
6. Chu JH. Investigating *in vitro* anticancer properties of Malaysian rainforest plants: *Acalypha wilkesiana* Müll, Arg. *Archidendron ellipticum* (Blume) Hassk. *Duabanga grandiflora* Walp. *Pseuduvaria macrophylla* (Oliv.) Merr. University of Nottingham 2014.
7. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, *et al*. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J Pharm. Res 2008;7(3):1019-24.
8. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. Separation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chem. Pharm. Bull 2003;51(5):595-8.
9. 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):516-22.
10. 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):205-11.
11. 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.
12. Debnath SL, Kundu P, Ahad MF, Saha L, Biswas NN, Sadhu SK. Investigation of phytochemical and pharmacological assessment of ethanol extract of *Stenochlaena palustris*-an edible fern of Sundarbans. J Med. Plant Stud 2021;9(3):226-32.
13. Saha L, Sultana T, Jahan T, Kundu P, Biswas B, Acharyya RN, *et al*. Phytochemical and pharmacological evaluation of ethanol leaves extract of *Hydnocarpus kurzii* (King) Warb. Trop. J Nat. Prod. Res 2021;5(2):324-30.
14. Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S *et al*. Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. Pharmacognosy Res 2010;2(4):258.
15. Dunn JS, McLetchie NG. Experimental alloxan diabetes in the rat. Lancet 1943;242(6265):384-7.
16. Lawag IL, Aguinaldo AM, Naheed S, Mosihuzzaman M. α-Glucosidase inhibitory activity of selected Philippine plants. J Ethnopharmacol 2012;144(1):217-9.
17. Sadeghi H, Mostafazadeh M, Sadeghi H, Naderian M, Barmak MJ, Talebianpoor MS *et al*. *In vivo* anti-inflammatory properties of aerial parts of *Nasturtium officinale*. Pharm. Biol 2014;52(2):169-74.
18. Yesilada E, Ustun O, Sezik E, Takaishi Y, Ono Y, Honda G. Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1 alpha, interleukin-1 beta and tumor necrosis factor alpha. J. Ethnopharmacol 1997;58(1):59-73.
19. Gupta BD, Dandiya PC, Gupta ML. A psycho-pharmacological analysis of behaviour in rats. Jpn. J. Pharmacol 1971;21(3):293-8.
20. Velu G, Palanichamy V, Rajan AP. Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. In Bioorganic Phase in Natural Food: An Overview 2018:135-56.
21. Singh S, Singh RP. *In vitro* methods of assay of antioxidants: an overview. Food Rev. Int 2008 16;24(4):392-415.
22. Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radical. Biochem. Pharmacol 1998;56(2):213-22.
23. Munira MS, Kabir MH, Bulbul IJ, Nesa ML, Muhit MA, Haque I. Pharmacological activities of *Youngia japonica* extracts. Annu. Res. Rev. Biol 2018;13:1-4.
24. Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, *et al*. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. Sci. Rep 2020;10(1):1-1.
25. Kwon YI, Apostolidis E, Shetty K. *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresour. Technol 2008;99(8):2981-8.
26. 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 2021;270:113781.
27. Nair SS, Kavrekar V, Mishra A. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Eur. J Exp. Biol 2013;3(1):128-32.
28. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evid Based Complement Alternat Med. 2014: Article ID 642942.
29. Saeidnia S, Manayi A, Gohari AR, Abdollahi M. The story of beta-sitosterol-a review. European J. Med. Plants, 2014, 590-609.
30. Singletary KW. Vanilla: potential health benefits. Nutr 2020;55(4):186-96.
31. Deraedt R, Jouquey S, Delevallée F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J Pharmacol 1980;61(1):17-24.
32. Altman R, Bosch B, Brune K, Patrignani P, Young C. Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology. Drugs 2015;75(8):859-77.
33. Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH, *et al*. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur. J Pharmacol 2000 ;387(1):111-8.
34. Griffin MR. Epidemiology of nonsteroidal anti-inflammatory drug–associated gastrointestinal injury. Am. J. Med 1998;104(3):23S-9S.
35. Pashmforosh M, Rajabi Vardanjani H, Rajabi Vardanjani H, Pashmforosh M, Khodayar MJ. Topical anti-inflammatory and analgesic activities of *Citrullus colocynthis* extract cream in rats. Medicina. 2018;54(4):51.
36. Bushra R, Aslam N. An overview of clinical pharmacology of Ibuprofen. Oman Med. J 2010;25(3):155.
37. Asif M. Mini review on important biological properties of benzofuran derivatives. J Anal. Pharm. Res. 2016;3(2):00050.
38. Usta C, Ozdemir S, Schiariti M, Puddu PE. The pharmacological use of ellagic acid-rich pomegranate fruit. Int. J Food Sci. Nutr 2013;64(7):907-13.
39. Mekonnen B, Asrie AB, Wubneh ZB. Antidiarrheal activity of 80% methanolic leaf extract of *Justicia schimperiana*. Evid Based Complement Alternat Med. 2018: Article ID 303712.
40. Dash PR, Nasrin M, Raihan SZ, Ali MS. Study of antidiarrhoeal activity of two medicinal plants of Bangladesh in castor-oil induced diarrhoea. Int. J. Pharm. Sci 2014;5(9):3864.
41. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. Phytother Res 2006;20(9):717-24.
42. Denenberg VH. Open-field behavior in the rat: What does it mean? Ann. N. Y. Acad. Sci 1969;159(3):852-9.
43. Datta BK, Datta SK, Chowdhury MM, Khan TH, Kundu JK, Rashid MA, *et al*. Analgesic, anti-inflammatory and CNS depressant activities of sesquiterpenes and a flavonoid glycoside from *Polygonum viscosum*. Pharmazie 2004;59(3):222-5.
44. Cordovilla-Guardia S, Lardelli-Claret P, Vilar-López R, López-Espuela F, Guerrero-López F, Fernández-Mondéjar E. The effect of central nervous system depressant, stimulant and hallucinogenic drugs on injury severity in patients admitted for trauma. Gac. Sanit 2019;33:4-9.