Exploration of Hypoglycemic, Analgesic, Antidiarrheal, and Antioxidant Properties of the Methanolic Extract of the Stems of *Gymnema inodorum* (Lour.) Decne.

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ABSTRACT: *Gymnema inodorum* (Lour.) Decne. (GI) is a climbing plant used as food. There are several different medicinal uses for this plant. Different fractions obtained from the GI stem's methanolic extract were subjected to pharmacological assays in this study. In the hypoglycemic activity assay, the chloroform fraction at both 200 mg/kg and 400 mg/kg body weight doses demonstrated potent hypoglycemic properties after 60, 120, and 180 minutes, whereas the ethyl acetate fraction showed significant activity after 120 and 180 minutes at both doses. With tail flicking responses elongating by 112.92% ($p<0.01$), 150.53% ($p<0.001$), and 257.23% ($p<0.001$) after 30, 60, and 90 minutes, respectively, the chloroform fraction (400 mg/kg) demonstrated the most prominent central analgesic activity among the test samples in the tail flicking method. Similarly, the chloroform fraction (400 mg/kg body weight) revealed strong peripheral analgesic activity with a 52.18% ($p<0.001$) writhing inhibition value in the acetic acid-induced writhing inhibition method. All other fractions at both doses had considerable antidiarrheal activity, with the exception of the n-hexane fraction. The n-hexane and ethyl acetate fractions' IC50 values in the DPPH free radical scavenging experiment were 31.5 µg/ml and 35.44 µg/ml, respectively. After subsequent phytochemical analysis, potential bioactive compounds for drug development might be obtained from the GI stems.

Key words: *Gymnema inodorum*; hypoglycemic; analgesic; antidiarrheal; antioxidant.

INTRODUCTION

Since ancient times, humans have used plants for medicinal and nutritional purposes. Plants with therapeutic properties have significantly contributed to human health. Every ancient civilization's history is linked to the usage of plants as therapeutic agents.[1] The earliest versions of these medications were applied in crude forms as poultices, tinctures, teas, and powders.[2] Because all plant parts contain bioactive substances, they have been demonstrated to have therapeutic uses.[3] Many diseases, such as diabetes, diarrhea, cough, gastritis, stomachache, vomiting, rheumatism, asthma infections, and more, are treated with medicinal plants.[4] Traditional plant-based medications are still used in primary healthcare in many parts of the world. This is particularly true for a large number of rural communities.[5] In comparison to conventional treatment systems, they are more readily available to locals, safer, and more reasonably priced.[1] The use of natural medicinal forms is becoming more relevant due to the declining effectiveness of synthetic drugs and the growing list of contraindications associated with their use.[6] Many of these conventional plant-based medications are

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being incorporated into mainstream healthcare systems in many countries through regulatory measures.[5] Additionally, there has been a shift in the global medical trend recently from synthetic to herbal therapy, dubbed as a "Return to Nature", implying the raising tendency of the medicinal plant's usage in healthcare nowadays.[7]

The plant *Gymnema inodorum* (Lour.) Decne. (GI) widely grows in Southern China, the Philippines, Indonesia, mainland Southeast Asia, and South Asia.[8] This plant can be used in food or consumed raw as a vegetable.[9] The plant belongs to the Asclepiadaceae subfamily of the Apocynaceae family. With over 4,600 species and 424 genera, it is one of the largest families. The plants of this family have been used for therapeutic purposes throughout the world.[10] Researchers have given *G. inodorum* considerable attention in recent years. The hypoglycemic, antidiabetic, antiadipogenesis, and antioxidant properties of different parts of the GI are found in some reports.[11,12] GI is used therapeutically in a number of clinical conditions, including gout, diabetes mellitus, rheumatoid arthritis, cataracts, liver cancer, and stomach cancer. The antipyretic and antiallergenic actions of the plant are also reported.[13] Bioactive phytocompounds, including glycosides, xanthophylls, vitamins, carotenes, tannins, and phenolics, are found in the GI leaves.[8]

All the previous reports mainly focused on the GI leaf's phytoconstituents and biological activities. As per our best knowledge, no study is available on the GI stems. So, the study was conducted to obtain the extracts from the stems of GI as well as to explore their hypoglycemic, analgesic, antidiarrheal, and antioxidant activities.

MATERIALS AND METHODS

Collection of plant material. In March 2021, the stems of the GI plant were collected from Bangladesh's Chattogram district. The taxonomist at the National Herbarium confirmed the plant sample's authenticity, and a voucher specimen was collected from the Bangladesh National Herbarium, Dhaka, Bangladesh (accession number DACB-89157).

Extraction and fractionation. The small pieces of the stems of GI were first dried and then bruised into rough powder. About 1.2 kg of the powder was taken in approximately 3 liters of methanol for a period of 21 days. The mixture was subjected to filtration to obtain the filtrate with the help of a cotton-plugged funnel and then through Whatman filter paper. The filtrate was condensed into a dry crude extract after the solvent was completely evaporated using a rotary evaporator operating at a low temperature (50 degrees) and pressure. The fractionation of the obtained crude extract was performed by using different solvents and water based on polarity and applying the modified Kupchan partitioning process.[14] Following another condensing of the liquid fractions, the n-hexane, chloroform, ethyl acetate, and aqueous fractions yielded 0.8 g, 1.7 g, 1.1 g, and 1.3 g of dry materials, respectively.

Drugs and chemicals. Analytical-grade reagents were procured for the study. Ascorbic acid, dimethyl sulfoxide, Tween 80, glucose, castor oil, and 2,2-diphenyl-1-picrylhydrazyl were among the reagents acquired from BDH Chemicals along with additional solvents used in extraction and fractionation (methanol, n-hexane, chloroform, and ethyl acetate). Moreover, Gonoshastho Pharmaceuticals Ltd. and Square Pharmaceuticals Ltd. provided morphine and diclofenac sodium, respectively. Loperamide and glibenclamide were collected from Incepta Pharmaceuticals Ltd.

Experimental animal. To conduct the antidiarrheal, central analgesic, and peripheral analgesic activity evaluations, Swiss-albino mice were employed. To evaluate the hypoglycemic properties, Wistar rats were used. The test mice and rats were of either sex, and their age was nearly 4 to 5 weeks. Before the experiment, these animals were kept in polypropylene cages. The recommended temperature (24±1°C) and other specified conditions were ensured to give them a proper housing period. The rodent food and water were given to the experimental animals. The research employed a reasonable number of animals in accordance with particular guidelines[15], and during the studies, no

unexpected mortality or aberrant behavior from the experimental animal was noticed. All animal experiments comply with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and follow the NIH (National Research Council) Guide for the Care and Use of Laboratory Animals.

Hypoglycemic activity. The hypoglycemic characteristics of the GI stem were assessed using a slightly modified form of the oral glucose tolerance test (OGTT).[16] Initially, test animals' blood glucose levels were measured using a glucometer; the animals' tails were used for blood collection. The blood glucose level was then raised by giving an oral 10% glucose solution (2 mg/kg of body weight). After 30 minutes, the negative control group (six rats in each group) was given a 1% tween 80 solution with normal saline, the positive control (standard) group was given glibenclamide orally (10 mg/kg), and the other groups were orally fed with test fractions at a dose of 200 mg/kg and 400 mg/kg of body weight. Ultimately, after 60, 120, and 180 minutes, the blood glucose levels of every rat were measured in an identical manner. The percentage decrease in blood glucose levels made it easier to evaluate the findings.

Central analgesic activity. The tail flicking method, in which a pain sensation in the mouse tail is produced by applying constant heat stress, was selected to judge the central analgesic properties of the GI stems.[17] Two doses of the extract and fractions of the GI stems (200 and 400 mg/kg) were given to the experimental mice, and around 5 cm of the tail end was sunk in warm (55±2℃) water. The tail's withdrawal time was observed at 0, 30, 60, and 90 minutes. The positive control (standard) group was subjected to a subcutaneous morphine injection (2 mg/kg). The activity was interpreted with the help of the time-prolongation response values.

Peripheral analgesic activity. The GI stem's peripheral analgesic property was evaluated by applying the acetic acid-induced writhing method.[18] "Writhing" refers to the contraction of the body brought on by the pain that intraperitoneal acetic acid administration produces. The experimental animals in the test sample groups were given two separate dosages (200 and 400 mg/kg) of the test fractions orally, while the standard group was given 50 mg/kg of diclofenac sodium. Following the administration of the control, standard, and test samples for 15 minutes, the quantity of twisting movements (writhing) was counted for 5 minutes. Then, the percent writhing inhibition was measured, and the values were utilized in interpreting the GI stem's peripheral analgesic properties.

Antidiarrheal activity. The anti-diarrheal effects of the GI stems were investigated using the castor oil-induced diarrhea method, in which the ricinoleic acid of castor oil induces diarrhea-like appearances in experimental animals.[19] Loperamide, a commonly used antidiarrheal drug, was used as a positive control (standard) at a dose of 50 mg/kg. The mice in the test sample groups received two different doses (200 and 400 mg/kg) of the fractions. After 30 minutes of the administration of control, standard, and test fractions, castor oil (1 ml) was administered orally. For a duration of 4 hours, the total number of fecal stools from each animal was noted. The test sample groups' percent defecation inhibition values were computed to justify the GI stems' anti-diarrheal properties.

Antioxidant activity. The *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used for determining antioxidant activity. The antioxidant properties of the GI stem's fractions were investigated using the method. The assay determines the samples' ability to scavenge DPPH-free radicals and gives a comparison with the standard ascorbic acid.[20] Concentrations ranging from 1 µg/ml to 500 µg/ml of the standard and the test samples were prepared for this assay. 3 ml of a methanol solution bearing DPPH (20 µg/ml) was combined with 2 ml of these solutions. For thirty minutes, these combinations were stored in a dark area. A UV

spectrophotometer was used to test the mixes' absorbance at a wavelength of 517 nm. The DPPH free radical inhibition percentage values were calculated, and by plotting these values against respective concentrations, 50% inhibitory concentration values (IC_{50}) were obtained.

Statistical analysis. Experimental data recorded from the biological assays were subjected to statistical analysis. MS Excel (version 10.0) was used for statistical analysis and constructing the graphs. The *in vivo* experiment's data were presented as mean ± SEM values. Using the student t-test, the p values of the assays were obtained. P values less than 0.05 indicated the statistical significance of the findings.

RESULTS AND DISCUSSION

Hypoglycemic activity. The fractions obtained from the methanolic crude extract of GI stems were investigated to explore whether they convey hypoglycemic properties utilizing oral glucose tolerance test in Wistar rats. Table 1 displays the recorded measurements at various time intervals. The standard (glibenclamide) resulted in 28.78% ($p<0.001$), 45.53% ($p<0.001$), and 53.93% ($p<0.001$) plasma glucose level reduction after 60, 120, and 180 minutes, respectively (Figure 1). The chloroform soluble fraction (CF) at both doses showed statistically significant glucose level reductions after 60, 120, and 180 minutes (Table 1). Besides, the ethyl acetate soluble fraction (EAF) at both doses produced significant results after 120 and 180 minutes.

Table 1. **Hypoglycemic properties of different fractions of methanolic extract of the GI stem in terms of average glucose level after loading the glucose sample.**

Groups	Average glucose level (mmol/l) after loading the glucose sample				
	0 min	30 min	60 min	120 min	180 min
CTL	5.68 ± 0.08	11.18 ± 0.34	10.02±0.23	9.58 ± 0.11	8.83 ± 0.15
STD	5.45 ± 0.11	10.98 ± 0.24	7.82 ± 0.13***	6.20 ± 0.16***	4.62 ± 0.12***
NHF-200	5.48 ± 0.11	11.32 ± 0.26	10.01 ± 0.28	9.58 ± 0.28	8.80 ± 0.21
NHF-400	5.03 ± 0.13	10.82 ± 0.27	9.67 ± 0.19	9.26 ± 0.27	8.55 ± 0.25
CF-200	5.87 ± 0.19	9.82 ± 0.24	9.32 ± 0.14*	7.72 ± 0.18***	5.95 ± 0.21***
CF-400	5.32 ± 0.17	10.78 ± 0.26	8.63 ± 0.22***	6.97 ± 0.17***	5.65 ± 0.18***
EAF-200	5.23 ± 0.09	11.08 ± 0.31	9.35 ± 0.24	8.22 ± 0.17***	6.85 ± 0.22***
EAF-400	4.88 ± 0.20	10.68 ± 0.19	9.52 ± 0.17	6.57 ± 0.18***	5.92 ± 0.19***
AQF-200	5.55 ± 0.07	10.72 ± 0.39	9.43 ± 0.28	9.07 ± 0.13	8.58 ± 0.10
AQF-400	5.45 ± 0.09	10.78 ± 0.26	9.50 ± 0.18	8.88 ± 0.27	8.37 ± 0.16

Data are represented as mean ± SEM for n=6. ***p<0.001, *p<0.05 versus control; CTL: Control, STD: Standard, NHF: n-Hexane fraction, CF: Chloroform fraction, EAF: Ethyl acetate fraction, AQF: Aqueous fraction

The GI stem's ethyl acetate and chloroform fractions both exhibited significant hypoglycemic effect. Previously, no study had been found on the hypoglycemic activity of the GI stems, but studies on the GI leaves revealed the existence of several antidiabetic compounds. Those phytochemicals with plasma glucose-lowering ability include triterpenoid saponins and pregnane glycosides. Various glucopyranosiduronic acid derivatives belong to triterpenoid saponins, and pregnane glycosides are gyminosides A and B, gymnepregoside F, tinctoroside B and C.[8] The bioactive antidiabetic compounds isolated from GI leaves showed the ability to decrease glucose absorption, suppress the α-glucosidase enzymes' activity, and stimulate insulin release from the pancreas.[21] The findings of the hypoglycemic activity evaluation indicated the presence of potent glucose-lowering compounds in the GI stems with probable similar types of mechanisms of action. Currently, the main pharmacological treatments for type II diabetes mellitus are biguanides, insulin sensitizers, sodium-

glucose co-transporter-2 (SGLT2) inhibitors, insulin secretagogues, alpha-glucosidase inhibitors, incretin mimetics, and so on. These first-line oral hypoglycemic medicines alone are not able to meet therapeutic goals sometimes; dual or triple medication treatments are frequently advised in those instances. Besides, these conventional antidiabetic medications offer several drawbacks, including frequent dosing, increased side effects, therapy ineffectiveness, and patient non-compliance.[22] Finding other sources of antidiabetic drugs is compelled by these concerns. Medicinal plants are a good means to obtain biologically active substances that have potent therapeutic benefits and no negative side effects. Many diabetic patients from developing countries use the plants for treatment purposes. It has become recommended to utilize medicinal plants for managing a variety of illnesses, including diabetes.[23,24] According to the findings, GI stems can be considered a reservoir of potential hypoglycemic compounds.

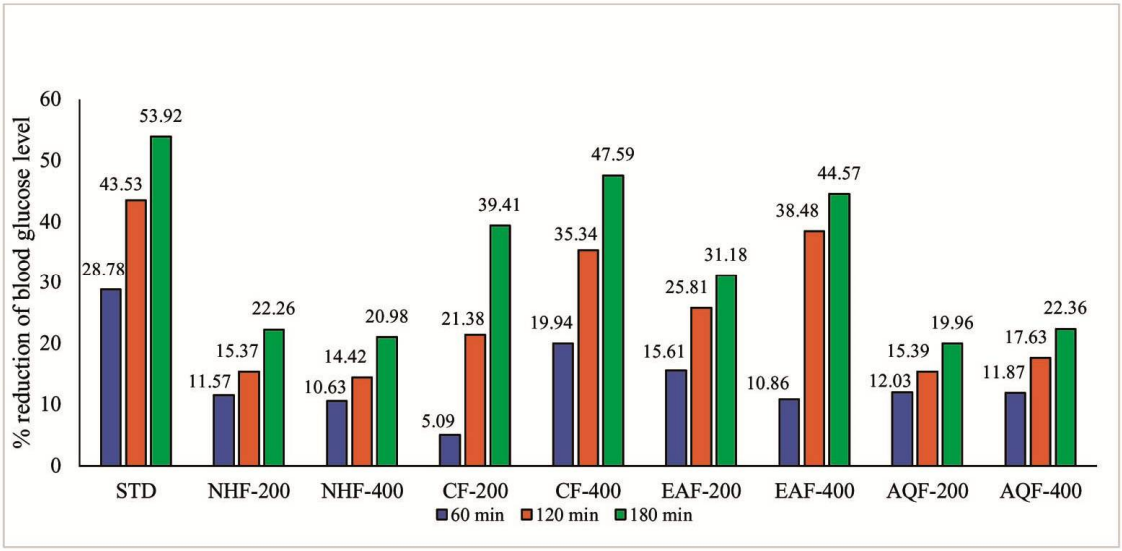

Figure 1. Hypoglycemic activity of the methanolic extract of different fractions of methanolic extract of the GI stems.

Central analgesic activity. By using Swiss-albino mice in the tail flicking method, the various fractions of GI stems have been examined for their central analgesic properties. The percent time elongation in tail flicking responses is presented in Table 2. 239.25% ($p<0.001$), 306.22% ($p<0.001$), and 434.76% ($p<0.001$) time elongation responses were observed in standard (morphine)-treated mice after 30, 60, and 90 minutes, respectively. Among the test fractions, the chloroform fraction (CF) at 400 mg/kg of body weight dose produced the highest activity with 112.92% ($p<0.01$), 150.53% ($p<0.001$), and 257.23% ($p<0.001$) time elongation responses after 30, 60, and 90 minutes, respectively. Except for the aqueous fraction (AQF) at 200 mg/kg of body weight dose, all other fractions revealed significant ($p<0.001$) central analgesic activity after 90 minutes, compared to the control group.

Peripheral analgesic activity. In the peripheral analgesic activity assay, diclofenac sodium was selected as a positive control (standard) in this assay. Compared to the control group, the standard inhibited 70.50% ($p<0.001$) of writhing (Table 3). Except for the n-hexane fraction at the 200 mg/kg dose and the aqueous fraction at both doses, all other fractions exhibited statistically significant peripheral analgesic properties compared to the control group. The most potent activity was produced by the chloroform fraction (CF) at a 400 mg/kg body weight dose, with 52.18% ($p<0.001$) writhing inhibition.

Table 2. Central analgesic activities of different fractions of the GI stems in terms of % time elongation in tail flicking responses.

Groups	% Time elongation in tail flicking responses		
	30 minutes	60 minutes	90 minutes
CTL	-	-	-
STD	239.25***	306.22***	434.76***
NHF-200	11.33	27.77	64.07***
NHF-400	13.47	87.07**	122.19***
CF-200	27.56	90.32**	101.22 ***
CF-400	112.92**	150.53***	257.23***
EAF-200	11.27	24.31	92.10***
EAF-400	22.29	105.16***	139.43***
AQF-200	9.80	21.44	25.03
AQF-400	13.29	45.74**	73.97***

n=6. ***p<0.001, **p<0.01, *p<0.05 versus control; CTL: Control, STD: Standard, NHF: n-Hexane fraction, CF: Chloroform fraction, EAF: Ethyl acetate fraction, AQF: Aqueous fraction

Table 3. Peripheral analgesic activities of different fractions of the GI stem in terms of the percent writhing inhibition.

Groups	% Inhibition of writhing
CTL	-
STD	70.50 ± 0.42***
NHF-200	13.56 ± 0.67
NHF-400	35.97 ± 0.48***
CF-200	41.34 ± 0.67***
CF-400	52.18± 0.33***
EAF-200	29.77 ± 1.05**
EAF-400	38.29 ±0.95***
AQF-200	10.51 ± 0.61
AQF-400	11.25 ± 0.87

Data are represented as mean ± SEM for n=6. ***p<0.001, **p<0.01, *p<0.05 versus control; CTL: Control, STD: Standard, NHF: n-Hexane fraction, CF: Chloroform fraction, EAF: Ethyl acetate fraction, AQF: Aqueous fraction

Globally, pain is a highly prevalent clinical condition affecting millions of people, contributing to their burden of illness and disability.[25] It seriously impairs people's health and, most importantly, their quality of life. In order to control pain, nonsteroidal anti-inflammatory medications (NSAIDs), opioids, and steroids are commonly used. However, the way pain is treated is far from sufficient, which results in ineffective pain management and distress.[26] NSAIDs are widely prescribed analgesics all over the world, but their gastrointestinal (GI) and cardiovascular adverse effects are well established.[27] Other available analgesics are also not free from negative effects, and those analgesics sometimes demonstrate lower effectiveness.[25] Due to these facts, there is a pressing need for advanced analgesics with low toxicity and enhanced efficacy. Phytocompounds have shown potential as promising lead molecules in the medication development process. It has been demonstrated that flavonoids, alkaloids, glycosides, and other related compounds have analgesic qualities with a variety of proposed mechanisms of actions.[28] Other parts of the GI plant were previously reported to possess many of these compounds with analgesic properties.[21,29] Two widely used methods were employed to explore the analgesic activity of GI stems in this work. The tail flicking method utilizes the tolerability of the mice tails to the heat of warm water after administration of the test samples as the principle, whereas in the acetic acid-induced writhing

approach, writing inhibition resulting from analgesic substance-induced diminution of pain perception was employed.[30] The chloroform fraction and the ethyl acetate fraction produced notable analgesic activities in those assays. These findings help to conclude that GI stems possess such effective analgesic compounds.

Antidiarrheal activity. The findings of the antidiarrheal activity assay indicated that the fractions of the GI stems were found to have significant antidiarrheal properties by comparing them to the control groups' data (Table 4). Standard loperamide (50 mg/kg of body weight) resulted in 64.10% ($p<0.001$) inhibition of defecation. Statistically significant findings were not produced by only the n-hexane fraction (NHF) at both doses. Both the aqueous fraction (AQF) and the chloroform fraction (CF) demonstrated 43.37% ($p<0.001$) and 49.04% ($p<0.001$) defecation inhibition at a dose of 400 mg/kg of body weight, respectively.

Table 4. Antidiarrheal properties of different fractions of the GI stem's methanolic extract.

Groups	Total number of fecal pellets after 4h	% inhibition of defecation
CTL	8.83 ± 0.48	-
STD	3.17 ± 0.31***	64.10
NHF-200	8.00 ± 0.45	9.40
NHF-400	7.50 ± 0.41	15.06
CF-200	5.33 ± 0.42***	39.64
CF-400	4.50 ± 0.50***	49.04
EAF-200	7.33 ± 0.21*	16.99
EAF-400	6.33 ± 0.49***	28.31
AQF-200	6.17 ± 0.17***	30.12
AQF-400	5.00 ± 0.37***	43.37

Data are represented as mean ± SEM for n=6. ***p<0.001, *p <0.05 versus control; CTL: Control, STD: Standard, NHF: n-Hexane fraction, CF: Chloroform fraction, EAF: Ethyl acetate fraction, AQF: Aqueous fraction.

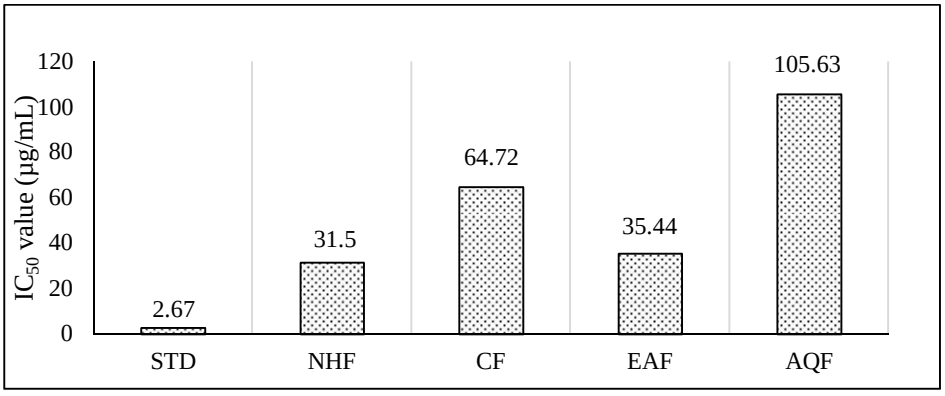

Figure 2. Antioxidant activity of different fractions of the GI stems in terms of the IC_{50} values. Here, STD: Standard, NHF: n-Hexane fraction, CF: Chloroform fraction, EAF: Ethyl acetate fraction, AQF: Aqueous fraction.

A large number of populations, especially those from developing countries, suffer from diarrhea. Children under 5 years old are more susceptible to this disease among the population. An estimated 1.3 million deaths are attributed to diarrheal illnesses each year.[31] Due to the emergence of antibiotic resistance, infectious diarrhea is becoming difficult to treat. Besides, many of the well-known traditional antidiarrheal medications have contraindications and unfavorable effects on health.[32] Plant extracts were found to inhibit gut motility, slow gastrointestinal transit, enhance water adsorption, and have antispasmodic properties.[33] These effects emphasize

the advantages of utilizing medicinal plants to treat diarrheal disease. As per our best knowledge, there are no previous reports on GI stem's antidiarrheal properties. The stems of GI, mainly its aqueous and chloroform fractions, exhibited antidiarrheal activity in this experiment. The findings indicate the presence of potential antidiarrheal substances in the stems of GI.

Antioxidant activity. The n-hexane fraction (NHF) had the highest antioxidant activity among the test fractions of the GI stems, with an IC_{50} value of 31.5 µg/ml. The ethyl acetate fraction (EAF) also demonstrated the next potent antioxidant activity, with an IC50 value of 35.44 µg/ml in the DPPH free radical scavenging method (Figure. 2). The IC_{50} value of the standard (ascorbic acid) was 2.67 µg/ml. The IC_{50} values of the chloroform fraction (CF) and the aqueous fraction (AQF) were 64.72 µg/ml and 105.63 µg/ml, respectively.

The human body produces reactive species and free radicals as part of regular metabolic processes. Additionally, external environmental stimuli such as ultraviolet radiation, cigarette smoking, and other pollutants aggravate this production.[34] Antioxidant molecules scavenge excessively produced free radicals and reactive species to keep a balance in free radicals' production and neutralization. When the generation of reactive species surpasses the antioxidant capacity, a condition known as oxidative stress is created.[35] In oxidative stress, excess free radicals target a number of endogenous molecules and damage membranes, enzymes, and DNA. These may cause an initiation of many clinical conditions, including cancer, atherosclerosis, malaria, rheumatoid arthritis, and neurological disorders.[36] The protective effects of antioxidant substances diminish the negative effects of oxidative stress.[37] Although synthetic antioxidants are available, interest in consuming natural antioxidants has significantly expanded due to their impact on longevity, greater antioxidant activity, and affordability.[38] GI leaves had previously been shown to contain antioxidant phytocompounds, including flavonoids, phenolics, quinic acids, and triterpenoid saponins.[29] The study's outcomes suggest that the n-hexane and ethyl acetate fractions obtained from the GI stems possess antioxidant properties. So, it can be concluded that antioxidant substances are also present in the stems of the GI plant. The n-hexane and ethyl acetate fractions are predicted to provide potent antioxidant substances after subsequent experimentation.

Limitation

Some notable pharmacological activities of the GI stems were examined through this study, but no phytocompounds were isolated from the plant part. Besides, the pharmacological properties were judged through single experimentation. Although there remain some limitations in the study, it provides several important pieces of information about the medicinal values of the GI stems.

CONCLUSION

Four fractions from the methanolic extract of the GI stems were subjected to different *in-vivo* and *in-vitro* experiments. The chloroform and ethyl acetate fractions exhibited notable activities in all hypoglycemic, analgesic, antidiarrheal, and antioxidant assays. The n-hexane fraction showed central analgesic and antioxidant properties at both doses, as well as peripheral analgesic activity at a higher dose. The aqueous fraction produced antidiarrheal activity at both doses but central analgesic activity at a higher dose. These results point to the presence of bioactive chemicals in the GI stems, which are expected to be important for potential therapeutic development.

AUTHORS' CONTRIBUTION

Md. Rasul Karim: Conceptualization, Formal analysis, Writing – original draft. Md. Sabbir Hossain: Writing – original draft, Validation, Data curation. Md. Saiful Islam: Methodology, Validation, Modified the draft. Muhammad Rabbin Hossen: Visualization, Validation. Mohammad Shawkat Ali: Conceptualization, Visualization, Supervision

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known conflict of interest

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