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Research Article

Phytochemical and pharmacological investigations of *Grewia serrulata* leaves methanol extracts and its different fractions against oxidative stress, inflammation and diarrhea: An *in vitro* and *in vivo* study.

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ABSTRACT

The Malvaceae (formerly Tiliaceae) family's *Grewia serrulata* DC has remarkable medicinal characteristics and its many parts are utilized in traditional medicine. However, scientific evidence supporting its pharmacological activities remains limited, particularly regarding the bioactivity of its leave extracts and solvent fractions. Therefore, the present study aimed to evaluate the phytochemical profile and antioxidant, anti-inflammatory, and antidiarrheal activities of the methanol extract of *G. serrulata* leaves and solvent fractions. To investigate the antioxidant effect, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay was conducted *in vitro*, while *in vivo* anti-inflammatory and antidiarrheal activities were evaluated using the carrageenan-induced paw edema model and the castor oil-induced diarrhea model in mice, respectively. *G. serrulata* methanol extract (GSME) and its n-Hexane fraction (GSNH) showed significant potential in DPPH scavenging at higher doses with showing IC_{50} values of 11.7886µg/mL and 89.86µg/mL, respectively. Also, GSME, GSNH & Dichloromethane fractions (GSDM) displayed significant (p <0.001) anti-inflammatory and antidiarrheal effects compared to control. GSME, GSNH & GSDM reduced inflammation at a rate of 88.48%, 66.03% & 58.46% during 4th hour of post-injection. GSME & GSNH demonstrated a highly significant (p <0.001) reduction (71.21% & 62.12% inhibition, respectively) in diarrhea, while Loperamide showed an inhibition of 72.73%. The findings indicate that *G. serrulata* leaves exhibit promising antioxidant, anti-inflammatory and antidiarrheal activities. The results provide preliminary pharmacological support for the traditional use of this plant and highlight its potential as a source of bioactive compounds for future drug discovery, though further investigations including bioassay-guided isolation, mechanistic investigations, and safety evaluations are required.

Keywords:
Methanol; Fractions; Antioxidant; Anti-inflammatory; Antidiarrheal.

1. INTRODUCTION

Numerous phytochemicals found in plants have shown promise as medicinal agents, and plants remain an abundant source of such compounds[1]. Around 80% of people in underdeveloped nations use plant-based traditional remedies to meet their essential healthcare requirements. Bangladeshi traditional medicines showcase a unique fusion of diverse ethnomedicinal influences[2]. Medicinal plants possess organic compounds

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that exert targeted biological impacts on the human body[1,3]. Secondary metabolites are extensively utilized in human therapy, scientific research, agriculture and numerous other areas[4].

Antioxidants counteract free radicals and mitigate the harmful effects of oxidants by breaking them down, thereby stopping chain reactions, or impeding the conversion of oxygen into highly reactive states[5]. Studies showed that about 29.3% of diabetic patients had HbA1c level < 7% and FPG level ≤ 7 mmol/L with higher concentration of NADPH oxidase and myeloperoxidase and inflammatory leukocyte markers the stimulator of reactive oxygen species (ROS)[6]. Antioxidants have recently earned considerable attention due to their promising roles as both preventive and therapeutic agents to treat conditions such as cancer, diabetes, heart disease, autoimmune problems, neurodegenerative disease, and aging has sparked a medical revolution and brought in a new age of healthcare[7]. Current efforts in antioxidant drug development focus on treating severe diseases that lack proven effective therapies[8,9].

Besides, inflammation is the body's response to detrimental stimuli, marked by vasodilation and the influx of fluids and cells into the affected tissue[10]. This mechanism is further distinguished by higher vascular permeability & the release of mediators[11], along with changes to the membrane and denaturation of proteins[12]. Inflammation turns on different kinds of cells that release inflammatory markers like cytokines (IL-1β, TNF-α, and IL-6), prostaglandin (PGE2) and nitric oxide (NO)[13]. While anti-inflammatory drugs, both steroidal and non-steroidal, are commonly employed to combat inflammatory conditions, the pursuit of new and safe anti-inflammatory agents remains a significant area of interest. Consequently, NSAIDs, alongside anti-infective agents, are among the leading causes of Drug-Induced Liver Injury (DILI). NSAIDs cause a wide variety of hepatotoxicity, from mild and temporary hyper-transaminasemia to severe liver failure[14].

Diarrhea is another major global health concern and is defined as the passage of 3 times or more loose or liquid feces daily[15]. It possesses a significant risk of morbidity & mortality, particularly among children and young animals in emerging countries. Medicinal herbs are a significant source for the improvement of antidiarrheal drugs[16]. The overall prevalence of chronic diarrheal diseases among children aged below 5 years was found to be 4.91%. Stunted children had 40.8% higher odds of diarrhea than normal children[17]. Results showed that the prevalence of diarrhea among under-five children is 13.5% in the southwestern coastal region[18]. The treatment strategy of acute diarrhea that is infectious typically involves antibiotics. Nevertheless, the use of antibiotics is frequently linked to the reduction of useful mucosal & gut microorganisms, immunosuppression, and allergic reactions[15].

The genus *Grewia*, belonging to the family Malvaceae (formerly Tiliaceae), comprises numerous species widely distributed across tropical and subtropical regions of the world[19]. Many *Grewia* species have been traditionally used in herbal medicine for the treatment of various ailments and are known to contain a variety of bioactive phytochemicals, including flavonoids, phenolic compounds, terpenoids, saponins, and glycosides[20,21]. These phytoconstituents are often associated with antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activities. Previous studies have reported that extracts from *Grewia serrulata* and related species such as *Grewia asiatica* exhibit several pharmacological effects, including analgesic, antinociceptive, antipyretic, hepatoprotective, antimicrobial, and antioxidant activities[22,23].

Grewia serrulata DC. is a small tree characterized by slender branches, deep grey bark, ovate to lanceolate serrated leaves, and drupaceous fruits that become fleshy and black upon ripening[24]. In traditional medicinal practices, different parts of the plant have been used for various therapeutic purposes, including the treatment of respiratory disorders, gastrointestinal problems, and skin ailments[23,25]. Phytochemical investigations have revealed the presence of several secondary metabolites in the aerial parts of the plant, including flavonoids, phenolics, saponins, glycosides, terpenes, and sterols[26], which may contribute to its pharmacological potential.

Despite several pharmacological reports on other *Grewia* species, the methanol extract and solvent fractions of *G. serrulata* leaves remain uninvestigated, representing a significant knowledge gap. Giving consideration in the enriched therapeutic properties of *Grewia* genus, lack of pharmacological data on *G. serrulata* and overall considering the prevalence of diarrhea, inflammation and other non-communicable diseases in Bangladesh region, the crude methanol extracts of leaves and its different soluble fractions have been taken into consideration for investigations into the qualitative analysis of phytochemicals, as well as the antioxidant, anti-inflammatory and antidiarrheal activities, which might pave the way to find out newer bioactive compounds through future research. Therefore, the present study aimed to evaluate the antioxidant, anti-inflammatory and antidiarrheal activities of *G. serrulata* leaves methanol extract and its n-hexane and dichloromethane soluble fractions.

2. MATERIALS AND MEDTHODS

2.1. Chemicals

Loperamide was sourced from Square Pharmaceuticals Ltd., situated in Gazipur, Bangladesh, and Indomethacin was procured from Opsonin Pharmaceuticals Ltd., Dhaka, Bangladesh, by the Department of Pharmacy of USTC. Chemicals used for

extracting plant parts, performing *in vitro* and *in vivo* pharmacological tests, and other related processes were purchased from Merck, Germany.

2.2. Plant sample identification and collection

Mature plant leaves were collected with the guidance of a recognized local traditional healer. An esteemed taxonomist later confirmed the leaves' identity and placed them in the herbarium with the designation KM-010320-16 at the department of Pharmacy, USTC.

2.3. Crude methanol extracts of *G. serrulata* Leaves preparation

Plant materials (leaves) were thoroughly cleansed and chopped, then subjected to semi-shade sun-drying. After the leaves were dried, they were mechanically pulverized by a high-speed multi-function comminutor (RRH-500A). A quantity of powdered (720g) *G. serrulata* leaves was thereafter soaked in around 7 litre of methanol. After a period of a week with intermittent shaking, the solution went through filtration. The resulting filtrate was then made concentrated using an evaporation technique under reduced pressure, maintaining temperatures less than 50°C, with the aid of a rotary evaporator (Stuart, UK).

2.4. Qualitative phytochemical screening

The primary phytochemical analysis was performed to assess the qualitative presence of various compounds including terpenoids, flavonoids, saponins, phenols, tannins, phlobatannins, steroids, alkaloids, glycosides, cardiac glycosides, anthraquinones, resins, carbohydrates, proteins, fats and oils and coumarins, following standard procedures[27].The analytical responses for these qualitative tests were determined by observing the color intensity or the appearance of precipitates.

2.5. Solvent–solvent fractioning

The *G. serrulata* leaves methanol extracts of were subjected to solvent-solvent partitioning following the protocol established by Kupchan[28], with modifications as described by Wagenen et al[29]. The solvents used in this sequential partitioning process were n-hexane and dichloromethane, repeating the separation cycle several times.

2.6. Experimental animals

The research employed male Swiss Albino mice aged 4 to 5 weeks, weighing between 25 and 35 grams. The mice were obtained from the Animal Resources Center of ICDDR,B, Dhaka. The animals had been kept in the animal facility of the University of Science and Technology Chittagong (USTC)'s Department of Pharmacy in dry & clean cages with a 12-hour light/dark cycle at a temperature of 25±2°C. The mice had been given a standard laboratory meal & water available ad libitum. The animals were allowed to acclimatize for a week and then the experiments commenced. Subjects abstained from meals for 12 hours both prior to and during the trial. All animal procedures were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985), and approved by the Institutional Ethical Review Committee of the University of Science and Technology Chittagong (Approval no IERC/USTC/24/007). For each model, mice were divided into groups of five at random. The sample size (n = 5 per group) was determined based on prior research and usual practice in similar pharmacological assessments. Although a formal power analysis was not performed, the sample size was deemed sufficient to detect impactful changes as this study was designed as a preliminary pharmacological evaluation, where n=5 per group are commonly used to identify potential biological actions before conducting larger confirmatory studies. This small sample size may limit statistical power; however it was chosen to minimize animal use in accordance with ethical principles. The subject animals were euthanized after finishing the study following ARRIVE guidelines[30].

2.7. Acute Toxicity study

Five Swiss albino mice per group were examined for acute toxicity of *G. serrulata* extracts following general principles of OECD guidelines 423[31] with minor modifications for preliminary assessment, similar to previously reported plant extract toxicity studies[32]. Oral extracts at doses of 1000, 2000, 3000, 4000, and 5000 mg/kg were administered to mice after an overnight period of starvation. Mice fasted for additional 3–4 hours after treatment. Animals were observed meticulously for first 30 minutes, periodically during the first 24 hours, then daily for 3 consecutive days. In order to identify toxicity, the mice were examined for several bodily functions, including the skin, fur, eyes, circulation, urination, breathing and neurological status. This study represents a preliminary safety assessment of the extract's safety over a short observation period. Long-term and chronic toxicity studies are suggested to thoroughly clarify the safety profile and therapeutic potential of the extracts.

2.8. *In vitro* study of antioxidant effect

2.8.1. DPPH scavenging assay

The antioxidant capacity of various chemicals and medicinal plants was evaluated by assessing their

free radical scavenging activity using the DPPH test. A solution of the sample (extractives/control) in methanol, with concentrations ranging 500 to 15.63 µg/mL, was prepared (n=3 independent experiments). Then, 2.0 mL of test solution was combined with 3.0 mL of a methanol solution containing DPPH (0.004% w/v). After incubating for 30 minutes at room temperature in the absence of light, the absorbance was calculated at 517 nm employing a UV spectrophotometer. Ascorbic Acid (AA) was used as the positive control. All measurements were performed in triplicate, and the experiments were repeated independently three times. The percentage of DPPH radical inhibition (%I) was calculated by the following equation[33]:

$$I\% = 1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100\%$$

The half-maximal inhibitory concentration (IC_{50}) was determined by plotting the percentage of inhibition against the concentration of the extractives and performing a linear regression analysis.

2.9. *In vivo* studies of anti-inflammatory effect

2.9.1. Carrageenan induced paw edema test

The experiment involved the utilization of carrageenan to serve as an inflammatory substance to make swelling in the paw of right hind limb of mice [34]. A total of eight groups, each consisting of five mice, were given different substances. First group was given 1% tween-80 at a dosage of 10 mL/kg. The mice in the second group were given indomethacin at a dosage of 10 mg/kg orally. The mice in the rest of the groups were given GSME, GSNH and GSDM, respectively, at dosages of 200 and 400 mg/kg orally. An hour after the samples were taken and thirty minutes after the positive control was administered, a subplantar injection of 100 µL of carrageenan (1% w/v in 0.9% normal saline) was delivered into the right hind paw. The paw edema was quantified prior to carrageenan injection and subsequently at 1, 2, 3 & 4 hours post-injection using Vernier calipers. The significant edema inhibition observed at 1 and 2h might interfere with early mediator release (e.g. histamine, 5-HT), whereas inhibition at 3–4h suggests that it may also suppress COX-mediated prostaglandin formation or downstream cytokine signaling [35]. Paw edema inhibition was measured by calculating the alteration in paw circumference (mm) following the specified method[36]:

$$\text{Inhibition of edema (\%)} = \frac{\text{Cc} - \text{Ct}}{\text{Cc}}$$

Here, Cc = the variation in paw circumference before and after carrageenan injection for the control group at various time intervals, Ct = the variation in paw circumference before and after carrageenan injection for the test groups at various time intervals.

2.10. *In vivo* studies of antidiarrheal effect

2.10.1. Castor oil-induced diarrhea test

A total of forty male Swiss Albino mice, weighing 25 to 35 gm, were separated into eight groups, with each group consisting of five animals at random. Group I administered a 1%Tween 80 solution (10 ml/kg) orally and marked as the control group. Group II received a standard treatment of 50 mg/kg of Loperamide delivered orally. The test groups, which comprised Group III to Group VIII, were administered concentrations of 200 and 400 mg/kg of GSME, GSNH, and GSDM, respectively. The solvent for the preparation of all dosages was tween 80 in saline. The standard, control, and test extract samples were administered to them at zero hour. Subsequently, 1.0 ml of castor oil per mouse was administered orally to each mouse in each group after 30 minutes for diarrhea induction. During fecal collection, each mouse was individually placed in a separate mice cage lined with clean white filter paper. The duration of induced diarrheal effect was measured over a four hour period. The consistency of feces was scored using a standard 3-point scale: 0 = normal, well-formed pellets; 1 = semisolid stools; 2 = watery diarrhea[37]. The following formulas were employed to determine the percentage inhibition of total defecation and diarrhea[38]:

$$\text{Inhibition of defecation (\%)} = \frac{\text{Total number of feces in control} - \text{total number of feces in treated mice}}{\text{Total number of feces in control}} \times 100$$

$$\text{Inhibition of diarrhea (\%)} = \frac{\text{Total number of diarrheal feces in control} - \text{total number of diarrheal feces in treated mice}}{\text{Total number of diarrheal feces in control}} \times 100$$

2.11. Statistical analysis

The results of this investigation were expressed using the mean ± SEM (Standard Error of Mean) notation. The data were subjected to statistical analysis by a one-way analysis of variance (ANOVA), followed by a post hoc Dunnett's "t" test. The Statistical Package for the Social Sciences (SPSS, version 16.0) was employed for this. Statistical significance was determined at the $*p<0.05$ and $**p<0.01$ levels and high significance was defined at the $***p<0.001$ level.

Table 1. Qualitative phytochemical screenings of *Grewia serrulata* methanol extract (GSME) and its n-hexane and dichloromethane solvent fractions (GSNH & GSDM).

Tests for phytoconstituents	Test name	Result of GSME	Result of GSNH	Result of GSDM
Alkaloid	a)Wagner"s Test	+	+	+
	b)Mayer"s Test	-	+	+
Tannin	a)Ferric chloride Test	+	+	+
Phlobatannins	a) Hydrochloric acid test	+	+	-
Triterpene	a)Liebermann-Burchard"s Test	+	+	+
Flavonoids	a)Zinc-hydrochloric acid reduction test	+	+	-
	b) Lead acetate test	+	+	+
Saponins	a)Shake or foam test	+	+	-
Resins	a) Resin test with acetone	-	-	+
Glycosides	a)Sodium hydroxide reagent test	+	+	+
Cardiac glycosides	a)Keller-Killiani test	-	-	-
Anthraquinone glycoside	a)Hydroxy-anthraquinone test	-	+	+
Phenol	a)Ferric chloride test	+	+	+
Reducing sugar	a)Fehling"s test	-	-	+
Carbohydrate	a)Molisch"s test	-	-	-
Protein	a)Biuret test	-	-	+
	b)HNO_3 test	+	-	-
Fats & fixed oils	a)Fats and fixed oil test with copper sulfate solution	-	-	+
	b)Spot test	-	+	+

Note: - means absent, + means present

3. Results

3.1. Extraction yield and phytochemical screening

The yield percentage of crude methanol extracts of *G. serrulata* leaves was 5.14% and the extract was partitioned to yield n-hexane (21.62%) and dichloromethane (29.73%) fractions. The qualitative phytochemical analysis confirmed the presence of terpenoids, tannins, flavonoids, alkaloids, glycosides, saponins, phenols, proteins and carbohydrates inside the GSME sample. And the examination revealed the negative result for resins, cardiac glycosides, anthraquinone, glycosides, reducing sugars, fat and fixed oils. The fractions of the extract were found to contain the phytochemicals listed in Table 1.

3.2. Acute toxicity study

In the acute toxicity test, no mortality or visible signs of toxicity was observed in mice treated with *G. serrulata* extracts at doses ranging from 1000 to 5000 mg/kg. The mice showed no indications of behavioral or physiological changes including decreased motor activity, restlessness, convulsions, salivation, diarrhea, tear production, loss of consciousness, and alterations in skin, fur, eyes, respirations and neurological status. No abnormal behavioral patterns or clinical signs of toxicity were detected in any of the treated groups. No significant changes of body weight in mice were observed during the observation period. The result led to the conclusion that the extract demonstrated low acute toxicity as no mortality occurred at doses up to 5000 mg/kg, suggesting a relatively wide safety margin for further pharmacological investigations.

3.3. Antioxidant activity

The findings of DPPH scavenging antioxidant assay of Standard, GSME, GSNH and GSDM are detailed Table 2 and Figure1-2.

Table 2. Antioxidant potential of the investigated extracts of *Grewia serrulata* leaves fractions through DPPH scavenging assay.

Group	Equation	R^2	IC_{50} (µg/mL)
Standard (Ascorbic Acid)	y = 0.1029x + 49.66	0.76	3.34
GSME	y = 0.0979x + 48.89	0.84	11.78
GSNH	y = 0.1175x + 39.44	0.81	89.86
GSDM	y = 0.1263x + 31.59	0.74	126.48

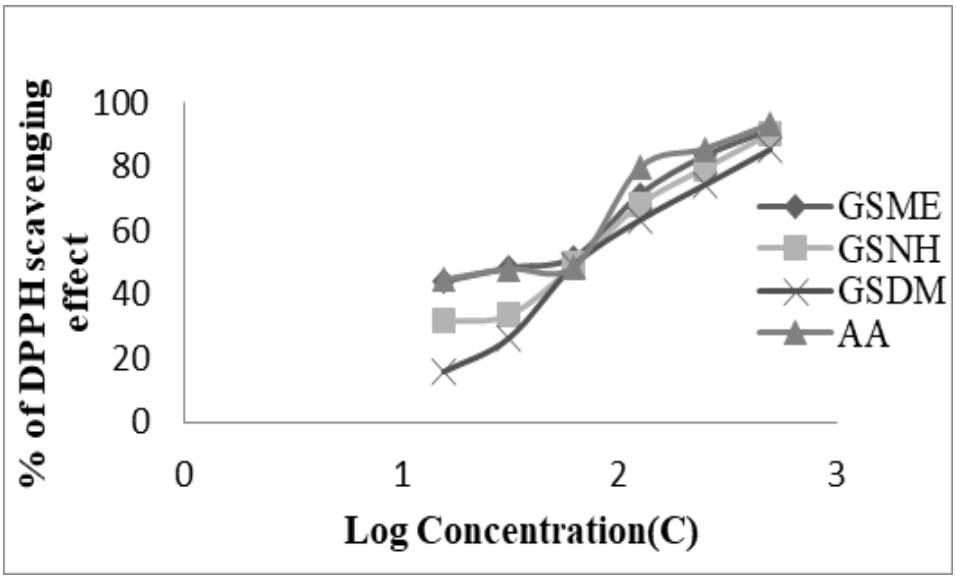

Figure 1. Graphical representation of percentage (%) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effect of different concentration (C) of methanol extract of *Grewia serrulata* and its solvent fractions.

3.4. Anti-inflammatory activity

The anti-inflammatory effect of GSME, GSNH, and GSDM on carrageenan induced paw edema in mice is shown in Table 3. The results suggest that all treatment groups (Indomethacin, GSME, GSNH and GSDM) exhibited significant (***p<0.001) reductions in paw circumference compared to control. The Standard (Indomethacin) group showed the most prominent effect (90.38% at 4th hour).

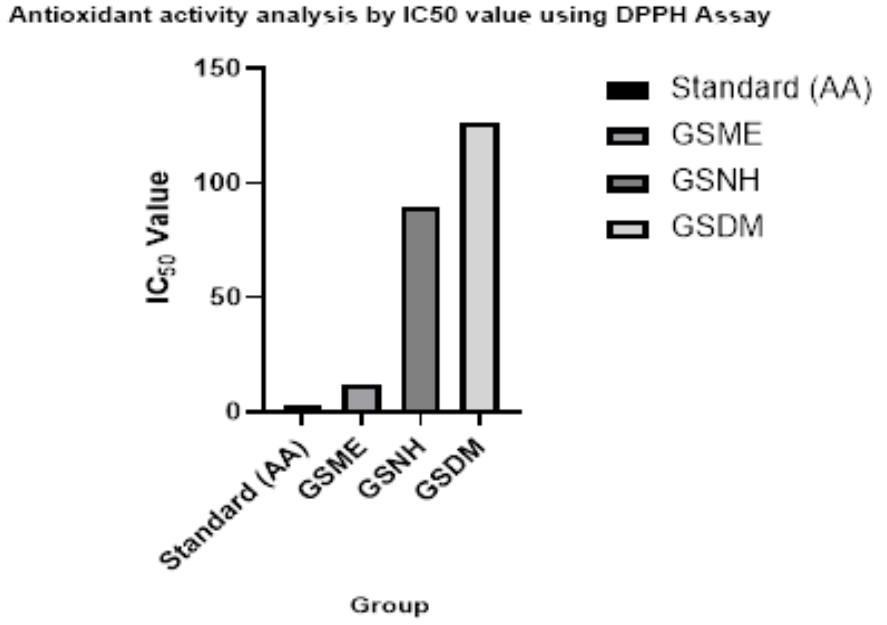

Figure 2. Effect of methanol extract of *Grewia serrulata* and its solvent fractions by comparing IC_{50} value using DPPH free radical scavenging assay; IC_{50}= half maximal inhibitory concentration.

Table 3. Effect of *Grewia serrulata* methanol extracts and its different solvent fractions on carrageenan-induced paw edema in mice.

Group	Pre-injection mean paw circumference (mm)	Post injection mean paw circumference (mm)			
		1hour	2hours	3hours	4hours
Control	9.80 ± 0.58	15.10 ± 0.51	15.00 ± 0.45	14.90 ± 0.29	15.00 ± 0.54
Standard	10.20 ± 0.37	13.40 ± 0.51*** (43.40%)	12.40 ± 0.51*** (57.69%)	11.30 ± 0.3*** (78.43%)	10.70 0 ± 0.25*** (90.38%)
GSME 200	11.80 ± 0.25	15.50 ± 0.16*** (30.19%)	14.60 ± 0.19*** (47.17%)	14.20 ± 0.25*** (54.72%)	13.40 ± 0.24*** (69.81%)
GSME 400	10.80 ± 0.37	13.80 ± 0.37*** (39.62%)	13.20 ± 0.56*** (53.85%)	12.20 ± 0.5*** (72.55%)	11.40 ± 0.24*** (88.46%)
GSNH 200	10.01 ± 0.75	14.50 ± 0.61** (16.98%)	13.40 ± 0.58*** (37.74%)	13.00 ± 0.47*** (45.28%)	12.20 ± 0.25*** (60.38%)
GSNH 400	10.00 ± 0.35	14.10 ± 0.68*** (22.46%)	13.80 ± 0.46** (28.30%)	12.90 ± 0.56*** (49.06%)	12.00 ± 0.47*** (66.03%)
GSDM 200	10.30 ± 0.39	15.10 ± 0.46** (13.56%)	14.03 ± 0.39** (34.30%)	12.80 ± 0.75*** (54.82%)	12.40 ± 0.30** (67.36%)
GSDM 400	11.20 ± 0.68	14.90 ± 0.72** (34.89%)	13.90 ± 0.87*** (40.56%)	13.20 ± 0.59** (48.39%)	11.60 ± 0.56*** (58.46%)

Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by followed by a post hoc Dunnett's "t" test $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared with control. GSME200= methanol extract at 200 mg/kg body weight; GSME 400= methanol extract at 400 mg/kg body weight; GSNH200= n-hexane soluble fraction of methanol extract at dose 200mg/kg body weight; GSNH400= n-hexane soluble fraction of methanol extract 400mg/kg body weight; GSDM400= dichloromethane soluble fraction of methanol extract 400mg/kg body weight; GSDM200= dichloromethane soluble fraction of methanol extract 200mg/kg body weight.

3.5. Antidiarrheal activity

Results regarding antidiarrheal activity of extracts of *G. serrulata* on castor oil induced diarrhea inhibition is depicted in Table 4. The results clearly shows that all treatment groups reduced diarrhea significantly compared to the control group, with varying degrees of inhibition observed at different time intervals.

4. DISCUSSION

The findings of this study demonstrate that leaves of *G. serrulata* hold significant free radical scavenging, anti-inflammatory and antidiarrheal activities, which seem being mechanistically interconnected through the modulation of oxidative stress and inflammatory mediators. Oxidative stress plays a vital role in the initiation and progression of inflammatory responses

Table 4. Effect of *Grewia serrulata* methanol extracts and its different solvent fractions on castor oil induced diarrhea in mice

Group	Mean ± SEM				
	1st hr (% of inhibition of defecation)	2nd hr (% of inhibition of defecation)	3rd hr (% of inhibition of defecation)	4th hr (% of inhibition of defecation)	Total (% of inhibition of defecation)
Control	3.40 ± 0.51	4.40 ± 0.25	3.20 ± 0.37	2.20 ± 0.58	13.20 ± 0.86
Standard	0.40 ± 0.25 88.24***	1.20 ± 0.20 72.73***	1.40 ± 0.25 56.25***	0.60 ± 0.40 72.73***	3.60 ± 0.68 72.73***
GSME 200	1.00 ± 0.32 70.59***	1.80 ± 0.20 59.09***	1.60 ± 0.40 50.00***	0.80 ± 0.37 63.64***	5.20 ± 0.37 60.61***
GSME 400	0.40 ± 0.25 88.24***	1.40 ± 0.40 45.45***	1.20 ± 0.20 62.50***	0.60 ± 0.25 72.73***	3.80 ± 0.49 71.21***
GSNH 200	1.00 ± 0.32 70.59**	2.40 ± 0.25 45.45***	1.60 ± 0.25 50.00***	0.56 ± 0.27 52.73***	5.60 ± 0.40 57.58***
GSNH 400	0.80± 0.37 76.47***	1.40 ± 0.25 68.18***	1.80 ± 0.37 43.75***	1.00 ± 0.32 54.55**	5.00 ± 0.45 62.12***
GSDM 200	1.00 ± 0.32 70.59**	1.40 ± 0.25 68.18***	1.60 ± 0.51 50.00**	1.60 ± 0.25 27.27	5.60 ± 0.51 57.57**
GSDM 400	0.80 ± 0.37 76.47**	1.60 ± 0.25 63.64***	1.60 ± 0.40 50.00**	1.20 ± 0.37 45.45	5.20 ± 0.73 60.60***

Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by followed by a post hoc Dunnett's "t" test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared with control. GSME200= methanol extract at 200 mg/kg body weight; GSME 400= methanol extract at 400 mg/kg body weight; GSNH200= n-hexane soluble fraction of methanol extract at dose 200mg/kg body weight; GSNH400= n-hexane soluble fraction of methanol extract 400mg/kg body weight; GSDM200= dichloromethane soluble fraction of methanol extract 200mg/kg body weight; GSDM400= dichloromethane soluble fraction of methanol extract 400mg/kg body weight.

by activating redox dependent signaling pathways that regulate pro-inflammatory cytokines and prostaglandin synthesis. Therefore, the observed antioxidant activity may contribute indirectly to the anti-inflammatory and antidiarrheal effects.

DPPH radical scavenging chemicals utilized for this purpose can boost antioxidant properties[7]. The current findings are consistent with earlier studies, where the ethanol extract of *G. serrulata* leaves exhibit strong free radical scavenging, as indicated by the low IC_{50} values (9.16 µg/mL) observed in DPPH assay[23]. In the present study, GSME and GSNH displayed prominent scavenging effects (IC_{50}=11.7886 µg/mL and 89.86 µg/mL respectively), while GSDM showed moderate effect (126.48 µg/mL) compared to control. The relative stronger activity of methanol extract may be attributed to its higher polarity, facilitating extraction of phenolic and flavonoid contents[39]. These phytocompounds have role in hydrogen donation and metal chelation, being involved in free radical neutralization[40]. Carrageenan-induced paw edema is a widely accepted experimental model to assess acute inflammatory responses and the efficacy of anti-inflammatory agents[36]. It occurs in two phases: an early phase mediated by histamine and serotonin, while a later phase associated with prostaglandin release. The results of this study indicated that GSME, GSNH and GSDM exhibited significant ($^{***}p<0.001$) inhibition of paw edema formation across various time points (shown in Table 3), suggesting their interference with prostaglandin-mediated pathways. These findings are in line with previous studies suggesting that *G. serrulata* extracts possess notable anti-inflammatory potential. The extracts of *G. serrulata* contain bioactive compounds like phenolic compounds, alkaloids, tannins, terpenoids that attribute to inhibiting the activity of COX enzymes or downregulate the production of inflammatory cytokines, thereby reducing the inflammatory response[41].

Similarly, the catalytic role of lipases on castor oil in the small intestine releases ricinoleic acid, which inflames and irritates the mucosa lining of digestive tract, leading to prostaglandin secretions in castor oil-induced method[42]. The effect of extracts during castor oil induced diarrheal method was also dose-dependent (shown in Table 4), where the comparison between GSME400 and Loperamide is noteworthy, suggesting its potential as a therapeutic agent for gastrointestinal conditions. This indicates that GSME may contain bioactive polar compounds capable of modulating intestinal motility and fluid secretion to a degree comparable with standard antidiarrheal drug Loperamide. While Loperamide acts as a µ-opioid receptor agonist reducing peristalsis and intestinal secretion[43], the mechanism underlying GSME's action is likely distinct and may involve phytoconstituents (flavonoids, alkaloids or tannins) exerting anti-secretory, anti-inflammatory or spasmolytic effects. GSDM and GSNH also demonstrated considerable antidiarrheal activity, although their effects were slightly less pronounced than GSME. The antidiarrheal effect of these extracts could be attributed to their ability to modulate intestinal motility and reduce inflammation in the gastrointestinal tract[16]. Flavonoids and phenolic compounds are known to modulate prostaglandin biosynthesis, providing a common pathway for the observed activities[44]. Solvent polarity significantly affects extraction efficiency and bioactivity of phytoconstituents. Polar solvents such as methanol are more effective at extracting phenolic acids, flavonoids and glycosides, which are commonly associated with antioxidant and anti-inflammatory effects[45]. Conversely, non-polar and mid-polar solvents (n-hexane, dichloromethane) may concentrate lipophilic terpenoids and sterols, which could contribute differently to anti-inflammatory activity. Such solvent-dependent variation in pharmacological activity has been observed in other plant species and supports the need for targeted phytochemical investigation[46]. However, this study did not include mechanistic assays such COX inhibition, cytokine profiling or oxidative stress biomarker analysis. Overall, these findings suggest that *G. serrulata* possesses a promising therapeutic potential, highlighting the necessity of further research to isolate and identify the specific phytoconstituents that cause these actions. Bioassay guided isolation, detailed mechanistic studies, toxicity assessment and eventually clinical validation are required to substantiate these preliminary findings and clarify their therapeutic relevance.

5. CONCLUSION

Grewia serrulata is a medicinal plant that has been used in ethno-pharmacological practices for the management of inflammatory and gastrointestinal disorders. In the present study, the methanol extract (GSME) and its solvent fractions demonstrated notable pharmacological activities. GSME exhibited strong antioxidant activity with an IC_{50} value of 11.78 µg/mL in the DPPH assay. Significant ($^{***}p<0.001$) anti-inflammatory effects were observed in the carrageenan-induced paw edema model, with 88.48% inhibition (at the 4th hour), while the extracts also showed marked antidiarrheal activity with up to 71.21% reduction in diarrheal episodes in the castor oil-induced model. The acute toxicity study indicated no mortality up to 5000 mg/kg, suggesting a favorable safety margin. These findings provide preliminary pharmacological support for the ethnomedicinal use of *G. serrulata*. However, further bioassay-guided isolation, mechanistic investigations, and clinical studies are required to identify the active constituents and confirm their therapeutic potential.

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Author contribution

Conceptualization: Nusrat Jahan; Data curation: Nusrat Jahan, Ummah Tasnim Nisat; Experimental design: Nusrat Jahan, Mohammed Kamrul Hossain; Formal analysis: Nusrat Jahan, Farjana Nawrin; Investigation: Nusrat Jahan, Ummah Tasnim Nisat, Tahiat Tasnia Tahi; Methodology: Nusrat Jahan, Tahiat Tasnia Tahi; Resource: Nusrat Jahan, Mohammed Kamrul Hossain; Software: Nusrat Jahan, Nizum Barua; Validation: Nusrat Jahan, Ummah Tasnim Nisat; Visualization: Nusrat Jahan; Writing- original draft: Nusrat Jahan, Nizum Barua; Writing- review & editing: Nusrat Jahan, Farjana Nawrin, Mohammed Kamrul Hossain.

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Conflict of interest

The authors affirm that they have no conflict of interests to this publication.

Ethics approval

All authors affirm that the guidelines outlined in "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985) were adhered to, along with relevant national laws where applicable. All experiments have been examined and received approval from the Institutional Ethical Review Committee of the University of Science and Technology Chittagong (IERC/USTC/24/007).

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