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Integrated Phytopharmacological Studies of *Wendlandia glabrata* DC.

Md. Shamiul Islam, Md. Al Amin Sikder, Sadia Tasnim Mina and Mohammad A. Rashid

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka
Dhaka-1000, Bangladesh

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Abstract

Wendlandia glabrata DC. (Family: Rubiaceae), locally known as "Bon Kafashi" in Bangladesh, is widely used as functional food in Indian subcontinent, where its tender shoots are believed to control blood sugar and reduce obesity. However, this plant has not been substantially studied in Bangladesh, and therefore it was selected for phyto-pharmacological investigations. The plant's methanolic extract, along with its Kupchan fractions, were assessed for *in-vitro* antioxidant, cytotoxic, thrombolytic and membrane stabilizing and *in vivo* analgesic, antidiarrheal and hypoglycemic properties. Four compounds, namely ursolic acid (**1**), β-sitosterol (**2**) stigmasterol (**3**) and lauric acid (**4**) were isolated and characterized from the *n*-hexane fractions by employing gel permeation chromatography and preparative TLC and NMR spectroscopy. All these compounds are first time reported from this species. The total phenolic content of the extract and its fractions was found in a range of 23.98 ± 0.65 to 80.74 ± 2.08 mg of GAE/gm of extractives. In DPPH assay, DCM fraction exhibited the highest antioxidant efficacy with an IC_{50} value of 14.29 µg/ml, in comparison to standard BHT (IC_{50}=13.56 µg/ml). The DCM solubles also showed the strongest cytotoxic activity (LC_{50} = 0.77 µg/ml) which was comparable to standard vincristine sulphate (LC_{50} = 0.58 µg/ml). Different solvent fractions exhibited thrombolytic activity ranging from 24.84% to 38.29%. Out of all, the methanolic extract showed considerable membrane stabilizing properties in hemolysis mediated by hypotonic solution and heat. The methanol extract showed weak analgesic activity as evident by acetic acid-induced writhing test. In castor oil-mediated diarrhea, the extract did not show potential antidiarrheal efficacy. The extract exhibited significant effect in the hypoglycemic test at 400 mg/kg, which decrease blood glucose levels by 24.82% ($p < 0.01$) and 27.35% ($p < 0.05$) during the 2nd and 3rd hour of study, respectively. Based on the findings, *W. glabrata* may be a viable source of potentially active secondary metabolites. Additional research should be undertaken for utilizing this plant in drug discovery and development.

Key words: *Wendlandia glabrata*, phytochemistry, pharmacological activity, cytotoxic, antioxidant, thrombolytic, membrane stabilizing, analgesic, antidiarrheal, hypoglycemic.

Introduction

Medicinal plants serve as rich reservoirs of natural compounds which can be applied in directing the development and synthesis of novel medications. People all over the world have been using a number of plants as traditional therapy for many years (Sofowora *et al.*, 2013). Information about the specialized medical uses of various herbs, shrubs and plants have been passing down from generation to generation (Khan, 2014). Since the start of the twenty-first century, researchers have presented an intriguing approach in drug discovery and development; a renewed focus on nature as a resource of prospective drug molecule (Lanzotti, 2014). Natural compounds from plant source which are believed to have considerable biological benefits includes flavonoids, polyphenols, alkaloids, organosulfur compounds, polysaccharides,

Corresponding author: Mohammad A. Rashid; E-mail: arpharm64@du.ac.bd
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glucosinolates, saponins, monoterpenes, stilbenoids, sesquiterpenes, capsinoids, capsaicinoids and essential oils (Wiseman, 2013; Xiao, 2015). Natural bioactive chemicals have been shown to be crucial in the preventive and curative aspects of various modern disease like cancer, diabetes, infectious diseases, age-related conditions, Alzheimer's disease and cardiovascular disorders according to epidemiological data-based evidence (Andrae-Marobela *et al*., 2013; Xiao and Jiang, 2015). They are also found to be effective as antibacterial, antiviral, antifungal, anti-oxidant and anti-inflammatory agents (Xiao, 2015).

The *Wendlandia* genus comprises of flowering plants under the Rubiaceae family. The Rubiaceae consists of 611 genera and over 13000 species and is one of the five major flowering plant families (Bremei, 2009). The *Wendlandia* genus is composed of 80 accepted species, commonly found from tropical and subtropical Queensland to Asia and in northeastern tropical Africa (POWO, 2025). *Wendlandia* species have numerous medicinal applications, including treating snake bites, scorpion stings, menstrual regulation, male child birth, colds and coughs, diarrhea, piles, intestinal parasites, fever, body aches, cardiovascular dysfunction, hypertension, mental disturbances, skin diseases and digestive disorders (Hossain *et al*., 2023; Maryam *et al*., 2019; Sindhe *et al*., 2015). Reported constituents include ixoside and iridoid glycosides, flavonoids, phenolic compounds, proanthocyanidins, terpenes, triterpenes, phytosterols, fatty acids, sugar alcohols, carotenoids, coumarin and ester compounds (Dinda *et al*., 2011, 2006; Farzana *et al*., 2022; Hossain *et al*., 2023). These structurally distinct phytochemicals contribute to the genus' claimed broad spectrum of pharmacological properties including antibacterial, antioxidant, analgesic, anti-asthmatic, anti-inflammatory, antidiabetic, anticancer, anthelmintic and antimutagenic activities (Farzana *et al*., 2022; Maryam *et al*., 2019; Sheikh *et al*., 2019; Sindhe *et al*., 2015).

Wendlandia glabrata DC. is a comparatively underexplored species within the genus. This is a flowering plant, usually, a shrub or small tree. The elliptic-lance-shaped leaves are smooth, hairless, and constricted into the stalk. The leaves are distantly and subtly nerved. They have greenish flowers that grow in wide, expanding panicles. Sepals lack hair. It is widely distributed in India, Borneo, Cambodia, Malaya, Sumatera, Vietnam, Myanmar including Bangladesh (Chittagong and Rangamati) (POWO, 2025; Sheikh *et al*., 2019). It is locally known as "Bon Kafashi" in Bangladesh. Traditionally, *W. glabrata* is used as ingredients of a salad called Singju which is believed to control blood sugar (Sheikh *et al*., 2015). Tender shoot and inflorescence of *W. glabrata* is used widely in dysentery and as an expectorant; it also helps to reduce obesity (Sarma *et al*., 2022; Sheikh *et al*., 2019; Yumnam and Tripathi, 2012).

Several compounds have been isolated from the previous studies on *W. glabrata.* Isolated and chemically characterized compounds from *W. glabrata* include maniposide, ixoside, cinnamtannin B-1, procyanidin A2, rutin, isoorientin-6"-O-glycoside, quercetin 3-O-β-D glucopyranoside, quercetin-3,7-O-diglucoside (Sarma *et al*., 2022; Sheikh *et al*., 2019). Procyanidin A2 isolated from *W. glabrata* acts as a potent antidiabetic agent which significantly inhibits glucose-6-phosphatase activities and downregulate mRNA level in diabetic mice and also improve glucose uptake in myoblast and hepatocytes cells (Sheikh *et al*., 2019). A fraction of *W. glabrata* enriched with novel iridoids significantly lessened blood glucose levels and exhibited amelioration in hepatic insulin resistance by modulating the AMPK/PEPCK/G6Pase signaling pathway involved in hepatic glucose metabolism (Sarma *et al*., 2022).

Biological analysis of the crude extract, different fractions, together with isolated molecules is crucial to identify the specific component that is responsible for any pharmacological activity. A comprehensive review of the available literature indicates a paucity of extensive phytochemical and pharmacological studies on Bangladeshi specimens of *W. glabrata*. Therefore, this study aimed to evaluate a broad range

of pharmacological activities of the crude methanolic extract and various fractions of *W. glabrata* aerial parts, including antioxidant, thrombolytic, membrane-stabilizing and cytotoxic potentials through *in vitro* assays, as well as peripheral analgesic, antidiarrheal and hypoglycemic activities using *in vivo* animal models. In addition, four compounds were characterized from its methanolic extract using phytochemical isolation followed by spectroscopic analysis.

Materials and Methods

General experimental procedures: Lipophilic Sephadex (LH-20) was loaded into gel permeation chromatography columns, and precoated (Keiselgel 60 PF_{254}) plates were used for TLC and PTLC (20x20 cm). The spots were observed using UV light (366 nm-UV long and 254 nm-UV short) and/or spray reagent of 1% vanillin-sulfuric acid. A Bruker Advance 400 MHz NMR spectrometer was employed to obtain the ^1H NMR spectra in deuterated chloroform ($CDCl_3$). All substances utilized for the experiments comprised of analytical quality. The reference drugs (vincristine sulphate, streptokinase, acetylsalicylic acid, diclofenac sodium, loperamide and glibenclamide) were obtained from local pharmaceutical industries.

Sample collection and preparation and extraction: Aerial parts (leaves and small stems) of *W. glabrata* were acquired from Sreemangal, Bangladesh, in June 2022 and verified by a botanist at the Bangladesh National Herbarium (DACB 83420). Samples were cleaned, chopped, shade-dried for 7 weeks with ventilation and then pulverized into a coarse powder (700 g). The powder was macerated in 2 litre methanol for 15 days with intermittent shaking, then filtrate passed through cotton and filtering paper (Whatman No. 1) and finally condensed through the use of a rotary evaporator to get methanol crude extract (ME). ME was fractionized following modified Kupchan technique (VanWagenen *et al.*, 1993) into n-hexane (HF), dichloromethane (DF), ethyl acetate (EF) and aqueous (AF) fractions, which were dried out and utilized for additional investigation.

Experimental animal: Young Swiss albino mice (male and female) were acquired from the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b). The animals were accommodated in a controlled laboratory environment (24 ± 2°C, 60-70% RH) and got uninterrupted availability of icddr,b-prepared feed and water. Before being employed in the research, these animals were housed in the same habitat for a minimum of three to four days to allow them to adjust in the surroundings. Every test procedure was carried out in compliance with regulations regarding the ethical handling of experimental animals (NIH, 2011; Zimmermann, 1983).

Total phenolic content: Total phenolic content (TPC) of *W. glabrata* extract and its partitionates was quantified by revised Folin-Ciocalteu test (Ahmed *et al.*, 2019). The extract solution (0.5 ml of a 2 mg/ml) was incorporated with 2.5 ml Folin-Ciocalteu reagent (1:10 diluted) and 2.0 ml 7.5% (w/v) Na_2CO_3, and subsequently underwent incubation for 20 min at standard room temperature. Following incubation, the extract's total phenolic content was assessed by obtaining the absorbance with UV-Vis spectrophotometry (760 nm). TPC was estimated employing a standard curve of gallic acid and presented as mg GAE/g extract.

DPPH assay: Free radical scavenging efficacy of *W. glabrata* extract was examined by employing the DPPH assay where 2.0 ml methanolic extract of various concentrations was incorporated with 3.0 ml methanolic DPPH (20 µg/ml), incubated for 30 minutes and its absorbance was taken at 517 nm by UV-Vis spectrophotometry. The antioxidant properties were assessed through comparing IC_{50} values of methanol extract and its various partitionates to that of BHT (Butylated hydroxy toluene) (Brand-Williams *et al.*, 1995; Kedare and Singh, 2011), as shown below.

$$\% \text{ inhibition} = (1 - Abs_{sample}/Abs_{blank}) \times 100 \quad \text{..... (1)}$$

Cytotoxicity test by brine shrimp lethality assay: The capability of the methanol extract and various

partitionates of *W. glabrata* to cause cellular death was examined *in vitro* by brine shrimp lethality assay (Meyer *et al*., 1982; Sarah *et al*., 2017). Simulated sea water was prepared to effectively hatch brine shrimp eggs for obtaining nauplii. To get the test sample to the appropriate concentration, a calculated amount of dimethyl sulphoxide (DMSO) was added. Vincristine sulfate (VS) was employed as standard. Visual examination was used to count the nauplii, which were then transferred into vials with 5 ml of simulated sea water. A micropipette was used to put varying concentrations of the sample preparations into pre-labeled vials. Following a 24 hour period, the total count of survived nauplii was documented.

Evaluation of thrombolytic activity: All the samples were examined for thrombolytic activity using streptokinase (SK) as a standard following established method (Prasad *et al*., 2006). A 30000 IU of SK was employed as standard, whereas distilled water acted as non-thrombolytic control.

Evaluation of membrane stabilizing activity: The test samples were exposed to hypotonic solution and heat-mediated hemolysis of human blood to investigate their membrane-stabilizing action (Islam *et al*., 2019).

Acetic acid-induced writhing test: The peripheral analgesic efficacy of *W. glabrata* was examined through acetic acid-mediated writhing test accordance with the procedure of Mondal *et al*. (2014) with slight modifications. Mice were assigned at random to four groups (n=6): control (distilled water p.o.), standard (diclofenac Na, 5 mg/kg p.o.), and test groups (ME at 200 and 400 mg/kg p.o.). Thirty minutes subsequent to the initial treatment, a dose of 0.6% acetic acid (10 ml/kg, i.p.) was delivered to the mice. After a latency period of 5 minutes, the acid-induced writhing, which included belly constrictions and hind limb stretching, were recorded for 30 minutes. The % analgesic effect was estimated according to the following:

$$\% \text{ analgesic effect} = 100 \times \frac{N - N_1}{N}$$

Here, N and N_1 represent mean number of writhing in control and test groups, respectively

Antidiarrheal activity test: Castor oil-mediated diarrheal method in mice was utilized to evaluate the antidiarrheal properties of the samples, as stated by Shoba and Thomas (2001). Here, mice (n=6/group) were randomized into four different groups: tests (ME at 200 and 400 mg/kg p.o.)., negative (1% tween 80 in water) and positive control (loperamide, 50 mg/kg p.o.). Diarrhea was triggered in all groups with 1 ml per oral administration of castor oil. Fecal output was monitored hourly for four-hour, and % reduction was measured as per the following equation.

$$\% \text{ reduction} = \frac{Mo - M}{Mo} \times 100$$

Here, Mo and M represent average number of feces in control and test groups, respectively.

Hypoglycemic activity test: The hypoglycemic efficacy of *W. glabrata* extract was evaluated using the approach reported in Islam *et al*. 2019. The animals were given oral doses of the samples (200 and 400 mg/kg) and the reference 10 mg/kg dose of glibenclamide. All groups (n=6) received a 10% solution of glucose at 2 g/kg dose 1 hour following the treatment. Blood was obtained from a mouse's tail vein and used to measure the blood glucose level 1, 2 and 3 hours after the glucose was administered. The hypoglycemic efficacy was determined by a % reduction in blood glucose.

$$\% \text{ Reduction of blood glucose} = [(G_c - G)/G_c] \times 100$$

Where, G and G_c represent mean blood glucose level in test and control, respectively.

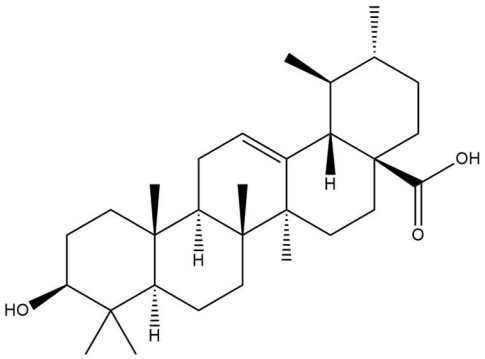
Statistical analysis: Microsoft Excel (version 2019) was employed for analyzing experimental data, with results represented as mean ± SEM. Data were evaluated against the control and subjected to statistical analysis ($^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$).

Results and Discussion

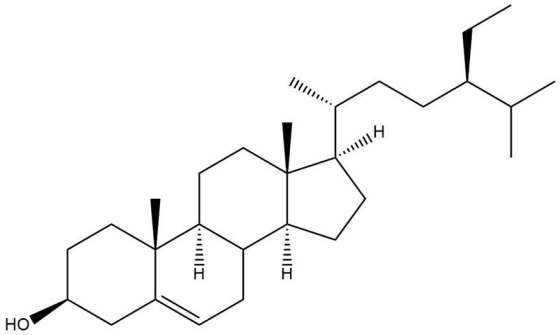
Characterization of compounds from *W. glabrata*

The n-hexane soluble fraction of the methanol extract of *W. glabrata* aerial parts was sequentially separated and purified, yielding a total of four compounds (**1-4**). These substances were

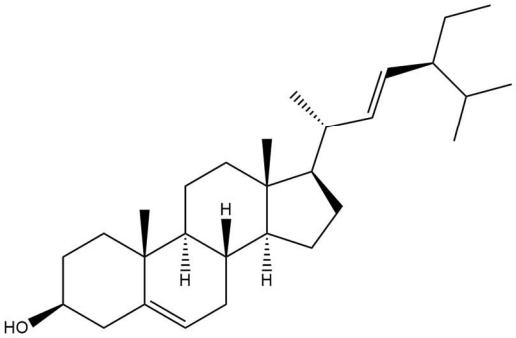
characterized as ursolic acid (**1**) (Migas *et al*., 2005), ß-sitosterol (**2**) (Chaturvedula and Prakash, 2012), stigmasterol (**3**) (Cayme and Ragasa, 2004) and lauric acid (**4**) (Priyadi and Saifudin, 2023) (Figure **1**). Extensive ^{1}H NMR spectral analysis was employed to confirm the structure of all of the purified compounds (data not shown), which were then substantiated by comparing with relevant published spectral data.



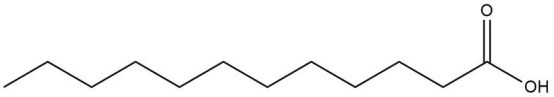
Ursolic acid (1)



ß-Sitosterol (2)



Stigmasterol (3)



Lauric acid (4)

Figure 1. Chemical structures of isolated compounds from *W. glabrata* aerial parts.

Total phenolic content: The TPC of *W. glabrata* was quantified by employing the gallic acid calibration curve (Y=0.0185x + 0.0197, R^2 = 0.9948). The methanolic extract possessed the highest phenolic content (80.74 ± 2.08 mg GAE/g extract), whereas the hexane fraction exhibited the lowest (23.98 ± 0.65 mg GAE/g extract) (Figure **2**).

The availability of phenolic substances including phenolic acids, flavonoids and phenolic diterpenes significantly enhances the antioxidant efficacy of various plant extracts (Sun and Shahrajabian, 2023). The anti-oxidant effects of phenolic compounds, which are mediated by a redox reaction, facilitate free radical absorption and neutralization, quenching of singlet and triplet oxygen along with peroxide breakdown (Pietta *et al*., 1998). The substantial phenolic content of *W. glabrata*'s methanol extract implied the plant's potential as a natural antioxidant.

DPPH assay: In DPPH assay with an IC_{50} value of 14.29 µg/ml, the DCM fraction (DF) exerted the strongest antioxidant activity among the extractives (Figures **3-4**), followed by methanolic extract (ME) (IC_{50} =15.89 µg/ml), whereas BHT, as standard, had a similar IC_{50} of 13.56 µg/ml.

The main components that were discovered to contribute to the antioxidant effects exerted by plant extracts were flavonoids, phenylpropanoids and phenolic acids. The scavenging capacity of these

molecules was attributed to their capacity to neutralize the free radicals as well as stabilize the resultant deactivated molecules (Kedare and Singh, 2011).

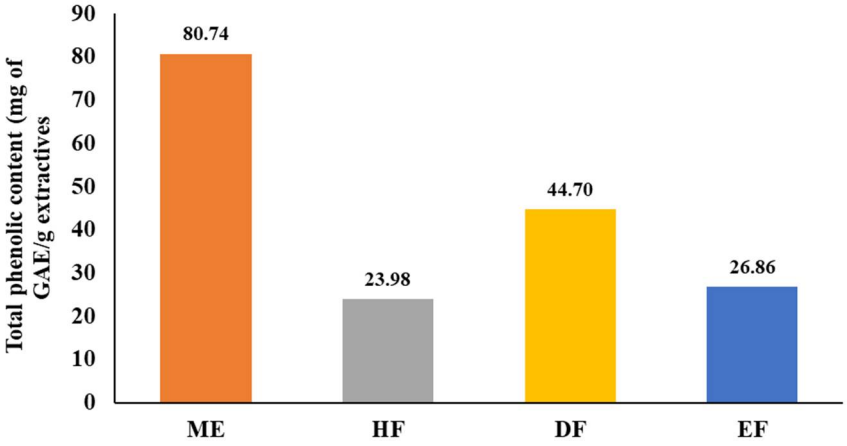


Figure 2. TPC values of *W. glabrata* extract and its fractions.

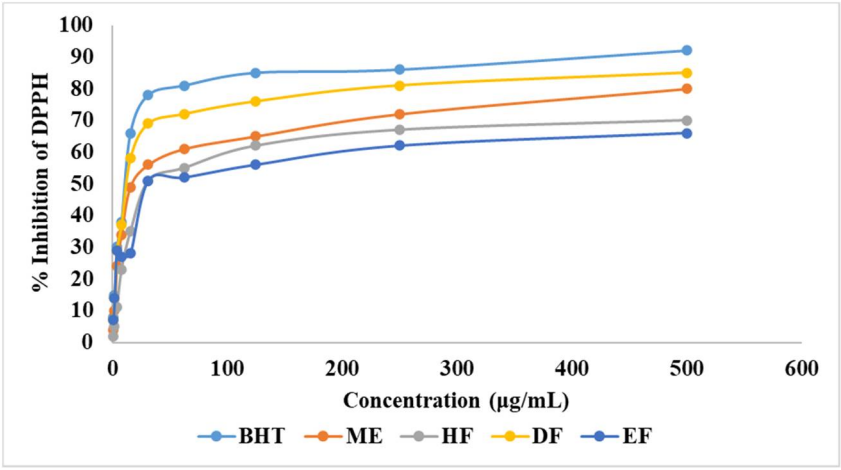


Figure 3. Percentage (%) inhibition of DPPH exerted by the standards and different fractions of *W. glabrata.*

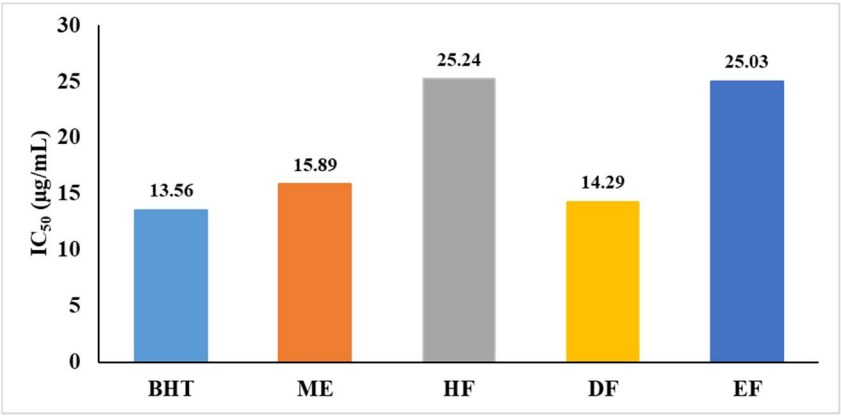


Figure 4. IC_{50} values of BHT (standard), methanolic extracts and different fractions of *W. glabrata.*

Cytotoxicity test by brine shrimp lethality assay: In cytotoxicity activity test, dose dependent cytotoxicity against brine shrimp nauplii was detected throughout the samples tested. Among the extract and the fractionates, the DCM fraction of the methanolic extract exerted the most powerful cytotoxic property (LC_{50}=0.77 µg/ml), in comparison with standard vincristine sulfate (LC_{50} = 0.58 µg/ml) (Figure **5**).

Cytotoxicity of any substance can be linked to its capability to facilitate cell death either by intracellular signaling activating programmed cell death or by disruption of cellular integrity (Kari *et al*., 2022). Thus, the capacity of extracts of plants to cause cellular death was examined *in vitro* with the help of brine shrimp lethality bioassay, which was first designed and reported by Meyer and co-workers and then modified thereafter (Meyer *et al*., 1982; Sarah *et al*., 2017). When compared to vincristine sulfate, the methanolic extract and the various partitionates of *W. glabrata*, especially its dichloromethane soluble fraction (LC_{50} = 0.77 µg/ml), demonstrated potent cytotoxic activity, suggesting the plant's potential in antitumor and anticancer drug research.

Evaluation of thrombolytic activity: In this test, the dichloromethane and ethyl acetate soluble fractions demonstrated potential thrombolytic properties of 38.29% and 32.64%, respectively, while standard streptokinase (SK) exhibited 70.65% thrombolytic activity (Figure **6**).

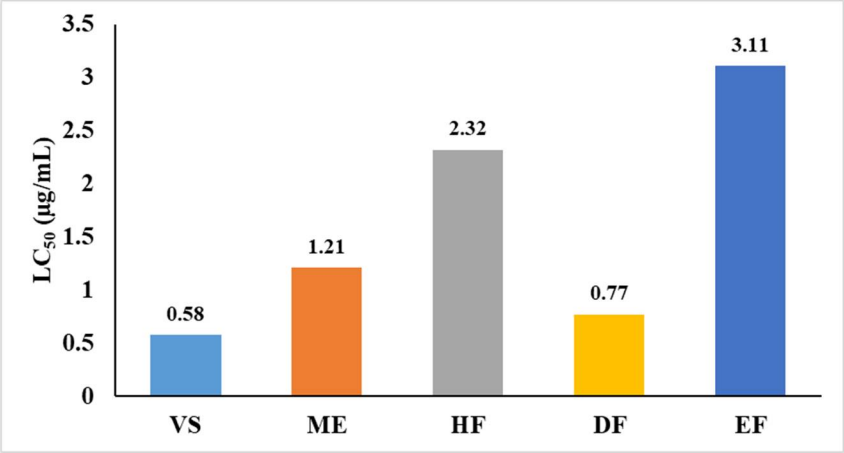


Figure 5. LC_{50} values of vincristine, methanolic extract and fractions of *W. glabrata* on the mortality of *shrimp nauplii*.

Thrombolytic medications such as streptokinase, urokinase, tissue plasminogen activator (t-PA) and others are applied widely for the management of a variety of vascular diseases like cerebral venous sinus thrombosis (Zuo *et al*., 2025). Thus, the potential of plant extracts to cause lysis of clots attracted interest within the scientific community. Among various assay methods devised, the *in vitro* approach by using streptokinase as the reference demonstrated higher constancy in test outcomes (Prasad *et al*., 2006). The dichloromethane soluble fractions in this study showed an effective thrombolytic activity of 38.29%; nevertheless, additional investigations are required to identify and characterize the pertinent phytocompounds.

Evaluation of membrane stabilizing activity: The membrane stabilizing assay measures a plant extract's capacity to prevent RBC membranes from lysis in adverse circumstances like hypotonic solution, heat, etc. in order to analyze the extract's *in vitro* anti-inflammatory efficacy (Mina *et al*., 2025). Acetylsalicylic acid, at 2.0 mg/ml, as standard, showed 79% and 73.71% prevention of hypotonic solution-induced and heat-induced hemolysis of RBC

membrane, respectively. However, ME and different fractions of *W. glabrata* exhibited minimal level of activity (Figure **7**) in both conditions, indicating limited therapeutic potential in this regard.

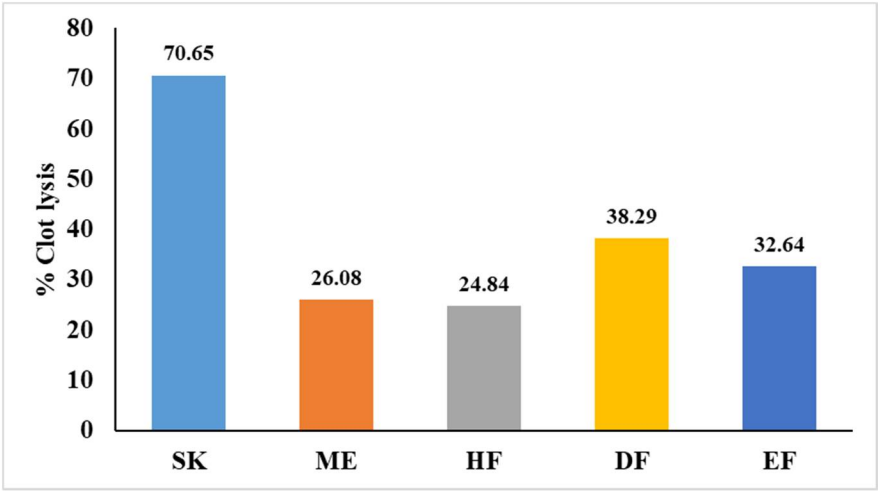


Figure 6. Thrombolytic activities of streptokinase, methanolic extract and fractions of *W. glabrata*

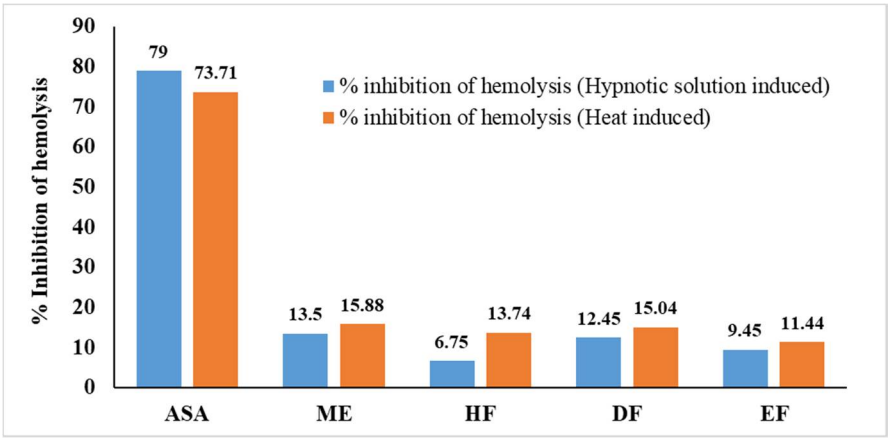


Figure7. Membrane stabilizing activity of acetylsalicylic acid, methanolic extract and fractions of *W. glabrata.*

Membrane stabilization of both erythrocytes and lysosomes represents a key mechanism for mitigating inflammation by blocking the secretion of lysosomal enzymes and other mediators of inflammation (Aidoo *et al*., 2021). In the present study, the *in vitro* membrane-stabilizing potency of *W. glabrata* extracts on erythrocytes relative to acetylsalicylic acid standard was evaluated in order to estimate their possible lysosomal membrane protection. The results implied that extracts of *W. glabrata* demonstrated weak membrane-stabilizing properties.

Acetic acid-induced writhing test: In acetic acid-mediated writhing test, *W. glabrata* extract showed minimal peripheral analgesic activity with respect to the standard, diclofenac Na (65.48%, $p < 0.001$) (Table **1**). However, at 400 mg/kg dose, ME inhibited the writhing by 11.90% compared to control.

The acetic acid-mediated writhing activity in an experimental model of mice has been utilized for assessing the peripheral analgesic properties of various samples (Gawade, 2012). Acetic acid is given intraperitoneally which generates nociception resulting in the mouse squirm periodically in pain.

The standard diclofenac and other analgesics reduce the number of writhing by raising the threshold level of nociception and peripherally inhibiting the release of pain modulators including PGE2 and PGF2a type prostaglandins (Mondal *et al.*, 2014). In the current investigation, the higher dose (400 mg/kg) of extract demonstrated minimal analgesic efficacy (11.90%) relative to the control group, suggesting limited potential of the plant extractives as a peripheral analgesic agent.

Castor oil induced antidiarrheal activity: The effects of *W. glabrata* extract on castor oil-mediated diarrhea was displayed in table **2**. Loperamide, as positive control, reduced the diarrheal condition. But ME extract of *W. glabrata* at the gradient 200 and 400 mg/kg doses did not exhibit any significant modifications in the parameters. The results indicated that this plant's methanolic leaf and stem extract did not show promising antidiarrheal properties.

Table 1. Effect of *W. glabrata* extracts on acetic acid-induced writhing.

Sample (mg/kg)	Writhing count within 30 minutes (Mean ± SEM)	% Inhibition
Control	21 ± 0.92	-
Standard (Diclofenac sodium 5 mg)	7.25 ± 0.41***	65.48
ME-200 mg	19 ± 0.63	9.52
ME-400 mg	18.5 ± 0.48	11.90

*p < 0.05, **p < 0.01, ***p < 0.001 against control

Table 2. Anti-diarrheal activity of *W. glabrata.*

Sample (mg/kg)	Total fecal count (Mean ± SEM)			
	1 h	2 h	3 h	4 h
Control	2.75 ± 0.12	5 ±1.34	10.5 ± 2.71	12.75 ± 5.14
Loperamide	0.5 ± 0.02** (81.82)	0.75 ± 0.31*** (85.00)	1.25 ± 0.05*** (88.10)	1.25 ± 0.03*** (90.20)
ME-200	2.5 ± 0.06 (9.09)	4.75 ± 0.18 (5.00)	9.75 ± 0.14 (7.14)	12.25 ± 0.04 (3.92)
ME-400	2.25 ± 0.05 (18.18)	4.20 ± 0.17 (16.00)	9.25 ± 0.06 (11.90)	11.90 ± 0.09 (6.67)

*p < 0.05, **p < 0.01, ***p < 0.001 against control; Parentheses represent the percent decrease in fecal count relative to control

The extract's anti-diarrheal efficacy was investigated utilizing castor oil-triggered diarrheal model in mice. Every animal was orally administered 1 ml of castor oil, after which the quantity of feces each mouse produced was noted and efficacy was assessed through comparing with the control (Shoba and Thomas, 2001). In this study, the results obtained did not appear significant when compared to the control, thus, implying a lack of antidiarrheal potential of the aerial parts of this plant. However, future studies might be redirected by evaluating the antidiarrheal potential of the tender shoot and inflorescence of *W. glabrata*, as these parts are used widely in dysentery (Yumnam and Tripathi, 2012).

Hypoglycemic activity test: At 400 mg/kg, the methanolic extract of *W. glabrata* showed significant hypoglycemic activity in mice (Table **3**), lowering

blood glucose levels by 24.82% ($p < 0.01$) and 27.35% ($p < 0.05$) at 2nd and 3rd hour, respectively, compared to the control. The standard drug, glibenclamide, reduced blood glucose by 40.54% ($p < 0.01$) and 62.96% ($p < 0.001$) during respective time points. These results indicate moderate hypoglycemic activity of the extract.

Table 3. Hypoglycemic activity of glibenclamide and different samples of *W. glabrata.*

Sample code and dose (mg/kg)	Blood glucose level (Mean ± SEM)		
	1 h	2 h	3 h
Control	11.2 ± 1.26	8.78 ± 0.68	7.02 ± 0.41
Glibenclamide	8.28 ± 0.72 (26.07)	5.22 ± 0.61** (40.54)	2.6 ± 0.24*** (62.96)
ME-200	9.9 ± 1.08 (11.61)	6.8 ± 0.52 (22.55)	5.28 ± 0.62 (24.79)
ME-400	9.45 ± 0.82 (15.63)	6.6 ± 0.43** (24.82)	5.1 ± 0.51* (27.35)

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ against control; Parentheses represent the percent decrease in blood glucose level relative to the control

Previous studies on *W. glabrata* demonstrated the isolation of a bioactive compound, Procyanidin A2, which exerted potent anti-diabetic activity (Sheikh *et al*., 2019). Another study revealed that the iridoid enriched fraction of this plant possessed significant hypoglycemic activity along with amelioration of resistance of hepatic insulin by modulating the AMPK/PEPCK/G6Pase signaling pathway involved in hepatic glucose metabolism (Sarma *et al*., 2022). The findings of current research aligned with the outcomes obtained from previous investigations and supported the traditional usage of *W. glabrata* for controlling blood sugar (Sheikh *et al*., 2015). However, further investigation is required to extensively isolate bioactive compounds that could be developed into therapeutic lead molecules for the management of diabetic conditions.

Conclusion

Four compounds were isolated, purified and structurally characterized from *n*-hexane fraction of *W. glabrata* methanolic extract. The pentacyclic triterpenoid ursolic acid was first obtained from this species, along with isolation of the steroids β-sitosterol and stigmasterol, and the fatty acid lauric acid. The methanolic extract alongside its subsequent *n*-hexane, DCM and ethyl acetate partitionates underwent evaluation for a variety of biological activities. The extracts displayed moderate thrombolytic and hypoglycemic and strong antioxidant and cytotoxic properties. Investigations into the fractions revealed that the *n*-hexane and dichloromethane soluble fractions are mainly responsible for these activities. In summary, *W. glabrata* may be regarded a promising reservoir with potentially active secondary metabolic compounds and further studies should be directed to utilize this plant in drug discovery and development.

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Conflict of interest

There are no conflicts of interest regarding publishing this work.

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