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Investigation of phytochemical and pharmacological assessment of ethanol extract of *Stenochlaena palustris*- an edible fern of Sundarbans

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Abstract

Stenochlaena palustris is a widely found fern in mangrove forests and other areas of different countries over the world. This plant has many uses in traditional medicine of many countries. This study was conducted on its leaves extract to investigate its different phytochemical and pharmacological properties based on its local medicinal uses. Phytochemical screening indicated the presence of different secondary metabolites in this plant. On silica coated TLC plates, the extract exhibited the presence of different antioxidative compounds. Total phenolic, flavonoid and tannin contents were determined (97 mg GAE/g, 90 mg QE/g and 23 mg GAE/g, respectively). This extract also scavenged DPPH, hydrogen peroxide and superoxide radicals ($SC_{50} = 80, 158$ and $236 \mu\text{g/ml}$, respectively). It also showed ferric ion reducing capability ($RC_{50} = 166 \mu\text{g/ml}$). This plant showed significant antihyperglycemic, peripheral analgesic (acetic acid induced), anti-inflammatory (formaldehyde induced) and laxative activities in mice. This plant also showed little cytotoxic activity in brine shrimp lethality bioassay. These results are also compared with respective standard drugs and our findings justified its traditional usage.

Keywords: *Stenochlaena palustris*, phytochemical, antioxidant, pharmacological activity

Introduction

Stenochlaena palustris locally named as Dhekilata is a green, climbing vegetable fern (Family-Blechnaceae) with long-creeping rhizome which are 0.5-1.0 cm in diameter (Figure 1). This adaptable species is normally found at hot springs edges, mangroves and fresh water at forest margins. Furthermore, this fern is also found growing on large trees. It is widely distributed throughout Malaysia, India, Myanmar, Yunnan in China, Laos, Thailand, Vietnam, India, Solomon Islands, south and northern Australia, islands of Fiji, Samoa and Tonga ^[1].

S. palustris have 2 types of fronds. Fertile fronds have thin, long pinnae that bear spores, and are seasonal and inedible. The young, sterile fronds of the fern have a crispy texture and are usually cooked with shrimp paste into a vegetable dish in Malaysia, Indonesia and Thailand while in Sumatra this plant is used as mild laxative. In Nicobar Islands and the central region of Papua New Guinea, the tender leaves of *S. palustris* are used as a contraceptive. In Malaysia and Indonesia, the fern is made into paste and applied on the ulcers, wound injuries and bacterial infected skin ^[1]. They are also used traditionally to treat fever, diarrhea, skin diseases, cutaneous disorders, and gastric ulcers ^[2]. Extract of *S. palustris* leaves is potent antioxidant, shows radical scavenging properties ^[3].

S. palustris is a good source of phosphorus and potassium. Moreover, its nutritional content is comparable or superior to some common leafy and fruit vegetables ^[4]. Five new O-acylated flavonol glycosides, stenopalustrosides A-E have been isolated from *S. palustris* leaves. Besides these, some other known compounds like kaempferol 3-O-(3''-O-E-p-coumaroyl)-(6'' - O-E-feruloyl)- β -D-glucopyranoside, kaempferol 3-O-(3''-6''-di-O-E-p-coumaroyl)- β -D-glucopyranoside, kaempferol 3-O-(3''-O-E-p-coumaroyl)- β -D-glucopyranoside, kaempferol 3-O-(6''-O-E-p-coumaroyl)- β -D-glucopyranoside and kaempferol 3-O- β -D-glucopyranoside have been also isolated from this plant. From GC-MS spectrum analysis, it also exhibits the presence of stigmaterol, stigmasta-dien-5.22-3-ol, acetate-(3 β), Ethyl linoleate, and Stigmast-

5- en-3-ol, oleic acid [3,5].

These compounds have many types of reported pharmacological activities.

Materials and Methods

Collection and identification of plant specimen

S. palustris leaves were collected from Dhansagor area of Sundarbans, Bangladesh in December, 2016. Plant samples were identified by the plant experts of Bangladesh National Herbarium, Mirpur, Dhaka and an accession no. was provided (DACB- 43822). After plant sample collection, all types of admixtures and unwanted substances were separated out and the pure leaves were kept in shade drying for 50 days. Then those were grinded to have fine powder cold extraction of 430 gm powder was carried out with 96% ethanol to have a gummy extract (Yield: 2.62%). This crude extract was used to conduct all of the experiments presented here.

Chemicals

To conduct the analytical experiments laboratory grade reagents were used such as acetic acid (Merck, Germany), formaldehyde (Merck, Germany), 2,2-diphenyl-1-picryl hydrazyl (DPPH) (Sigma, USA), FC reagent (Merck, India), Na₂CO₃ (Loba, India), NaNO₂ (Loba, India), AlCl₃ (Loba, India), NaOH (Loba, India), H₂O₂ (Merck, Germany), TBA (Sigma, USA), PMS (Sigma, USA), ascorbic acid (Merck, Germany) and NBT (Sigma, USA). Standard drugs such as Diclofenac Sodium and Glibenclamide were purchased from Square Pharmaceuticals Limited (Bangladesh) and Ibuprofen, Glucose powder, Bicacodyl were purchased from ACI Laboratories (Bangladesh), GSK Bangladesh Limited (Bangladesh) and Opsonin Pharmaceuticals Limited (Bangladesh), respectively.

Animals

For the assessment of the pharmacological tests, 4-5 weeks aged Swiss-albino mice (*Mus musculus*) having weights of 20–25 gm were bought from the Animal house, Jahangirnagar University, Bangladesh. For adaptation, these mice were placed in optimum environmental condition for 3 weeks in the Animal house of Khulna University, Bangladesh. All of the experimental procedures were performed in noiseless condition in accordance with the ethical permission of Animal Ethics Committee (AEC), Khulna University, Khulna-9208, Bangladesh [Ref: KUAEC-2020/09/19].

Phytochemical screening

A qualitative phytochemical investigation of the extract of *S. palustris* was performed according to the method of Ayoola, *et al.*, 2008 in order to determine the presence or absence of different secondary metabolites such as reducing sugars, polyphenols, flavonoids, glycosides, alkaloids, terpenoids, saponins, tannins etc [6].

In vitro qualitative antioxidant assay

This experiment was performed according to the method of Sadhu *et al.*, 2003 [7]. Crude *S. palustris* extract was diluted in methanol and then it was spotted on 3 pieces of pre-coated silica gel with Thin Layer Chromatography (TLC) plates with capillary tube. Then, these 3 plates were placed in polar, medium polar and non-polar solvent systems and chromatogram was let to be developed. After developing, the plates were dried and kept under UV light at both 254 nm and 366 nm to observe the UV active and fluorescent compounds. Finally, 0.02% DPPH solution was sprayed on the plates to

check the existence of yellow spots that indicate the presence of antioxidant compounds.

In vitro quantitative antioxidant assay

1. Radical scavenging assay

a. DPPH free radical scavenging assay

DPPH is a very common oxidant molecule having stable free radicals. Free radicals of DPPH were scavenged according to Goyal *et al.*, 2010 with slight modifications [8]. Solution of *S. palustris* extract of various concentrations (1-4096 µg/mL) were properly mixed with 0.007886% methanol solution of DPPH. Using Thermo Scientific Multiskan Ex microplate photometer, the absorbance was taken at 517 nm. From the curve of log concentration versus percent inhibition, free radical scavenging activity was calculated at expressed in SC₅₀ (µg/ml, concentration of sample required to scavenge 50% of DPPH free radical formation).

b. Hydrogen peroxide radical scavenging assay

Hydrogen peroxide is an oxidizing agent that dissociates to Hydrogen (H⁺) and Hydroxyl (OH⁻) radicals [9]. The ability of the *S. palustris* extract to scavenge hydrogen peroxide radical was determined according to Goyal *et al.*, 2010 with slight modifications [8]. An aliquot of hydrogen peroxide (40 mM) was added with various concentrations (6.25-800 µg/mL) of plant extract solution. After incubating for 10 minutes, then absorbance was measured at 230 nm. From the curve of log concentration versus percent inhibition, Hydroxyl and Hydrogen radical scavenging activity was calculated at expressed in SC₅₀ (µg/ml, concentration of sample required to scavenge 50% of radical formation).

c. Superoxide radical scavenging assay

When PMS couples with NADH, it yields Superoxide anion (O²⁻). Superoxide radical scavenging of *S. palustris* extract was performed according to the method described by Debnath *et al.*, 2020 [10]. In this assay, *S. palustris* extract solutions of different concentrations (6.25-800 µg/mL) were prepared. NBT (312 µM), NADH (936 µM) and PMS (120 µM) were prepared to create superoxide radical. Then superoxide was added into solutions of different concentrations (6.25-800 µg/mL) of *S. palustris* extract. After incubating for 5 minutes, absorbance was taken at 560 nm. From the curve of log concentration versus percent inhibition, superoxide radical scavenging activity was calculated at expressed in SC₅₀ (µg/ml, concentration of sample required to scavenge 50% of superoxide radical formation).

2. Reducing power assay

Due to the capability to act as reducing agent, antioxidants molecules can convert ferric iron to ferrous iron. The reducing ability of *S. palustris* extract was performed according to the method described in Debnath *et al.*, 2020 [10]. Solution of *S. palustris* extract of various concentrations (6.25-800 µg/mL) were properly mixed with 0.2 M phosphate buffer and 1% potassium ferricyanide and incubated at 50°C. After that of 10% trichloroacetic acid (TCA), H₂O, 0.1% FeCl₃ were mixed with each concentration. Finally, absorbance was taken at 700 nm and percentage of reduction was calculated from the calibration curve and was expressed as RC₅₀ (µg/ml, concentration of sample required to reduce 50% Fe³⁺).

3. Determination of total content of secondary metabolites

a. Total phenolic content (TPC) assay

Total content of phenolic compounds in *S. palustris* extract

was determined using Folin Ciocalteu reagent where analytical grade gallic acid was used as the standard [10]. TPC was expressed as mg of gallic acid equivalent per gram of dry extract using the calibration curve.

b. Total flavonoid content (TFC) assay

Total flavonoid content in the *S. palustris* extract was determined using Aluminum Chloride colorimetric assay [10]. TFC was expressed as mg of quercetin equivalent per gram of dry extract using the calibration curve.

c. Total tannin content (TTC) assay

Total tannin content in the *S. palustris* extract was determined using the Folin Ciocalteu reagents [10]. TTC was expressed as mg of gallic acid equivalent per gram of dry extract using the calibration curve.

Evaluation of antihyperglycemic activity

Oral glucose tolerance test is a test that addresses the time dependent clearance of exogenous glucose from blood. For the *S. palustris* extract, oral glucose tolerance was screened according to the method described by Saha *et al.*, 2021 where Glibenclamide at 5 mg/kg was administered as standard drug [11]. At first, *S. palustris* extract at 250 mg/kg and 500 mg/kg body weight doses and glibenclamide solution were administered orally mice group (each group consists of 5 mice). After 30 minutes, glucose solution at 2 gm/kg body weight was administered orally and from then glucose level (mmol/L) of blood of each mouse were measured at the 0, 30, 60, 90, 120 and 150 minutes using a glucometer.

Assessment of analgesic activity

Acetic acid-induced writhing method is a common method for the assessment of peripheral analgesic activity in animal model. Analgesic activity of *S. palustris* extract was evaluated by acetic acid induced writhing method [10]. *S. palustris* extract was given at doses of 250 mg/kg and 500 mg/kg body weight. Diclofenac sodium was used as standard drug at the dose of 25 mg/kg body weight. After oral administration of *S. palustris* extract and Diclofenac Sodium to each mice group (each group consists of 5 mice), intraperitoneal administration of 0.7% acetic acid induced writhing (constriction of abdomen, turning of trunk and extension of hind legs). After 15 minutes later for each mouse, no. of writhing was counted up to 5 minutes. Percent inhibition of writhing in comparison to the control group was taken as an index of analgesia and was calculated by the following equation:

$$\text{Inhibition of writhing (\%)} = [(Wc - Wt) \times 100] / Wc$$

where,

Wc = average number of writhing reflexes in control group

Wt = average number of writhing reflexes in test group

Evaluation of anti-inflammatory activity

Inflammation is the cellular response mediated by cytokines, histamine, bradykinin, prostaglandins, leukotrienes [12]. Anti-inflammatory activity of *S. palustris* extract was evaluated by the formaldehyde induced paw edema model in mice [10]. Ibuprofen at oral dose of 100 mg/kg was used as standard drug. *S. palustris* extract (at 250 mg/kg and 500 mg/kg body weight) and Ibuprofen solutions were administered orally in each mice group (each group consists of 5 mice). 30 minutes later, linear circumference of the right hind paw was measured by a slide calipers. Then 0.1 mL 0.2% formaldehyde solution was injected into the right hind paw of

each mouse. After 1 hr, 2 hr, 3 hr and 4 hr interval, the linear circumference of the injected paw was measured and % inhibition of inflammation of paw edema was calculated as: % Inhibition of inflammation = 100% - [(I_s/I_c) x 100] where,

I_s = inflammation of the test group

I_c = inflammation of the control group

Evaluation of laxative activity

Laxatives are widely used drugs to treat hard stool by softening it with increasing water content [13]. The ability of *S. palustris* extract as laxative was evaluated according to the method described by Saha *et al.*, 2021 [11]. Bisacodyl at oral dose of 10 mg/kg body weight was used as standard drug. All of the mice were kept fasting for 10 hours before starting the experiment. *S. palustris* extract (at 250 mg/kg and 500 mg/kg body weight) and Bisacodyl solution were orally administered to the mice of each group (consisting of 6 mice) and they were kept in metabolic cages for next 16 hours. After that period, the stool excreted by mice were collected & weighed. This experiment was performed in duplicate.

Brine Shrimp lethality bioassay

In order to evaluate the cytotoxic nature of any component, Brine shrimp lethality bioassay is a very common preliminary assay. Brine shrimp lethality bioassay of *S. palustris* extract on *Artemia salina* nauplii was performed according to the method described by Debnath *et al.*, 2020 [10]. Vincristine sulphate was used as standard drug. The eggs of *A. salina* were hatched in order to have live nauplii. Solution of different concentrations of *S. palustris* extract (5-640 µg/mL) and Vincristine sulphate (0.1562-10 µg/mL) were prepared by serial dilution. 10 live nauplii of *A. salina* were put in each concentration of plant extract and standard and were kept for 24 hours. After day 1, live nauplii were counted. Then percent mortality was calculated to determine the LC₅₀ (concentration needed to kill 50% live nauplii) value according to the following equation:

$$\% \text{ Mortality} = [(L_c - L_s) / L_c] \times 100$$

where,

L_c = average number of alive shrimps in control group

L_s = average number of alive shrimps in test group

Statistical analysis

SPSS Statistics version 25 was used to analyze the obtained data. All of the values were expressed as mean ± standard deviation. An analysis of variance (ANOVA), and then Tukey's *post hoc* test were used to determine significant differences, where *p* < 0.05 was considered significant [10, 11].

Result and Discussion

Plants are monstrous ultimate repository of phytochemicals which are divided as primary and secondary metabolites depending on their chemical structure and biosynthetic derivation. Secondary metabolites are further classified based on their chemical structure and functional groups present in it. These exhibit diverse pharmacological activities which are beneficial to both plant itself and human. Human rely on these medicinal plants from the very ancient period [14]. Our phytochemical assessment exhibited that, leaves of *S. palustris* contain different types of phytochemical groups such as reducing sugars, flavonoids, glycosides, steroids, terpenoids, tannins, saponins etc (Table 1). These phytochemicals are said to be the main compounds for significant biological activities.

Almost each plant contains various antioxidant compounds. In our qualitative antioxidant test, the development of yellow spots after DPPH spray exhibited the presence of antioxidant molecules. Then we led to perform the quantitative measurement of some antioxidant groups and the content of total phenolics, flavonoids and tannins in *S. palustris* extract were found to be 97 mg gallic acid equivalent (mg GAE)/g, 90 mg quercetin equivalent (mg QE/g) and 23 mg GAE/g respectively (Table 2). It is an unavoidable fact that, free radicals are continuously produced in our body by different oxidase enzymes. These free radicals are responsible for causing different types of pernicious effects to our body such as lipid peroxidation, stimulating oxidation of sulfhydryl groups and then leading to bind to our cellular components. As a result, these further lead to different diseases such as atherosclerosis, muscular dystrophy, inflammation, cancer, diabetes and different types neurological, cardiovascular and respiratory disorders [15]. To combat these free radicals associated damages, antioxidants play a very crucial role. Mangrove plants possess different types of antioxidant compounds (such as phenolics, flavonoids, polyphenolic groups, tannins, alkaloids, vitamins, terpenoids, glycosides) [16]. Our results indicate that *S. palustris* possess different antioxidant contents. Thus, it can be said that these compounds may be helpful to fight against different oxidative reactions and then prevent our body from oxidative damages and their associated diseases.

After quantitative measurement of different antioxidant content in *S. palustris* leaves, we then focused our effort on different radical scavenging testing. DPPH is a very well-known stable radical that can quickly attack to nearby molecules. Antioxidants donate one or more hydrogen atoms to DPPH and then DPPH get bleached. Thus, DPPH lost its original purple color and forms yellow color [17]. In our test, both *S. palustris* and ascorbic acid scavenged DPPH free radicals and with the obtained results, the calculated SC₅₀ values were 80 µg/ml and 15 µg/ml for *S. palustris* and ascorbic acid, respectively (Table 2). Although hydrogen peroxide itself does not act as an oxidant, but when it gets dissociated in aqueous solution, it yields hydroxyl radical that can readily attacks to nearby molecules and accelerates chain reaction [9]. In our body, these severely attacks proteins, lipids and nucleic acids and leads cellular death. Both *S. palustris* and ascorbic acid scavenged the hydroxyl radicals in hydrogen peroxide scavenging assay and with those obtained results, the calculated SC₅₀ values were 158 µg/ml and 11 µg/ml for *S. palustris* and ascorbic acid, respectively (Table 2). Superoxide radical can act as a precursor of the more reactive oxygen species, contributing to damage the tissue and leads organ failure. These harmful superoxide radicals can be scavenged by taking food rich in antioxidant contents [18]. Both *S. palustris* and ascorbic acid scavenged superoxide radicals and with these results, the calculated SC₅₀ values were 236 µg/ml and 111 µg/ml for *S. palustris* and ascorbic acid, respectively (Table 2). However, antioxidants not only act as free radical scavengers but also show reducing properties that is also beneficial for our health. *S. palustris* also showed its reducing activity in ferric chloride reducing reaction. The absorbance was measured for different concentrations for both *S. palustris* and ascorbic acid. The calculated RC₅₀ (50% reducing concentration) values were 166 µg/ml and 28 µg/ml, respectively where maximum absorbance (for 800 µg/ml) for those were 1.653 and 1.112, respectively (Table 2)

Diabetes mellitus (type II diabetes) is the most common

metabolic disorder that affects many people worldwide. People with diabetes have a 2–3 folds risk of all-cause mortality and presence of diabetes is also associated with increased cardiovascular disease, stroke, cancer, renal and hepatic disease [19]. Abnormal rise in blood glucose level (hyperglycemia) is associated to onset of type II diabetes in human [20]. Oral glucose tolerance test (OGTT) is an index of clearance of glucose from blood. In our OGTT experiment, the *S. palustris* extract significantly reduced blood glucose within the observed time. (Average values are plotted in figure 2). *S. palustris* contains different types of stigmasterol, kaempferol, stenopalustrosides and their many derivatives. These compounds are already reported for their hypoglycemic property [21-24]. So, we can assume that these compounds are responsible for showing antihyperglycemic activity.

Pain and inflammation are cellular responses to external stimuli provoked by sudden rupture, injury, allergen and different types of antigens. These cellular responses are mediated by some endogenous compounds such as vasopressin, vasoactive intestinal peptide (VIP), oxytocin, catecholamines, endorphin / enkephalin, prostaglandins, prostacyclin by cyclooxygenase pathways [25]. Intraperitoneal administration of 0.7% acetic acid induces some endogenous pain mediators (such as prostaglandins, histamine, serotonin (5-HT), bradykinin) which initiate sensitizing pain nerve endings [26]. *S. palustris* extract showed inhibition of writhing at 26% and 60% at 250 mg/kg and 500 mg/kg doses, respectively where Diclofenac Na (at 25 mg/kg body weight dose) showed 78.52% inhibition of writhing (Table 3). After the evaluation of analgesic activity of *S. palustris*, it also showed its effect to reduce the swelling or edema of mice in both 250 mg/kg and 500 mg/kg doses (Figure 3) which was induced by formaldehyde. As pain and inflammatory mediators are mostly same, so it can be said that compounds showing analgesic activity might also show anti-inflammatory activity. Compounds like stenopalustrosides, stigmasterol, ethyl linoleate, kaempferol and their different derivatives present in this *S. palustris* extract might be responsible for these activities [21-24, 27].

Constipation is one type of gastrointestinal problem that is due to hardening of stool. Sometimes patients feel unbearable pain during stooling. Chronic constipation may be a predisposing factor to some other colorectal disorders such as piles, enlarged hemorrhoids and colorectal cancer [28]. Laxatives are used frequently to treat constipation as they add bulk to intestinal contents by retaining water and increasing the frequency and cause ease of defecation [29]. In the laxative test, we observed that *S. palustris* extract increased weight of stool significantly. At 250 mg/kg and 500 mg/kg body weight doses, the increase in weight were found 29.31% and 51.72%, respectively where Bisacodyl at the dose 10 mg/kg increased the stool weight 115.56% (Table 4). The probable reason for *S. palustris* showing the laxative activity may be the presence of terpenoids, sterols, flavonoids, phenolic compounds, tannins [28].

In the recent few decades, cancer has become one of the major causes for mortality of people around the world. Genetic modification, exposure to radiation and pollution are the predisposing factors to cancer. Brine shrimp lethality bioassay is a very common technique used to kill the *Artemia salina* nauplii. After performing this assay, the *S. palustris* extract showed the LC₅₀ at 184.7 µg/ml where Vincristine sulphate showed LC₅₀ at 0.584 µg/ml (Figure 4). The probable reason for *S. palustris* to show this cytotoxic property may be the presence of stigmasterol, its derivatives, ethyl linoleate

and other cytotoxic compounds [22, 27]. Considering the result, little cytotoxic property. we can assume that although this *S. palustris* extract showed a

Table 1: Presence or absence of different phytochemical groups in *S. palustris* leaves

Phytochemical groups	Reducing sugars	Tannins	Flavonoids	Saponins	Gums	Steroids	Alkaloids	Glycosides	Xanthoproteins	Terpenoids	Acidic compounds
<i>S. palustris</i> extract	+	+	+	+	+	+	-	+	-	+	-

'+' indicates presence and '-' indicates absence

Table 2: Total content of secondary metabolites (phenolics, flavonoids and tannins) of *S. palustris* leaves and approximate data of quantitative antioxidant assays

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg GAE/g)	DRSA (SC ₅₀ µg/mL)	HPSA (SC ₅₀ µg/mL)	SRSA (SC ₅₀ µg/mL)	RPA (RC ₅₀ µg/mL)
<i>S. palustris</i> extract	97	90	23	80	158	263	166

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

Table 3: Representation of effects of *S. palustris* leaves on acetic acid induced writhing in mice

Treatment group	Dose (mg/kg)	Mean writhing	% Inhibition of writhing
Negative control	-----	27±2 ^{θ▲}	-----
Standard (Diclofenac Na)	25	5.8±0.84*▲	78.52±3.1*▲
<i>S. palustris</i> extract	250	20±2.24* ^θ	25.92±8.28* ^θ
<i>S. palustris</i> extract	500	10.8±2.86* ^{θ▲}	59.99±10.6* ^{θ▲}

Data are means of five replicates ± SD (standard deviation); * P < 0.05 vs. Control (Dunnett's t test); ^θ P < 0.05 vs. Diclofenac Na 25 mg/kg; ▲ P < 0.05 vs. *S. palustris* 250 mg/kg; ▲ P < 0.05 vs. *S. palustris* 500 mg/kg (pair-wise comparison by Post Hoc Tukey test)

Table 4: Representation of effects of *S. palustris* leaves on laxative test

Treatment group	Dose (mg/kg)	Mean stool weight (gm)	% Increase of stool weight
Negative control	-----	0.58±0.028 ^{θ▲}	-----
Standard (Bisacodyl)	10	1.09±0.042*▲	87.931±7.314*▲
<i>S. palustris</i> extract	250	0.75±0.028* ^θ	29.315±4.869* ^θ
<i>S. palustris</i> extract	500	0.88±0.042* [▲]	51.724±9.76* [▲]

Data are means of two replicates ± SD (standard deviation); * P < 0.05 vs. Control (Dunnett's t test); ^θ P < 0.05 vs. Bisacodyl 10 mg/kg; ▲ P < 0.05 vs. *S. palustris* 250 mg/kg; ▲ P < 0.05 vs. *S. palustris* 500 mg/kg (pair-wise comparison by Post Hoc Tukey test)



Fig 1: *Stenochlaena palustris* leaves

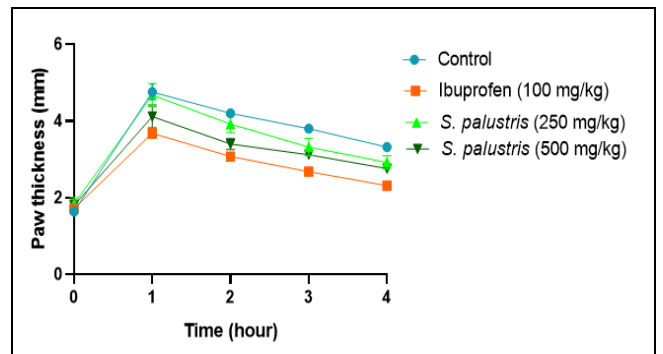


Fig 3: Comparison of paw thickness at different times for control, standard and *S. palustris* extract in the formaldehyde-induced anti-inflammatory activity test

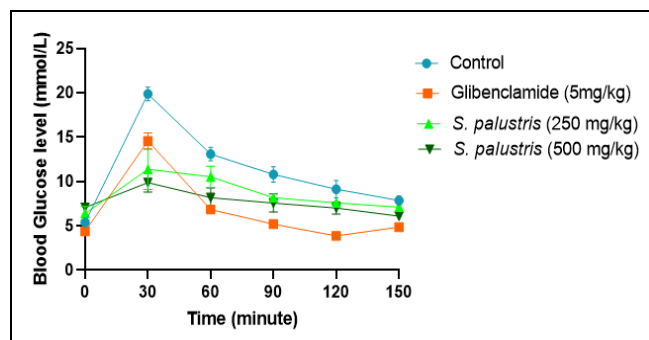


Fig 2: Comparison of blood glucose levels (mmol/L) at different time points for control, standard and *S. palustris* extract in the oral glucose tolerance test

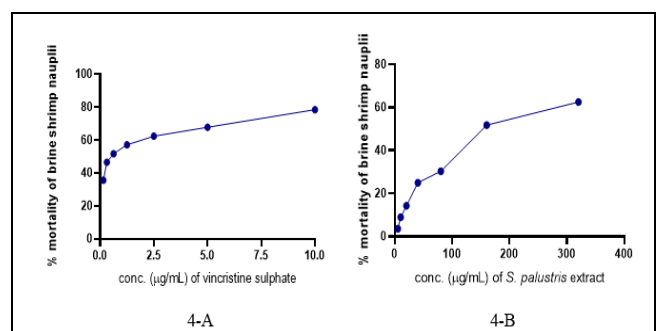


Fig 4: Graphical presentation of brine shrimp lethality bioassay of vincristine sulphate (4-A) and *S. palustris* extract (4-B)

Conclusion

This present study was conducted on *S. palustris* leaves to investigate its some phytochemical and pharmacological properties based on its traditional uses. In this study, we observed the presence of different secondary metabolites. This extract contains different types of antioxidative components hence showed prominent radical scavenging activity. The extract also exhibited significant antihyperglycemic, analgesic, anti-inflammatory and laxative effects with minimal cytotoxicity and these findings supported its traditional usage. Considering these above-mentioned findings, we suggest that the leaves of *S. palustris* might be a vital source of bioactive compounds.

Conflict of Interest

The authors hereby declare that there is not any type of conflict of interest among them.

Authors' Declaration

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

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