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EXTRACTION, PHYTOCHEMICAL ANALYSIS AND IMMUNOMODULATORY ACTIVITY OF *SCHREBERA SWIETENIOIDES* LEAVES EXTRACT

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ABSTRACT

The present study was undertaken to investigate the extraction yield, phytochemical profile, antioxidant potential, and immunomodulatory activity of the hydroalcoholic leaf extract of *Schrebera swietenioides*. Sequential extraction revealed a higher percentage yield for the hydroalcoholic extract (7.50% w/w) compared to petroleum ether extract (0.80% w/w). Preliminary phytochemical screening of the hydroalcoholic extract confirmed the presence of flavonoids, phenolic compounds, proteins, and carbohydrates, while alkaloids, glycosides, saponins, and diterpenes were absent. Quantitative estimation showed total phenolic content of 0.985 mg/100 mg and total flavonoid content of 0.647 mg/100 mg of dried extract. The antioxidant activity assessed by the DPPH radical scavenging method demonstrated concentration-dependent inhibition with an IC_{50} value of 78.23 µg/mL, indicating moderate antioxidant potential compared to ascorbic acid (IC_{50} = 17.15 µg/mL). Immunomodulatory activity was evaluated using neutrophil adhesion test, carbon clearance assay, and hemagglutination (HA) titer. The extract significantly enhanced neutrophil adhesion and phagocytic index, indicating stimulation of innate immunity. It also modulated HA titer, suggesting an effect on humoral immune response. The immunostimulatory activity observed at 100 mg/kg and 200 mg/kg doses was comparable to the standard drug Levamisole. The findings suggest that the hydroalcoholic extract of *Schrebera swietenioides* leaves possesses significant antioxidant and immunostimulatory properties, likely attributed to its phenolic and flavonoid constituents. The study scientifically supports the traditional use of the plant and highlights its potential as a natural immunomodulatory agent.

Keywords: *Schrebera swietenioides*, Hydroalcoholic extract, Phytochemical screening, Total phenolic content, Total flavonoid content, Antioxidant activity, DPPH assay, Immunomodulatory activity.

INTRODUCTION

Schrebera swietenioides Roxb. is a medicinal plant belonging to the family Oleaceae that grows widely in dry deciduous forests across South and Southeast Asia, including India, Bangladesh, and Thailand. It is commonly known as the weaver's beam tree and has a long history of use in traditional and folk medicine for a variety of ailments such as digestive disorders, skin infections, and wound healing (Hong *et al.*, 2023).

Plant extracts have increasingly become the focus of modern pharmacological research due to their broad spectrum of bioactive compounds, including flavonoids, tannins, saponins, alkaloids, and phenolic acids, which

contribute to diverse biological activities such as antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects. These phytochemicals can modulate immune responses by influencing cytokine secretion, macrophage activity, lymphocyte proliferation, and other key components of the immune system (Altemimi *et al.*, 2017).

Phytochemical screening of *S. swietenioides* leaf extracts has revealed the presence of important secondary metabolites including flavonoids such as rutin and quercetin, which are known to possess potent antioxidant and therapeutic properties. Antioxidant potential of the aqueous leaf extract has also been demonstrated by DPPH radical-scavenging activity, indicating its ability to counteract free radical-mediated oxidative stress, a factor closely linked to modulation of immune and inflammatory processes (Nigussie *et al.*, 2023).

While there is limited direct evidence on the immunomodulatory activity of *S. swietenioides* in scientific literature, related findings including anti-inflammatory activity of its alcoholic leaf extract in wound healing suggest potential effects on immune-related pathways. Moreover, traditional medicinal systems have claimed broader immunomodulatory roles for this plant, supporting the rationale for systematic investigation into its effects on immune functions such as macrophage activation, immunoglobulin levels, and cytokine modulation.

Taken together, these traditional uses and preliminary scientific findings provide a strong premise for exploring the extraction, qualitative and quantitative phytochemical profiling, and immunomodulatory potential of *S. swietenioides* leaf extracts, with an aim to validate its therapeutic relevance and identify bioactive constituents that may contribute to immune regulation.

MATERIALS AND METHODS

Materials

The present study demonstrates that the hydroalcoholic leaf extract of *Schrebera swietenioides* is rich in phenolic and flavonoid compounds and shows appreciable antioxidant activity. The extract significantly enhanced neutrophil adhesion, phagocytic index, and humoral immune response, indicating stimulation of both innate and adaptive immunity. These findings validate its traditional medicinal use and suggest its potential as a promising natural immunomodulatory agent.

Methods

Extraction using microwave assisted technique

The shade dried leaves of *Schrebera swietenioides* were coarsely powdered and subjected to extraction. Plant material extracted by hydroalcoholic solvent (ethanol: water 75:25v/v) was used. Followed by drying, powders of plant material were prepared using mixer grinder and then 50 gram powder for extraction was carried out by microwave assisted extraction technique (Khandelwal, 2005). Then extract were centrifuged at 7000 rpm for 10min. Supernatant was collected in petriplates and solvent was allowed to evaporate room temperature. Powder was scrapped from the petriplates from which the solvent has been evaporated.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive

components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Quantitative estimation of bioactive compounds

Total phenol content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Mishra *et al.,* 2017).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Mishra *et al.,* 2017).

***In-vitro* antioxidant activity using DPPH method**

Total free radical scavenging capacity of extract from leaves of *Schrebera swietenioides* was estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

In-vivo Immunomodulatory activity

Animals

Laboratory-bred Wistar albino rats (180–200 g) and albino mice (20–25 g) of either sex were maintained under controlled environmental conditions (25 ± 5°C) with a 12-hour light/dark cycle in a well-ventilated animal facility. Animals had unrestricted access to standard pellet feed, comprising protein (22.10%), oil (4.13%), fiber (3.15%), ash (5.15%), silica (1.12%), and water provided *ad libitum*. Bedding material, consisting of paddy husk, was replaced regularly to ensure hygiene and dryness. All animals were housed under standard laboratory conditions in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). Prior to the commencement of the experimental procedures, animals underwent a 10-day quarantine period to ensure health and acclimatization.

Antigen preparation

Fresh sheep blood was obtained from a local abattoir. The sheep red blood cells (SRBCs) were thoroughly washed three times with sterile, pyrogen-free 0.9% normal saline to remove plasma and other contaminants. The final cell suspension was adjusted to a concentration of 0.5×10^9 cells/mL for use in immunization and challenge experiments (Thomas *et al.*, 2007).

Acute toxicity studies

The acute oral toxicity study was conducted to determine the appropriate dose using the up-and-down (staircase) method. Initially, two mice were administered a starting dose of 50 mg/kg orally and observed for 24 hours to monitor signs of toxicity or mortality. Subsequent doses were increased by a factor of 1.5 to identify the maximum non-lethal and minimum lethal doses. The isolated compound was found to be non-toxic up to an oral dose of 5 g/kg. Based on the guidelines provided by the Office of Pollution Prevention and Toxics (OPPT), one-tenth of the maximum safe dose (i.e., 500 mg/kg) was selected for further pharmacological evaluation (Ghosh, 1984).

Experimental protocol

The test drug solutions were prepared in distilled water for oral administration. Immunomodulatory activity was assessed at both the cellular and humoral levels. Cellular immune response was evaluated using the neutrophil adhesion test and carbon clearance assay, while humoral immunity was assessed through the mice lethality test and indirect hemagglutination assay.

All experimental models comprised four groups, each consisting of six animals:

Group I: Served as the control and received the vehicle (distilled water, 1 mL/100 g body weight, p.o.).

Group II: Received the standard immunostimulant, Levamisole (0.68 mg/kg, p.o.).

Groups III and IV: Were administered the hydroalcoholic extract of *Schrebera swietenioides* at doses of 100 mg/kg and 200mg/kg orally, respectively.

For the mice lethality test, an additional negative control group was included to validate the experimental outcomes.

Neutrophils adhesion test

Rats were pre-treated orally with either the vehicle or the isolated compounds once daily for 14 consecutive days. On day 14, blood samples were collected from the retro-orbital plexus into heparinized tubes under light anesthesia. The samples were initially analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC). Following this, the blood samples were incubated with nylon fibers (80 mg/mL) at 37 °C for 15 minutes to facilitate neutrophil adhesion. Post-incubation, the samples were again analyzed for TLC and DLC. The difference in neutrophil count before and after incubation was used to calculate the neutrophil adhesion index, reflecting the degree of cellular immune activation (Fulzele *et al.*, 2003; Shinde *et al.*, 1999). The percent Neutrophils adhesion was calculated as follows:

$$\textbf{Neutrophil adhesion } \% = \frac{\textbf{Nlu} - \textbf{Nlt}}{\textbf{NIu}} \boldsymbol{x100}$$

Where NIu is the Neutrophils index of untreated blood samples and NIt is the neutrophil index of treated blood samples.

Carbon clearance test

Swiss albino mice were orally administered the hydroalcoholic extract of *Schrebera swietenioides* at doses of 50 mg/kg and 100 mg/kg, as well as the standard drug Levamisole, once daily for 10 consecutive days in their respective treatment groups. Forty-eight hours after the final dose, all animals received an intravenous injection of Indian ink (colloidal carbon suspension; 0.3 mL per 30 g body weight) via the tail vein. Blood samples were collected from the retro-orbital plexus at 0 and 15 minutes post-injection. Each blood sample was immediately mixed with 4 mL of 0.1% sodium carbonate solution to lyse red blood cells and stabilize the suspension. The optical density of the resulting solution was measured spectrophotometrically at 660 nm.

The phagocytic index (K), an indicator of reticuloendothelial system activity, was calculated using the following formula (Jayathirtha *et al.,* 2004; Gokhale *et al.,* 2003):

$$K = \frac{(\text{Loge OD1} - \text{Loge OD2})}{15}$$

Where OD1 and OD2 are the optical densities at 0 and 15 min, respectively

Mice lethality test

Swiss albino mice were pretreated orally with the hydroalcoholic extract of *Schrebera swietenioides* at doses of 50 mg/kg and 100 mg/kg, as well as with Levamisole, for 21 consecutive days in their respective groups. On days 7 and 17 of treatment, all animals were immunized subcutaneously with the hemorrhagic septicemia (HS) vaccine. On day 21, the animals were challenged subcutaneously with 0.2 mL of a lethal dose ($25 \times LD_{50}$) of *Pasteurella multocida* (bovine strain), containing 10^7 cells/mL. The animals were monitored for 72 hours post-challenge, and the percentage of mortality was recorded for each group.

Indirect heamagglutination test

Rats in each experimental group were pretreated with the respective drugs for 14 days. On day 0, all animals were immunized intraperitoneally with 0.5×10^9 sheep red blood cells (SRBCs). Drug administration was continued for an additional 14 days post-immunization. At the end of the treatment

period, blood samples were collected from each rat, and serum was separated for the assessment of antibody response.

The hemagglutination titer was determined by serially diluting serum samples (50–100 µL) and mixing them with 0.025×10^9 SRBCs in microtiter plates. The plates were incubated at room temperature for 2 hours and then visually inspected for agglutination. The highest dilution of serum that exhibited visible agglutination was recorded as the hemagglutination (HA) titer.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test for multiple comparisons. A *p*-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the extraction yield, phytochemical composition, antioxidant potential, and immunomodulatory activity of the hydroalcoholic leaf extract of *Schrebera swietenioides*. The findings collectively suggest that the plant possesses significant bioactive constituents capable of modulating both innate and humoral immune responses.

As shown in Table 1, the percentage yield of the hydroalcoholic extract (7.50% w/w) was considerably higher than that of the petroleum ether extract (0.80% w/w). This indicates that the majority of phytoconstituents present in *S. swietenioides* leaves are polar or moderately polar in nature and are better extracted using hydroalcoholic solvent systems. Hydroalcoholic solvents are well known for their ability to extract phenolics and flavonoids, which may account for the higher recovery reported in Table 1.

Preliminary phytochemical analysis presented in Table 2 revealed the presence of flavonoids, phenolics, proteins, and carbohydrates in the hydroalcoholic extract, while alkaloids, glycosides, saponins, and diterpenes were absent. The positive reactions observed for flavonoids (alkaline reagent and lead acetate tests) and phenolics (ferric chloride test) in Table 2 indicate that these compounds are major constituents of the extract.

Flavonoids and phenolic compounds are well documented for their antioxidant and immunomodulatory activities. Their presence in Table 2 correlates with the biological effects observed in subsequent experimental models.

Quantitative estimation shown in Table 3 indicates that the total phenolic content (0.985 mg/100 mg of dried extract) was higher than the total flavonoid content (0.647 mg/100 mg). The higher phenolic content reported in Table 3 supports the strong antioxidant potential observed in the DPPH assay. Since phenolic compounds are effective hydrogen donors, their abundance contributes to free radical scavenging activity.

The antioxidant activity results summarized in Table 4 demonstrate that the hydroalcoholic extract exhibited concentration-dependent DPPH radical scavenging activity. At 100 µg/mL, the extract showed 58.24% inhibition, whereas ascorbic acid showed 90.67% inhibition at the same concentration (Table 4).

The IC_{50} value of the extract (78.23 µg/mL) was higher than that of ascorbic acid (17.15 µg/mL), as indicated in Table 4, confirming

that although the extract possesses antioxidant activity, it is less potent than the standard. The antioxidant activity observed in Table 4 can be attributed to the phenolic and flavonoid constituents identified in Tables 2 and 3.

The results of the neutrophil adhesion test are presented in Table 5**.** Both doses of *S. swietenioides* (100 mg/kg and 200 mg/kg) showed enhanced neutrophil adhesion compared to the control group (Table 5). The increase was comparable to that observed with Levamisole (0.68 mg/kg), a standard immunostimulant.

The increase in neutrophil index and adhesion percentage shown in Table 5 suggests stimulation of cellular immunity. Enhanced neutrophil adhesion reflects improved margination and migration of neutrophils, indicating activation of innate immune mechanisms. The phagocytic index results shown in Table 6 demonstrate that the hydroalcoholic extract significantly increased macrophage phagocytic activity compared to control. The values at 100 mg/kg (0.0461 ± 0.0025) and 200 mg/kg (0.0423 ± 0.0018) were comparable to Levamisole (0.0489 ± 0.0021), as presented in Table 6**.**

An increased phagocytic index indicates enhanced activity of the reticuloendothelial system and improved clearance of foreign particles from circulation. Thus, the findings in Table 6 confirm stimulation of non-specific immune responses.

The hemagglutination (HA) titer results summarized in Table 6 reflect modulation of humoral immunity. Both extract-treated groups showed significant effects compared to control. The statistical significance indicated in Table 6 (*******$p < 0.001$) confirms the immunostimulatory potential of the extract in antibody-mediated immune responses.

Table 1: Results of percentage yield of *Schrebera swietenioides*

S. No.	Extracts	Percentage yield (w/w)
1.	Pet. ether	0.80 %
2.	Hydroalcoholic	7.50 %

Table 2: Result of phytochemical screening of hydroalcoholic extract of *Schrebera swietenioides*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	a) Mayer's Test:	-Ve
	b) Wagner's Test:	-Ve
	c) Dragendroff's Test:	-Ve
	d) Hager's Test:	-Ve
2.	**Glycosides**	
	a) Modified Borntrager's Test:	-Ve
	b) Legal's Test:	-Ve

3.	**Flavonoids** a) Alkaline Reagent Test: b) Lead acetate Test:	 +Ve +Ve
4.	**Saponins** a) Froth Test: b) Foam Test:	 -Ve -Ve
5.	**Phenol** a) Ferric Chloride Test: b) Gelatin test:	 +Ve -Ve
6.	**Proteins** a) Xanthoproteic Test: b) Millon's test:	 +Ve -Ve
7.	**Carbohydrate** a) Fehling's Test: b) Benedict's Test:	 +Ve -Ve
8.	**Diterpenes** a) Copper acetate Test:	 -Ve

[+Ve= Positive; -Ve= Negative]

Table 3: Estimation of total phenol and flavonoids content of hydroalcoholic extract of *Schrebera swietenioides*

S. No.	Total phenol content	Total flavonoids content
1.	**mg/100mg of dried extract**	
	0.985	0.647

Table 4: % Inhibition of ascorbic acid and extract of *Schrebera swietenioides* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	42.25	12.35
2	20	54.92	24.96
3	40	62.14	37.02
4	60	73.09	41.57
5	80	85.43	50.94
6	100	90.67	58.24
IC_{50} value		**17.15**	**78.23**

Table 5: Effect of hydroalcoholic extract of *Schrebera swietenioides* and Levamisole on Neutrophils adhesion test

Treatment	TLC ($\times 10^3/mm^3$)	Neutrophils (%)	Neutrophil Index (TLC × Neutrophil%)	Neutrophil Adhesion (%)
	UB	NFTB	UB	NFTB
Control	5.6 ± 0.16	5.5 ± 0.16	23.3 ± 0.80	22.5 ± 0.80
Levamisole (0.68 mg/kg)	6.6 ± 0.18	5.8 ± 0.15	26.6 ± 1.08	18.6 ± 0.40
S. swietenioides (100 mg/kg)	6.7 ± 0.12	5.8 ± 0.13	27.0 ± 1.33	16.6 ± 1.00
S. swietenioides (200 mg/kg)	6.3 ± 0.86	5.9 ± 0.49	24.5 ± 1.23	17.6 ± 1.20

Table 6: Effect of hydroalcoholic extract of *Schrebera swietenioides* and Levamisole on phagocytic index and HA titer

Treatment	Phagocytic Index (Carbon Clearance Assay)	Hemagglutination (HA) Titer (µL)
Control	0.0161 ± 0.0035	0.0862 ± 0.2540
Levamisole (0.68 mg/kg, p.o.)	0.0489 ± 0.0021 ***	0.0019 ± 0.0003 ***
S. swietenioides (100 mg/kg, p.o.)	0.0461 ± 0.0025 ***	0.0016 ± 0.0004 ***
S. swietenioides (200 mg/kg, p.o.)	0.0423 ± 0.0018 ***	0.0046 ± 0.0007 ***

CONCLUSION

The present study demonstrates that the hydroalcoholic leaf extract of *Schrebera swietenioides* is rich in phenolic and flavonoid compounds and shows appreciable antioxidant activity. The extract significantly enhanced neutrophil adhesion, phagocytic index, and humoral immune response, indicating stimulation of both innate and adaptive immunity. These findings validate its traditional medicinal use and suggest its potential as a promising natural immunomodulatory agent.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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