RESEARCH PAPER

Exploring the Immunomodulatory Potential of Sesbania sesban (L.) Merr.

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ABSTRACT

The present study was undertaken to evaluate the immunomodulatory potential of Sesbania sesban (L.) Merr. stem extracts using established in vivo experimental models. The plant material was extracted using n-butanol (Soxhlet method) and aqueous (maceration method) solvents, followed by qualitative phytochemical screening and estimation of total flavonoid content (TFC). Phytochemical analysis confirmed the presence of important bioactive constituents, including flavonoids, alkaloids, tannins, phenolic compounds, carbohydrates, and proteins. The n-butanol extract showed comparatively higher flavonoid content than the aqueous extract.

Acute oral toxicity studies demonstrated that both extracts were safe up to 3000 mg/kg. The immunomodulatory activity was assessed using the Carbon Clearance Test (CCT), Neutrophil Adhesion Test (NAT), and Delayed-Type Hypersensitivity (DTH) response in albino rats. The n-butanol stem extract exhibited a significant and dose-dependent enhancement in phagocytic index, neutrophil adhesion, and DTH response, with maximum activity observed at 300 mg/kg, comparable to the standard drug levamisole. In contrast, the aqueous extract showed moderate immunostimulatory effects.

Overall, the results indicate that Sesbania sesban possesses significant immunomodulatory activity, particularly in the n-butanol extract, which may be attributed to its higher flavonoid and phenolic content. These findings support the traditional use of the plant and suggest its potential as a natural immunotherapeutic agent. Further studies are warranted to isolate and characterize the active constituents responsible for this activity.

Keywords: sesbania sesban, immunomodulatory activity, carbon clearance test, delayed-type hypersensitivity, flavonoids, neutrophil adhesion

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INTRODUCTION

The immune system plays a crucial role in maintaining physiological homeostasis by defending the body against infections, foreign agents, and abnormal cell proliferation. However, dysregulation of immune responses can lead to increased susceptibility to infections, autoimmune disorders, and chronic inflammatory diseases. Therefore, modulation of the immune system through natural or synthetic agents has become an important therapeutic strategy in modern medicine [1,2].

In recent years, there has been growing interest in plant-based immunomodulators due to their safety, affordability, and minimal side effects compared to synthetic drugs. Medicinal plants are rich sources of bioactive phytoconstituents such as flavonoids, alkaloids, saponins, tannins, and glycosides, which are known to influence both innate and adaptive immune responses [3,4]. Several herbal formulations have demonstrated the ability to enhance immune function by stimulating phagocytosis, antibody production, and cell-mediated immunity [5].

Sesbania sesban (L.) Merr., belonging to the family Fabaceae, is a traditionally used medicinal plant widely distributed in tropical and subtropical regions. It has been used in traditional systems of medicine for the treatment of various ailments such as inflammation, microbial infections, and fever. Phytochemical investigations of *Sesbania sesban* have revealed the presence of important constituents including flavonoids, saponins, tannins, and phenolic compounds, which may contribute to its pharmacological activities [6,7]. Previous studies have reported antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties of this plant, suggesting its potential role in immune modulation [8].

Despite its traditional use and reported pharmacological activities, scientific validation of the immunomodulatory potential of *Sesbania sesban* using well-established

experimental models remains limited. In vivo models such as the Carbon Clearance Test (CCT), Neutrophil Adhesion Test (NAT), Delayed-Type Hypersensitivity (DTH), are widely employed to evaluate different aspects of immune function, including phagocytic activity, neutrophil activation, cell-mediated immunity, and humoral immune response, respectively [9,10].

Therefore, the present study aims to explore the immunomodulatory potential of *Sesbania sesban* (L.) Merr. using a battery of in vivo experimental models, including CCT, NAT and DTH to provide scientific evidence supporting its traditional use and to identify its potential as a natural immunotherapeutic agent.

MATERIALS AND METHODS

Plant material

The plant material was collected from the Sangli district of Maharashtra and subsequently authenticated by the Botanical Survey of India, Western Regional Centre, Pune.

Preparation of n-butanol and aqueous extracts

For phytochemical investigation, the plant material was thoroughly cleaned, washed, shade-dried, and coarsely powdered. The n-butanol stem extract was prepared using a Soxhlet apparatus, while the aqueous stem extract was obtained by the maceration method. The resulting extracts were subsequently subjected to qualitative phytochemical analysis.

Fig. 1 Soxhlet Extraction Process

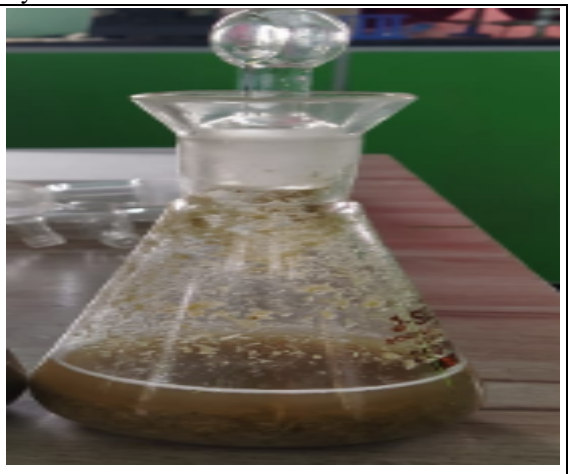

Fig. 2 Maceration Extraction Process

Qualitative phytochemical analysis

Using standard procedures, qualitative tests were performed on the concentrated extracts to detect different phytochemical ingredients. [11]

Estimation of Total Flavonoid Content:

Aluminum chloride method: (Standard: Quercetin)

The different plant extracts and Quercetin were dissolved in ethanol at a 1 mg/ml concentration. In test tubes, initially, 0.9 ml of distilled water and 0.1 ml of extract were mixed. After 75 µl, 5% sodium nitrite solution was added. 6 minutes later 150 µl, 10% aluminum chloride solution was added, and the mixture was allowed to stand for 5 minutes. Then, 0.5 mL of sodium hydroxide (1M) was added. After adding distilled water to bring the reaction mixture's volume upto 2.5 ml, it was well mixed. Immediately with the help of spectrophotometer (510nm) the absorbance was measured. There were three copies of each determination made. Each and every reagent used in the quercetin standard solution or extract was substituted with 0.1 mL of ethanol in the blank solution. A quercetin equivalent in milligrams per gram of dry extract weight was used to express the results. [12]

Animal study: Albino rats weighing between 170 and 350 grams were kept in a well-conditional animal house with a equally dark and light cycle at 27° ± 3 °C. The animals were provided food pellets and water on-needed basis, with fresh rice husk bedding material added to ensure cleanliness and dryness. Before the experiments began, the animals had a seven-day period of acclimatization. Experimental animal study was approved from Institutional Animal Ethics Committee (IAEC/ABCP/22/2023-24), and the animals were kept in an animal house that met the standards set by the Committee for the Purpose of Control and Supervision of Experiments on Animals. Acute toxicity was performed by using OECD TG 420.

Experimental design: Rats were split up into 8 groups, each group contains 6 animals. The treatments received by all animals were *per oral* route of administration.

Table 1: Experimental design

Group	Treatment	Group	Treatment
I Control	Normal saline, 10 ml/kg	V Test	SSNBSE, 300 mg/kg
II Standard	Levamisole, 50mg/kg	VI Test	SSASE, 100 mg/kg
III Test	SSNBSE, 100 mg/kg	VII Test	SSASE, 200 mg/kg
IV Test	SSNBSE, 200 mg/kg	VIII Test	SSASE, 300 mg/kg

SSNBSE: *Sesbania sesban* (L.) Merr. N- Butanol Stem Extract, **SSASE:** *Sesbania sesban* (L.) Merr. Aqueous Stem Extract

Immunomodulatory activity: [13-16]

Carbon Clearance Test: On the seventh day of study, each experimental animal was divided into its designated group and given treatment. Three hours later, each group's animals got an intravenous injection of carbon ink suspension. Following that, the blood samples (25µl) were removed at 5 and 15 minutes from retro-orbital plexus and lysed in a solution of sodium carbonate (3ml). Using a spectrophotometer set to 660nm, the optical density was determined. The following formula was used to get the phagocytic index:

Phagocytic index = OD at 5 min. – OD at 15 min. / $T_2 – T_1$

Where, T1= optical density at 5 min. & T2 = optical density at 15 min.

Neutrophil adhesion test: All groups had blood samples taken via retro-orbital plexus puncture on 14th day of treatment. Total and differential leukocyte count were determined after blood was collected in vials treated with disodium EDTA. After the preliminary counts, the samples were incubated at 37°C (15 min.) at a concentration of 80 mg/mL with nylon fiber. TLC and DLC were re-evaluated after incubation. Multiplying the TLC by the neutrophil percentage yielded the neutrophil index (NI). The following formula was used to get the % neutrophil adhesion:

% neutrophil adhesion =

NI before incubation – NI after incubation / NI before incubation X 100

Delayed type hypersensitivity Test:
Preparation of Sheep RBCs (SRBCs):

Table 2: Composition of Alsever's solution

For 1000mL	Content
20.5gm	Dextrose / Glucose
8gm	Sodium Citrate
0.55gm	Citric acid
4.2gm	Sodium chloride
Up to 1000mL	Distilled water

Table 3: Composition of Phosphate buffered saline

For 1000mL	Content
8 gm	Sodium chloride
0.2 gm	Potassium chloride
1.44 gm	Disodium phosphate
0.24 gm	Mono-potassium phosphate
Up to 1000mL	Distilled Water
By using Hcl / NaoH, pH adjusted to 7.4	

Blood from sheep was drawn and placed in sterile Alsever's solution in a 1:1 ratio with newly made Alsever's solution. After being refrigerated, the blood was processed to create the batch of SRBCs. Centrifugation at 2000 rpm for 10 minutes was required for this, and then 4-5 washes with physiological saline were performed. After that, the SRBCs were preserved in buffered saline for later use. All groups received an i.p. inj. of 0.1 mL of a 20% SRBC suspension in normal saline on 14th day of therapy as a form of sheep red blood cell (SRBC) immunization. A 0.03 mL 20% SRBC suspension was injected into right hind paw's sub-plantar region on 21st

day, presenting a challenge to every animal in each group. The left hind paw was simultaneously injected with the same volume of normal saline. Through the induction of foot pad edema, this approach was used to quantify the cellular immune response. Following a 24-hour period, or on the 22nd day, by using vernier caliper the foot pad reaction was assessed by measuring the increase in thickness of right hind foot pad that was brought on by the hypersensitive reaction and ensuing edema. The foot pad reaction was measured as the thickness (mm) difference between left footpad (injected with normal saline) and right footpad (injected with SRBC).

Statistical analysis: All the expressed data were presented as mean ± SEM. The data were statistically analysed by one-way ANOVA (Dunnett's test) compared to control.

RESULTS

Qualitative analysis: Medicinal plants derive their therapeutic efficacy from bioactive chemical constituents known as secondary metabolites. These compounds, including flavonoids, glycosides, alkaloids, essential oils, gums, resins, mucilage, and tannins, play a vital role in conferring their pharmacological properties.

Preliminary phytochemical screening: All extracts underwent screening to identify various Phytoconstituents and the findings are presented in the table below.

Table 4: Preliminary phytochemical screening

Sr. No.	Test	Stem	
		Aqueous	N- Butanol
1	**Carbohydrates**		
	Molisch Test	+	+
	Fehling's Test	–	–
	Benedict 's Test	+	+
	Barfoed's Test	–	–
2	**Proteins**		
	Biuret Test	+	+
	Millon's Test	–	–
	Xanthoprotein Test	–	–
	Test for sulphur	–	–
3	**Amino acids**		
	Ninhydrin Test	+	–
	Test for Tyrosine	–	–
	Test for Tryptophan	–	–
	Test for Cysteine	–	–
4	**Alkaloids**		
	Dragendorff's Test	+	+
	Mayer's Test	+	+
	Hager's Test	+	–
	Wagner's Test	–	–
	Murexide Test	–	–
	Tannic acid Test	+	+
	Picrolonic acid Test	+	+

5	**Test for Tannins and Phenolic compounds**		
	Ferric chloride Test	+	+
	Lead acetate Test	+	+
	Gelatin Test	+	+
	Bromine water Test	–	+
	Acetic acid Test	–	–
	Potassium dichromate Test	+	+
	Dil. Iodine Test	+	+
	Dil. Nitric acid Test	+	+
	Potassium ferricyanide Test	–	–
	Potassium permanganate Test	+	+
6	**Test for Flavonoids**		
	Shinoda Test	+	+
	Sulphuric acid Test	–	–
	Lead acetate Test	+	–
	Sodium Hydroxide Test	+	–
7	**Test for Glycosides**		
	Legal's Test	–	–
	Keller –Killiani Test	–	–
	Borntrager's Test	–	–
	Foam Test	+	+

+ indicates Present and – indicates Absent

Estimation of Total Flavonoid content

Table 5: Estimation of TFC

Sample	Absorbance at 510nm			Mean
Blank	0.109	0.108	0.109	0.108
Standard	0.903	0.914	0.907	0.908
n-Butanol – Stem Extract	0.652	0.651	0.651	0.651
Aqueous – Stem Extract	0.111	0.110	0.112	0.110

Acute oral toxicity study:

The rats were starved overnight before receiving the extracts. Each extract was administered orally to each animal group at a dose of 3000 mg/kg body weight. After extract administration, animals were watched for the first 30 minutes, then constantly for the first 24 hours and thereafter for three days. The animals were checked daily for changes in their respiratory rate, fur, skin, eyes, mucous membranes, circulatory rate, and central and autonomic nervous systems. There were no reported deaths, indicated that 3000 mg/kg dose was acceptable. Therefore, for the comparative analysis of various plant extracts, we selected doses of 100, 200, and 300 mg/kg.

Statistical analysis:

All the expressed data were presented as mean ± SEM. The data were statistically analysed by one-way ANOVA (Dunnett's test) compared to control.

Immunomodulatory activity

Table 6: Results of Immunomodulatory activity

Group	Treatment	CCT	NAT	DTH
		Phagocytic Index	% Neutrophil adhesion	Mean Difference of paw edema (mm)
I Control	NS, 10ml/kg, p.o.	0.0048±0.0002 (100%)	51.155±0.0054 (100%)	1.335±0.052 (100%)
II Std.	Levamisole, 50mg/kg, p.o.	0.0077±0.0001**** (↑160.40%)	82.35**** (↑160.99%)	3.950±0.657 **** (↑296.99%)
III Test	SSNBSE, 100 mg/kg, *p.o.*	0.0050±0.0001 (↑104.16%)	53.01±0.004**** (↑103.63%)	1.36±0.01 (↑102.25%)
IV Test	SSNBSE, 200 mg/kg, *p.o.*	0.0062±0.0001**** (↑129.16%)	65.51±0.004**** (↑128.07%)	3.14±0.12*** (↑236.09%)
V Test	**SSNBSE, 300 mg/kg, *p.o.***	0.0070±0.0002**** (↑145.83%)	75.45±0.005**** (↑147.50%)	4.45±0.25**** (↑334.58%)
VI Test	SSASE, 100 mg/kg, *p. o.*	0.0042±0.0001**** (87.5%)	42.75±0.004**** (83.57%)	0.99±0.02 (74.43%)
VII Test	SSASE, 200 mg/kg, *p. o.*	0.0053±0.0003** (↑110.41%)	52.66±0.010**** (↑102.95%)	2.08±1.39 (↑156.39%)
VIII Test	SSASE, 300 mg/kg, *p. o.*	0.0059±0.0002**** (↑122.91%)	61.75±0.004**** (↑120.72%)	2.36±1.22* (↑177.44%)

The values were expressed in Mean ± SEM. The one-way ANOVA followed Dunnett's test (n=6) and analyzed the results statistically. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 as compared to control group.

SSNBSE: *Sesbania Sesban* n-Butanol Stem Extract, SSASE: *Sesbania Sesban* Aqueous Stem Extract

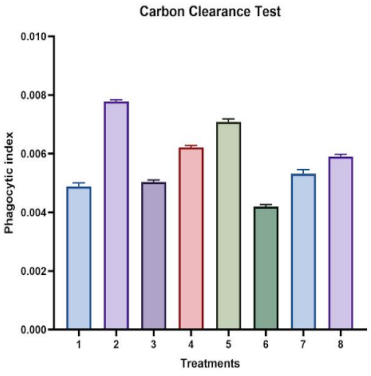

Fig.3 Graph of Carbon Clearance Test

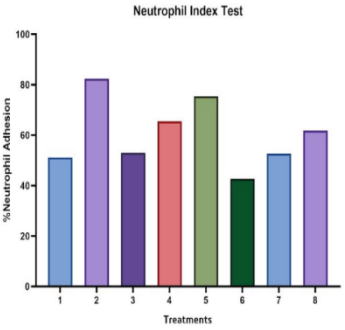

Fig.4 Graph of Neutrophil Adhesion Test

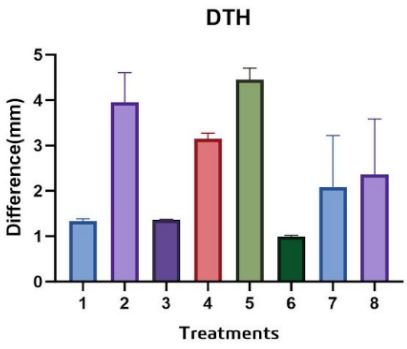

Fig.5 Graph of Delayed Type Hypersensitivity Test

DISCUSSION

The present study aimed to evaluate the immunomodulatory potential of Sesbania sesban (L.) Merr. using well-established in vivo models, including the Carbon Clearance Test (CCT), Neutrophil Adhesion Test (NAT), and Delayed-Type Hypersensitivity (DTH). These experimental models are widely employed to assess various components of the immune system, such as phagocytic activity, neutrophil function, and cell-mediated immune responses [17,18].

Preliminary phytochemical screening of both aqueous and n-butanol stem extracts revealed the presence of several bioactive constituents, including flavonoids, alkaloids, tannins, phenolic compounds, carbohydrates, and proteins. These secondary metabolites are known to play a significant role in modulating immune responses. In

particular, flavonoids and phenolic compounds have been reported to enhance immune function through their antioxidant and free radical scavenging activities, thereby protecting immune cells from oxidative damage [19,20]. The comparatively higher total flavonoid content observed in the n-butanol extract may account for its superior pharmacological activity.

The acute oral toxicity study demonstrated that both extracts were safe up to a dose of 3000 mg/kg, as no mortality or observable behavioral changes were recorded. This finding indicates a favorable safety profile and supports the traditional therapeutic use of the plant [21]. In the Carbon Clearance Test, the n-butanol stem extract produced a significant and dose-dependent increase in the phagocytic index, particularly at doses of 200 and 300 mg/kg, indicating enhanced activity of the reticuloendothelial system. This enhancement suggests improved macrophage-mediated clearance of foreign particles from the bloodstream. In contrast, the aqueous extract exhibited relatively lower activity, especially at lower doses, indicating weaker stimulation of phagocytic function [22].

Similarly, the Neutrophil Adhesion Test revealed that the n-butanol extract significantly increased neutrophil adhesion in a dose-dependent manner, with effects comparable to the standard drug levamisole. This increase reflects enhanced neutrophil activation and margination, which are critical components of innate immunity. The aqueous extract, however, demonstrated only moderate to minimal activity, indicating comparatively lower immunostimulatory potential [23]. The Delayed-Type Hypersensitivity response, a measure of cell-mediated immunity, was also significantly enhanced by the n-butanol extract, particularly at the highest dose (300 mg/kg), where the response exceeded that of the standard drug. This finding indicates potent stimulation of T-lymphocyte–mediated immune responses. The aqueous extract showed a moderate increase in DTH response, further suggesting its comparatively reduced efficacy [24].

Overall, the findings of the present study indicate that the n-butanol stem extract of Sesbania sesban exhibits pronounced immunostimulatory activity across multiple immune parameters, whereas the aqueous extract demonstrates moderate effects. The superior activity of the n-butanol extract may be attributed to its higher content of flavonoids and phenolic compounds, which are known to influence both innate and adaptive immune mechanisms [25].

CONCLUSION

The present study concludes that stem extracts of Sesbania sesban (L.) Merr. exhibit significant immunomodulatory activity, with the n-butanol extract demonstrating a highly significant effect at a dose of 300 mg/kg.

FUTURE PROSPECTIVE

The *Sesbania sesban* (l.) Merr. showed immunomodulatory activity however isolation and characterization are needed to identify the exact phytoconstituent/s causing the enhancement of immunity.

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Competing interests

Nil

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