

RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF AQUEOUS SEED EXTRACT OF *SESBANIA SESBAN* (L) MERR.S. Kathiravan^{a & b}, Shwetha.V.Kalava^{a,*}^a Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore-641 029, Tamil Nadu, India.^b Department of Biochemistry, PSG College of Arts & Science, Coimbatore – 641014, Tamil Nadu, India.

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ABSTRACT

The study was designed to explore the phytochemicals present in the aqueous seed extract of *Sesbania sesban* (L) Merr. *Sesbania sesban* is a traditional and native plant of India. The aqueous extract of the plant seed was subjected to qualitative and quantitative phytochemical analysis. The qualitative analysis revealed the presence of many phytochemicals. Phenols, flavonoids, flavonols, tannins and condensed tannins were estimated quantitatively and their values were found to be 28.10 ± 0.18 mg/g (Gallic Acid Equivalent) & 33.69 ± 0.06 mg/g (Catechin Equivalent), 17.57 ± 0.10 mg/g (Quercetin Equivalent) & 20.68 ± 0.09 mg/g (Rutin Equivalent), 6.08 ± 0.21 mg/g (Caffeic Acid Equivalent), 12.34 ± 0.07 mg/g (Caffeic Acid Equivalent) and 3.84 ± 0.11 mg/g (Caffeic Acid Equivalent) respectively. The primary metabolites such as total carbohydrates, total proteins and starch were also estimated and recorded as 166.0 ± 10.07 mg/g, 25.6 ± 1.50 mg/g, and 147.6 ± 6.73 mg/g respectively. The aqueous extract was analysed with FTIR, which revealed the presence of potential functional groups. The GCMS analysis revealed the presence of valuable phyto compounds with significant role in disease prevention.

Keywords: *Sesbania sesban*, aqueous extract, phytochemical, antioxidant, FTIR.

Introduction

Medicinal plants have been used by humans for several decades to treat diseases in both humans and animals through traditional medicinal and healing practices [1]. Plants contain enormous antioxidants which help to provide protection against free radicals associated diseases. The antioxidant compounds are mostly produced in plants in the form of secondary metabolites which counteract the free radicals. Phytochemicals classified as primary constituents includes the common sugars, amino acids, chlorophyll's, purines and pyrimidines of nucleic acids and proteins etc. Others classified as the secondary constituents are the chemicals consisting of alkaloids, flavonoids, terpenes, phenolics, lignans, plant steroids, curcumines, saponins, glucosides. They are the non-nutritive phyto components that possess numerous health benefits and disease prevention properties [2].

As per WHO, there are 21,000 potential medicinal plants and a substantial majority of people worldwide (80 %), rely on herbal remedies to fulfill their principal healthcare requirements. Over three- quarters of individuals globally depend predominantly on medicinal plants or plants compounds to alleviate their illnesses and ailments. A huge percentage of all plant species, were utilized

for medicinal purposes at some point in history. As per WHO estimations, phytomedicines make up 25 % of all treatment in wealthy nations like the USA, while they make up 80 % of all medications in under- developed nations like India. The biggest advantage of plant derived drugs is their synchronization with nature and also can be used across all age groups. For these reasons, herbal treatment is gaining popularity all over the world [3].

Sesbania sesban (L.) Merr., a fast-growing leguminous plant widely distributed in tropical and subtropical regions, has attracted considerable research interest due to its diverse medicinal properties. Traditionally, different parts of *S. sesban* have been used for treating inflammatory conditions, infections, liver disorders, and oxidative stress-related ailments [4,5]. Although several studies have reported the phytochemical and pharmacological potential of its leaves, bark, and flowers, the seeds of *S. sesban* remain relatively underexplored despite their potential nutritional and medicinal value.

Currently, there is an emerging significance on the use of aqueous extracts in plant compounds screening as water is the most commonly used solvent in traditional medicine and it is considered as safe and acceptable in all aspects. Aqueous

extracts are particularly relevant as water-soluble phyto compounds such as phenolics, flavonoids, glycosides, carbohydrates and proteins can be obtained in it. Preliminary phytochemical screening of aqueous extracts serves as a vital step in identifying the phytochemicals which will guide through bioactivity based investigations [6].

From a future perspective, comprehensive phytochemical profiling of *Sesbania sesban* seeds can open new avenues for the development of plant-based therapeutics, functional foods, and natural antioxidants. Integrating traditional knowledge with modern phytochemical research supports sustainable utilization of medicinal plants and contributes to biodiversity conservation. Therefore, the present study aims to investigate the phytochemical constituents of the aqueous seed extract of *Sesbania sesban* (L.) Merr., providing scientific insight into its chemical composition and establishing a foundation for future pharmacological and nutraceutical research.

2. Materials and Methods

2.1. Plant material and Preparation of the extract:

The seeds of *Sesbania sesban* were purchased from the local market, Coimbatore. The seeds were shade dried and healthy seeds were selected and grinded well to a coarse powder. 10g of the powdered sample was extracted in 300 ml of water by heating at 80°C. The extract that was obtained was condensed in an oven and was preserved in an air tight container and stored at 4°C for further use.

2.2 Qualitative Phytochemical analysis

The extract was analysed for phytochemicals qualitatively according to the published standard methods [7].

2.3 Quantitative phytochemical analysis

2.3.1 Determination of total phenolic content [8]

The amount of phenol in the extract was determined spectrophotometrically using the modified method of Zovko with the Folin-Ciocalteu reagent. An aliquot of the extract (1 mg/ml) was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for color development. Absorbance was then measured at 765 nm using the AJI-C03 UV-VIS spectrophotometer. Results were expressed as mg/g of gallic acid equivalents.

2.3.2 Estimation of flavonoids [9]

The total flavonoids were determined using the method of Ordonez *et al.* A volume of 0.5 ml of 2% AlCl₃ ethanol solution was added to 0.5 ml of extract solution. The mixture was incubated for 1 h at room temperature for yellow color appearance; the

absorbance was measured at 420 nm. Plant extracts were evaluated at a final concentration of 0.1 mg/ml. Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation obtained from the curve: $Y = 0.255x$, $R^2 = 0.9812$, where x is the absorbance and Y is the quercetin equivalent.

2.3.3 Determination of Flavanols [10]

One volume of the sample diluted in methanol was mixed with 2.5 volumes of vanillin and 2.5 volumes of HCl. The mixture was incubated for 20 min at 35 °C. For each sample, a blank was used in which each vanillin solution was replaced with methanol alone. A standard curve was constructed with catechin. The flavanols was expressed as catechin equivalents mg/g sample.

2.3.4 Determination of Tannins [11]

Pipetted 1ml of the extract and added 5ml of vanillin hydrochloride solution. Read in a spectrophotometer at 500nm after 20 minutes. Prepared a blank with reagent alone. Tannins in the sample were expressed as catechin equivalents mg/g sample.

2.3.5 Determination of Condensed Tannins [12]

A volume of 0.5ml of 0.1mg/ml extract solution was mixed with 3ml of 4% vanillin- methanol solution and 1.5 ml hydrochloric acid. The mixture was allowed to stand for 15min while the absorbance was measured at 500nm. Total condensed tannin content was expressed as catechin equivalents(mg/g).

2.3.6 Estimation of total carbohydrate by anthrone method [13]

0.5 and 1.0 ml aliquots was taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard. '0' served as blank. Made up the volume in all the tubes to 1ml including the sample tubes by adding the distilled water. 4ml of anthrone reagent was added to all the tubes and heated in a boiling water bath for eight minutes. They were cooled rapidly, read at 630 nm and a standard graph was drawn. From the graph the amount of carbohydrate present in the sample was calculated.

2.3.7 Estimation of starch by anthrone method [13]

0.1 and 0.2 ml of the processed sample was pipette out and made up the volume to 1ml with water. Standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard and made up the volume to 1ml in each tube with water. 4ml of anthrone reagent was added to all the tubes and heated for eight minutes in a boiling water bath. The tubes were cooled rapidly and read at 630nm. The amount of starch present in the sample was found by multiplying the glucose content by a factor 0.9.

2.3.8 Estimation of protein [14]

Pipetted out 0.2 to 1.0 ml working standard solution. 0.1 ml of the processed sample was taken. The volumes in all the tubes were made up to 1.0 ml with distilled water. Added 5.0 ml of alkaline copper reagent to each tube. Mixed well and allowed to stand for 10 min. Added 0.5 ml of Folin-Ciocalteu reagent. Mixed well and incubated at room temperature for 30 minutes. A reagent blank was also prepared. After 30 minutes, the blue colour developed were read at 660 nm.

2.4 FTIR spectral analysis

The analysis was made using Shimadzu FT-IR spectrophotometer at South India Textile Research Association (SITRA), Coimbatore.

2.5 GCMS analysis

The analysis was done at South India Textile Research Association (SITRA), Coimbatore with the following specifications. Equipment: Thermo GC – trace Ultra Ver 5.0, Thermo MS DSQ II, Column: DB5 - MS capillary standard non-polar column, Dimension: 30 Mts, ID: 0.25 mm, Film: 0.25 µm, Carrier gas: He, Flow: 1.0 ml/min, Temp prog: oven temp 40° C raised to 250° C at 5°C /min, Injection Volume: 1microliter, Low Mass(m/z): 50, High Mass(m/z): 650, Instrument Name : DSQ, Run Time(min): 43.17.

3. Results and Discussion

3.1 Qualitative phytochemical analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

Qualitative analysis was carried out to screen the phytochemicals present in the aqueous extract

of *Sesbania sesban*. The results of the phytochemical analysis are presented in table 1. Dragendroff test and Meyer's test for alkaloids showed negative and Wagner's test for alkaloids showed positive. Flavonoids were found to be present in the extract and saponins showed no presence. Benedicts test and Fehling's test gave presence for the carbohydrates in the extract. Molisch's reaction also confirmed the presence of carbohydrate in the extract. Millon's reaction was positive with the extract and biuret test also showed positive reaction for the presence of protein. Ferric chloride test and libermann reaction were negative while lead acetate test showed presence of phenols. Salkowski reaction gave a positive result indicating the presence of steroids in the aqueous extract while Libermann-Burchards test showed negative reaction for steroids. Glycosides and resins were found to be absent in the aqueous extract. Thiols were also not detected in the extract. Ferric chloride and lead acetate tests showed presence in extract for the presence of tannins.

The presence of various bioactive compounds that defend the traditional medicinal properties of the plant was recorded. Overall, the qualitative phytochemical analysis confirmed the presence of phenolics, flavonoids, tannins, steroids, carbohydrates and proteins. Flavonoids and phenolics are commonly recognized for their antioxidant and anti-inflammatory properties reported in earlier studies on this plant and other leguminous plants [15,16]. The presence of tannins in the extract may play a role as antimicrobials and astringents [17].

Table 1: Qualitative phytochemical analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

Phytochemical		Aqueous extract
Alkaloids	Dragendroff test	-
	Wagner's test	+
	Meyer's test	-
Flavonoids		+
Saponins		-
Carbohydrates	Fehling's reaction	+
	Benedicts test	+
	Molisch's reaction	+
Protein	Millon's reaction	+
	Biuret test	+

Phenols	Ferric chloride test	-
	Lead acetate test	+
	Liebermann reaction	-
Steroids	Liebermann-Burchards	-
	Salkowski reaction	+
Glycosides		-
Resins		-
Tannins	Ferric chloride	+
	Lead acetate	+
Thiols		-

3.2 Quantitative phytochemical analysis in aqueous seed extract of *Sesbania sesban* (L) Merr.

The aqueous extracts of seeds of *Sesbania sesban* were analysed for the secondary metabolites

such as total phenols, flavonoids, flavonols, tannins & condensed tannins and primary metabolites carbohydrate, protein & starch content. The results of the phytochemical analysis are presented in table 2 & 3.

Table 2 Quantitative secondary metabolites in aqueous seed extract of *Sesbania sesban* (L) Merr.

Total phenols		Flavanoids		Flavonols	Tannins	Condensed Tannins
mg /g (GAE)	mg/g (CE)	mg /g (QE)	mg /g (RE)	mg/g (CAE)	mg/g (CAE)	mg/g (CAE)
28.10 ± 0.18	33.69 ± 0.06	17.57 ± 0.10	20.68 ± 0.09	6.08 ± 0.21	12.34 ± 0.07	3.84 ± 0.11

Values are expressed as mean ± SD (n=3)

Table 3. Quantitative primary metabolites in aqueous seed extract of *Sesbania sesban* (L) Merr.

Carbohydrate	166.0 ± 10.07 mg/g
Protein	25.6 ± 1.50 mg/g
Starch	147.6 ± 6.73 mg/g

Values are expressed as mean ± SD (n=3)

Quantitative analysis demonstrated appreciable levels of total phenols, flavonoids, flavonols, and tannins. High phenolic and flavonoid contents are strongly correlated with free radical scavenging and antioxidant capacity, which play a crucial role in preventing oxidative stress-related disorders [18]. Flavonoids play important roles in preventing diseases associated with oxidative stress. It has the capacity to transport electrons to free radicals,

inhibit oxidases, reduce radicals of alpha tocopherol, activate antioxidant enzymes and chelate metals [19]. The presence of condensed tannins enhances the antimicrobial and anti-inflammatory properties of the extract. Additionally, the significant carbohydrate and starch content observed in the primary metabolite analysis highlights the nutritional value of *S. sesban* seeds, while the detectable protein content supports their use as a

supplementary protein source [20]. Overall, the findings validate the medicinal and nutraceutical importance of *Sesbania sesban*.

3.4 FTIR spectral analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the major functional groups present in the aqueous seed extract of *Sesbania sesban*. The spectrum exhibited a broad absorption band around 3330–3270 cm^{-1} , corresponding to O–H stretching vibrations, indicating the presence of hydroxyl groups associated with carbohydrates, phenolic compounds, and polysaccharides. The peaks observed in the

region of 2920–2850 cm^{-1} were attributed to C–H stretching vibrations of aliphatic chains, suggesting the presence of lipids and alkanes. A prominent absorption band at 1632 cm^{-1} corresponds to C=O stretching and N–H bending vibrations, characteristic of amide groups, confirming the presence of proteins. The bands near 1515–1450 cm^{-1} represent aromatic C=C stretching and C–H bending vibrations, indicating phenolic constituents. Strong peaks around 1220 and 1033 cm^{-1} are associated with C–O and C–O–C stretching vibrations, characteristic of carbohydrates and starch. Overall, the FTIR spectrum confirms the presence of primary metabolites such as carbohydrates, proteins, and starch in the aqueous seed extract.

Figure.1 FTIR spectral analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

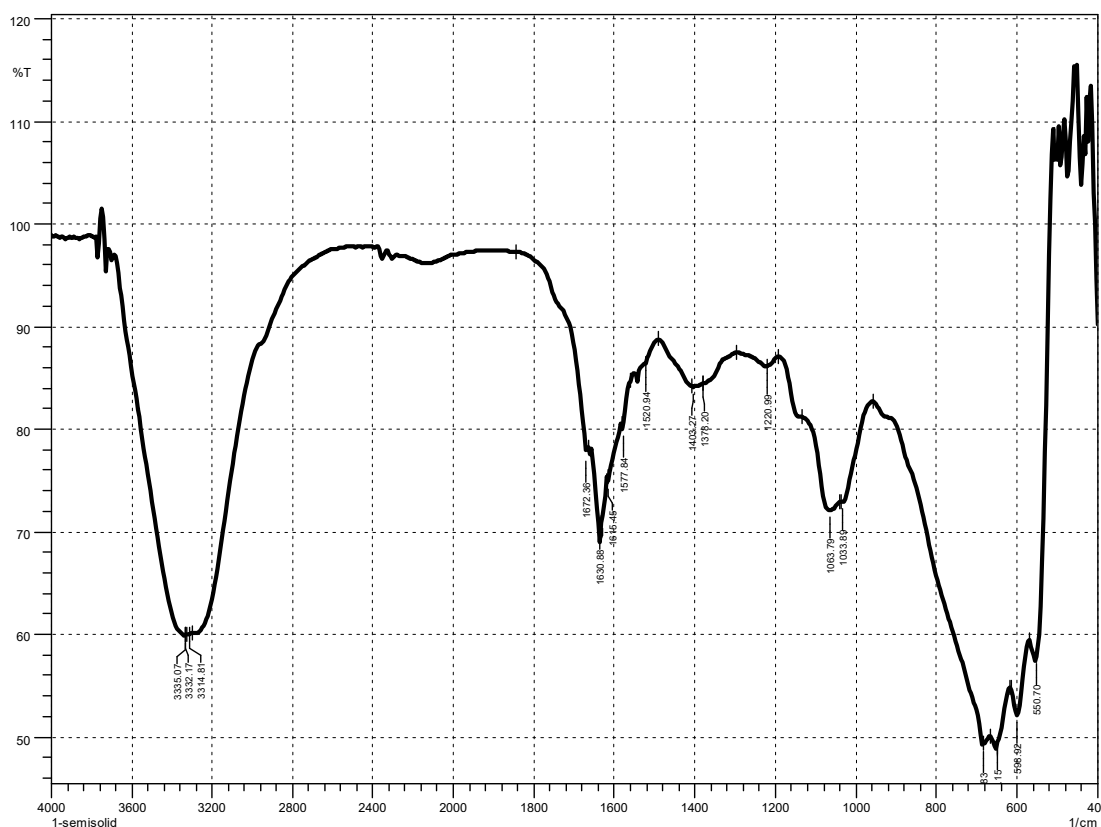


Table 4. FTIR spectral analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

Peak Value (cm^{-1})	Type of Bond / Functional Group	Type of Compound
3330–3270	O–H stretching	Alcohols, phenols, carbohydrates
2920–2850	C–H stretching	Alkanes, lipids
1632	C=O stretching / N–H bending	Amides, proteins
1580	N–H bending	Proteins, amines
1515	Aromatic C=C stretching	Phenolic compounds
1450	C–H bending	Alkanes, lipids
1382	C–H bending	Carboxylic acids, alkanes
1220	C–O stretching	Polysaccharides, phenols

1033	C–O–C stretching	Carbohydrates, starch
890	β-glycosidic linkage	Polysaccharides
670	C–H out-of-plane bending	Aromatic compounds
600	C–X stretching	Halogenated compounds / fingerprint region

3.5. GCMS analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

GC–MS analysis was employed to identify and characterize volatile and semi-volatile phytochemicals present in the plant extract. The

technique enables efficient separation and precise mass-based identification of bioactive compounds, providing valuable insights into the chemical composition and potential biological activities of the extract.

Figure.2. GCMS analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

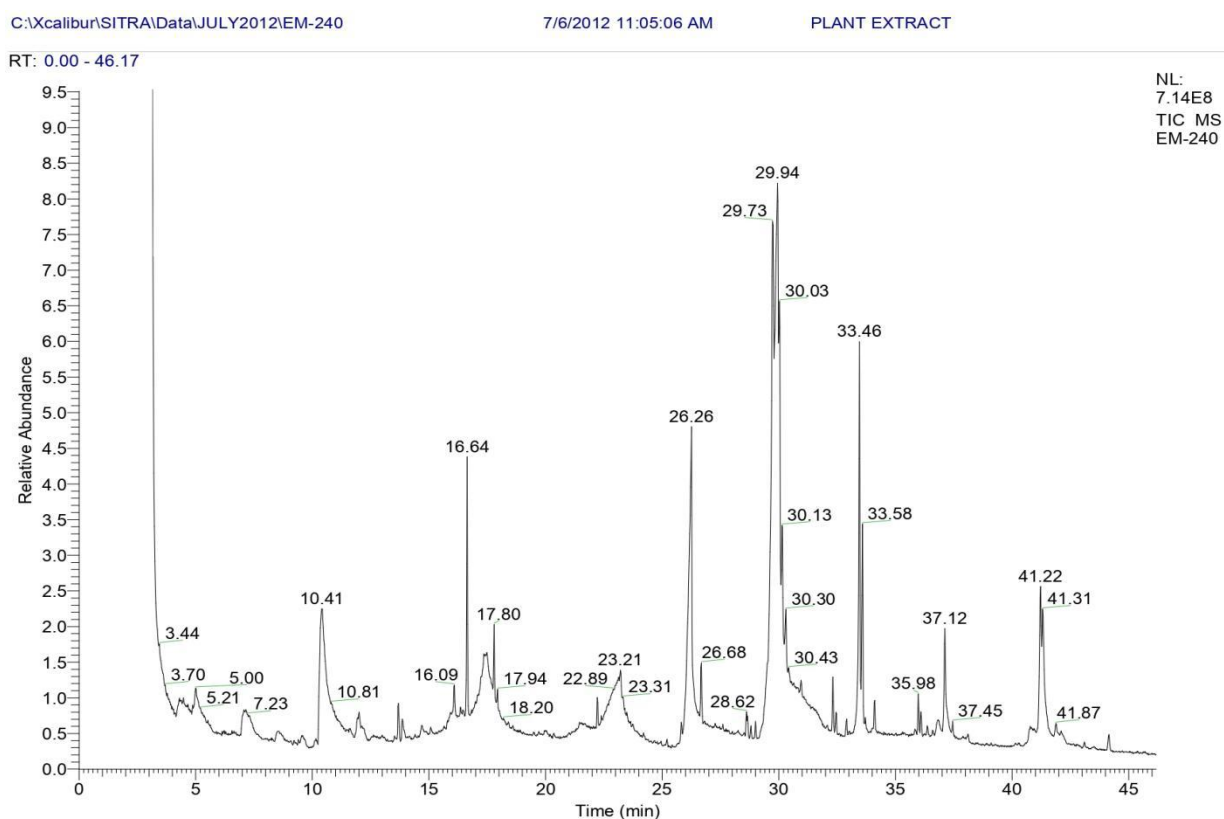


Table.5 GCMS analysis of aqueous seed extract of *Sesbania sesban* (L) Merr.

Retention Time (min)	Compound Name	Molecular Formula	Peak Area (%)	Bioactivity Potential
3.04	Ethanol	C ₂ H ₆ O	16.32	Solvent residue; antimicrobial carrier
10.41	5-(Hydroxymethyl)-2-furaldehyde	C ₆ H ₆ O ₃	4.26	Antioxidant, anti-inflammatory
17.80	1-Tetradecanol	C ₁₄ H ₃₀ O	0.80	Antimicrobial
23.16	Azacyclopentanone derivative	C ₉ H ₁₅ NO ₂	4.26	Bioactive alkaloid derivative
26.68	Ethyl stearate	C ₂₀ H ₄₀ O ₂	0.80	Anti-inflammatory
32.32	Imidazole thione derivative	C ₁₂ H ₁₆ N ₄ O ₂ S	0.59	Antimicrobial
34.11	Artemisia alcohol	C ₁₀ H ₁₈ O	0.59	Antioxidant
37.12	2-Monostearin	C ₂₁ H ₄₂ O ₄	0.59	Anti-inflammatory
40.78	Oxoapomorphine	C ₁₇ H ₁₃ NO ₂	0.43	Neuroprotective

GC–MS analysis of *Sesbania sesban* plant extract revealed a diverse profile of bioactive compounds including alcohols, fatty acid esters, aldehydes, and nitrogen-containing heterocycles. The presence of hydroxymethyl furfural and long-chain alcohols indicates antioxidant and antimicrobial potential. Fatty acid esters such as ethyl stearate and monostearin contribute to anti-inflammatory activity. Alkaloid and heterocyclic compounds suggest pharmacological relevance. Overall, the GC–MS profile supports the therapeutic potential of *Sesbania sesban* and validates its traditional medicinal use.

4. Conclusion

The plants belonging to *sesbania* species was reported to have rich phytochemical diversity and many plants with most parts have a significant role in traditional medicine. The present study gave a broad phytochemical characterization of phytochemicals both qualitatively and quantitatively followed by FTIR and GCMS analysis. Qualitative phytochemical analysis confirmed the presence of phenols, flavonoids, steroids, tannins, carbohydrates and proteins revealing the phytochemical richness of the plant sample. Quantitative estimation of phytochemicals further confirmed the quantities of phytochemicals which can be inferred to the antioxidant and therapeutic effect of the seed sample. FTIR analysis exposed characteristic absorption bands matching to functional groups such as hydroxyl, carbonyl, amine, and aliphatic groups, which are often related with phenolic compounds, flavonoids, proteins, and other secondary metabolites. GC–MS analysis enabled the identification of several volatile and semi-volatile compounds with known pharmacological relevance, including antioxidant, antimicrobial, anti-inflammatory, and metabolic regulatory properties.

On the whole, the outcomes authenticate the medicinal significance of *Sesbania sesban* seeds and highlight the suitability of aqueous extraction for isolating biologically active phytochemicals. The study delivers a scientific basis for further in-depth investigations, including bioactivity-guided fractionation, in vitro and in vivo pharmacological evaluations, and the development of plant-based therapeutic formulations.

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Conflict of interest:

The authors declares no conflict of interest.

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