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RESEARCH ARTICLE

GC-MS Profiling and Neuroprotective Potential of *Sida mysorensis*: *In Vitro* Antioxidant and Acetylcholinesterase Inhibitory Activities for Alzheimer's Disease Management

Kokila Kuppusamy[1] and Hemnath Elango[2*]

[1]Karpagam Academy of Higher Education, Eachanari, Coimbatore - 641021, Tamil Nadu, India
[2]Drug Testing Laboratory, Karpagam Academy of Higher Education, Coimbatore - 641021, Tamil Nadu, India; hemnathelango@kahedu.edu.in

Abstract

Background: Oxidative stress is a major contributing factor in the pathogenesis of Alzheimer's Disease (AD). Medicinal plants are increasingly explored as sources of natural antioxidants and cholinesterase inhibitors to mitigate oxidative damage and manage AD. *Sida mysorensis* has shown notable therapeutic potential due to its rich phytochemical composition. **Aim:** To investigate the pharmacognostical and phytochemical characteristics, antioxidant potential, and anti-cholinesterase activity of chloroform and ethanolic extracts of *S. mysorensis*. **Methods:** Antioxidant activity was evaluated using ABTS, hydrogen peroxide (H_2O_2), and DPPH radical scavenging assays. Acetylcholinesterase (AChE) inhibition was assessed to determine neuroprotective potential. Total phenolic and flavonoid contents were quantified. The chloroform extract (CESM), which exhibited superior activity, underwent GC-MS analysis to identify its phytochemical constituents. **Results:** CESM was rich in phenols (63.90 mg GAE/g) and flavonoids (37.68 mg QE/g) and demonstrated potent antioxidant activity with IC_{50} values of 21.08 µg/mL (ABTS), 30.98 µg/mL (H_2O_2), and 35.06 µg/mL (DPPH), surpassing the standard. CESM also showed significant AChE inhibitory activity (IC_{50} = 132.46 µg/mL), suggesting potential in AD-related neurodegeneration management. GC-MS profiling identified over 38 phytochemicals with retention times ranging from 3.96 to 37.5 minutes. **Conclusion:** The study confirms *S. mysorensis*, particularly its chloroform extract, as a promising source of natural antioxidants and cholinesterase inhibitors. Its potent in vitro bioactivities highlight its potential for AD treatment, warranting further research to isolate and characterize the active compounds responsible for these effects.

Major Findings: The Chloroform Extract of *S. mysorensis* (CESM) demonstrated significant antioxidant activity (IC_{50}: 21.08-35.06 µg/mL) and potent acetylcholinesterase inhibition (IC_{50}: 132.46 µg/mL), correlating with its high phenolic and flavonoid content. GC-MS profiling revealed 38 bioactive phytochemicals, supporting its Fpotential as a natural therapeutic candidate for Alzheimer's disease.

Keywords: Alzheimer's Disease, Antioxidant Potential, Cholinesterase, DPPH, GC-MS, *S. mysorensis*

1. Introduction

Dementia has become a significant health issue worldwide. As outlined in the World Health Organization's 2022 dementia research plan, it is estimated that 55.2 million people around the world are impacted[1] Alzheimer's Disease (AD), noted to be the predominant mode of dementia, demonstrates comparable epidemiological patterns and poses a significant and increasing global challenge[2]. AD is a condition that leads to nerve cell degradation and progressive cerebrum atrophy, resulting in memory loss and various cognitive difficulties, including declines in various skills[3]. The AD pathology is noted to be the

*Author for correspondence

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extracellular plaques made of β-amyloid (Aβ) peptide and neurofibrillary tangles caused by tau protein (hyperphosphorylated) within neurons[4].

Since the early 1970s, basal forebrain cholinergic neuron loss has been documented in the patient's brain with AD. This finding has contributed to the formulation of the cholinergic hypo thesis, which addresses age-related memory dysfunction[5]. The equilibrium of various neurotransmitter systems, including Acetylcholine (ACh), noradrenaline, dopamine, serotonin, and glutamate, is crucial for optimal function of the brain[6]. The cholinergic system, while not the sole neurotransmitter system, plays a notable part in the pathophysiology of learning as well as memory deficits associated with adult-onset dementia disorders. A deficiency in cholinergic neurotransmission is a critical factor contributing to these cognitive impairments[7]. Therapeutic approaches aimed at improving the cholinergic neurotransmission have primarily focused on the identification of Acetyl Cholinesterase (AChE) inhibitors. These inhibitors function by preventing the degradation of ACh, resulting in increased neurotransmitter levels in the brain and thereby enhancing deficient cholinergic neurotransmission[5,8].

Previous reports have evidenced oxidative stress as one of the causes for AD, establishing it as a significant therapeutic strategy[9]. Overproduction of Aβ in the brain is attributed to a disruption amongst synthesis and degradation of Aβ within self-regulatory pathways. This imbalance is primarily driven by oxidative stress and neuroinflammation[10]. Owing to its intricate etiopathology, existing pharmacological treatments have been unable to achieve a complete cure for the disease. Current research endeavours focus on therapeutic strategies are at slowing or halting the progression of AD[11,12]. Extensive research has been conducted on natural compounds as alternative therapies. A range of herbs with beneficial neurochemical constituents has been identified, including plant-derived molecules that possess anti-neurodegenerative and neuroprotective properties[13].

The *Sida* genus is recognized as the most diverse species within the *Malvaceae* family, encompassing approximately 200 species that are disseminated globally[14]. Phenolic compounds present in *S. mysorensis* are recognized as biologically active constituents with notable biological properties. These include the capacity for free radical scavenging, the inhibition of oxidative enzymes, and the demonstration of anti-inflammatory effects[15]. Our goal is to examine the preliminary pharmacognostical studies, phytochemical profiling, antioxidant activity, and anti-cholinesterase activity of *S. mysorensis*.

1.1 Novelty and Comparison with other *Sida* Species

Sida mysorensis differs both chemically and pharmacologically from other species like *Sida cordata* and *Sida rhombifolia*. GC-MS profiling of *S. mysorensis* revealed a unique and rich diversity of 38 phytoconstituents, spanning phenolics (notably resorcinol), fatty acids (n-hexadecanoic acid, cis-vaccenic acid), sugar derivatives (3-O-methyl-D-glucose), sterols (stigmasterol, β-sitosterol, campesterol), triterpenoids (lupeol, glutinol), and neuroactive compounds (levodopa, valproic acid). While *S. rhombifolia* and *S. cordata* also contain phenolics and flavonoids, *S. mysorensis* showed higher total phenolic and flavonoid content, and the chloroform extract demonstrated stronger acetylcholinesterase inhibition (IC_{50} = 132.46 µg/mL) and antioxidant activity than reported for *S. rhombifolia (e.g.*, 58.5% AChE inhibition at 100 µg/mL). These differences in chemical profile and biological potency, particularly the dual antioxidant and neuroprotective action in *S. mysorensis*, underscore the novelty and value of this study compared to prior work on other *Sida* species.

2. Materials and Methods

2.1 Materials

Dragendorff's reagent was procured from MOLYCHEM (Global Scientific Company, Erode). Mayer's reagent obtained from ISOCHEM (Chemico Glass and Scientific Company, Erode). Hager's reagent was sourced from Ozone, and Molisch reagent was supplied by Spectrum. Folin-ciocalteu reagent was purchased from HIMEDIA. Chemicals assessed in the study are of analytical grade quality.

2.2 Plant Materials

2.2.1 Collection and Identification

During the flowering period in September 2023, *S. mysorensis* was collected in the Erode, Tamil Nadu and

authenticated and identified by M. U. Sharief (Scientist 'F'), from Botanical Survey of India, Coimbatore (No.: BSI/SRC/5/23/2023/Tech) dated 25th September 2023. The obtained plant material was air dried and examined for macroscopic and microscopic characteristics. Finally, the dried whole plant was finely powdered and then passed through a sieve. 40 to for homogeneous powder. The homogeneous powder was further used for the investigations. The fresh leaf sample was utilized for microscopical identification.

2.3 Pharmacognostical Evaluation

2.3.1 Plant Description and Macromorphology

Sida mysorensis was evaluated for its organoleptic and macroscopic characteristics, utilizing its leaves, stems, and roots. This assessment focused on the odour, colour, and shape of the various plant parts. Additionally, the arrangement and morphology of the leaves were meticulously examined.

2.3.2 Plant Anatomy, Quantitative Microscopy and Powder Microscopy

Using a sterile razor, sections of transversely cut *S. mysorensis* fresh leaf, stem, and root midribs were prepared for the analysis. Microscopic sections were prepared to examine the distinctive features of the plant. The specimens were mounted using phloroglucinol in hydrochloric acid, Sudan IV, and N/50 iodine to facilitate thorough analysis. The images of the TS were captured using a binocular under bright field illumination. The cellular characteristics were also carefully observed and documented through photomicrography.

2.4 Methods for Physicochemical Parameters

Physicochemical parameter was carried out using the standard procedures[16].

2.5 Plant Preparation

The dried and coarsely powdered entire plant (1000 gm) was extracted progressively with chloroform and ethanol by continuous hot percolation in a Soxhlet extractor. The crude extracts were evaporated until dry and then weighed. Preliminary qualitative phytochemical screening of chloroform and ethanol extracts for the identification of several active components was performed using typical conventional techniques.

2.6 Phytochemical Profiling

2.6.1 Total Flavonoids Estimation

The method of Karamian and Ghasemlou was followed to estimate the total flavonoids[17]. To CESM and EESM (1 mg/mL), ethanol and 0.5 mL 2% $AlCl_3$ were added and made to stand for 1 hour, and at 420nm the absorbance was measured. A calibration curve was plotted with quercetin as a reference. The quantification was based on the calibration curve, where y = 0.0136x + 0.0335, R^2 = 0.9994, and x was absorbance.

2.6.2 Total Phenols Estimation

The total phenols were quantified by following Singleton and Rossi method, with minor modifications. To CESM and EESM (1mg/mL), add water and Folin-ciocalteu reagent (0.5 mL and 0.2 mL) (1:1). After 5 minutes, 1 mL of saturated Na_2CO_3 (8% w/v in H_2O) was added, and made up to 5 mL with deionized water. The absorbance was noted at 765 nm after 30 minutes of incubation. Total phenols were estimated as GAE/g of extract by the standard curve, y = 0.0098x + 0.0194, R^2 = 0.9995.

2.7 ABTS Radical Scavenging Assay

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity of CESM and EESM was assessed by following Re *et al.,*[18]. To produce ABTS•+ radicals, 140 mM potassium persulfate (440 µL) was added to the ABTS stock (7 mM) in deionized water to oxidize it. ABTS was incubated for 12-16 hours in the dark.

The tests were prepared in 5 to 320 µg/mL concentrations in methanol. In a 96-well plate, add 2.0 mL of the ABTS stock standard and 200 µL of each test or standard (ascorbic acid). After gently shaking the reaction mixtures, incubate for 20 minutes, and note the absorbance at 734 nm.

$$\% \text{ ABTS scavenging} = (A_0\text{-}A_1/A_0) \times 100$$

A_0 and A_1 are the absorbance of the control and sample.

2.8 Hydrogen Peroxide (H_2O_2) Scavenging Assay

Fresh H_2O_2 solution was prepared in phosphate buffer (pH - 7.4) to measure the activity. H_2O_2 solution (0.6

mL) was mixed with CESM and EESM of 5 - 320 µg/mL concentration. After 10 minutes incubation, at 230 nm, the absorbance was noted. Blank was employed to account for background absorbance. The IC_{50} value was calculated and contrasted with a standard drug, tocopherol.

$$\% \text{ Scavenged } (H_2O_2) = (A_c - A_s/A_c) \times 100$$

A_c and A_s is the absorbance of the control and sample[19].

2.9 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Scavenging Assay

The DPPH scavenging experiment was performed to evaluate the antioxidant capacity of CESM and EESM. Stock DPPH (0.135 mM) was prepared in methanol and stored in the dark. The concentrations of the samples were 5 - 320 µg/mL. Each sample and standard were added with 100 µL of DPPH. To ensure a complete reaction, the reaction mixture was gently stirred and made stand in for half an hour in dark, and at 517nm the absorbance was measured.

$$\% \text{ inhibition of DPPH} = (A_0 - A_1/A_0) \times 100$$

A_0 and A_1 are the absorbance of the control and sample[20].

Antioxidant assays: IC_{50} values

For all antioxidant assays (ABTS, H_2O_2, DPPH), standard reference compounds were used alongside plant extracts. The IC_{50} values of these reference standardsfor example, ascorbic acid and α-tocopherolwere determined and are presented consistently for comparison with extract IC_{50} within all results tables and corresponding figure captions

2.10 Inhibition Assay for AChE

The AChE inhibitory activity of CESM and EESM was assessed by following Ellman *et al.* method[21]. To the well, add 140 µL of potassium phosphate buffer (0.1 M) with a pH of 8.0, 20 µL of freshly prepared 5,5′-Dithiobis-(2-Nitrobenzoic acid) (DTNB) (3 mM), and 20 µL of Acetylthiocholine Iodide (AChI) (15 mM) as the substrate. Concentrations of 25 - 400 µg/mL of CESM and EESM were prepared in buffer, and 10 µL of each was added to individual wells. Initiated the reaction by adding 10 µL of AChE solution (0.16 U/mL), except for the blank, which received buffer instead of enzyme. The plate was gently shaken to ensure mixing and incubated for 5 minutes. Hydrolysis of AChI by AChE produces thiocholine, which reacts with DTNB to form 5-thio-2-nitrobenzoate, yielding a yellow colour measurable at 405 nm.

$$\% \text{ AChE inhibition} = (A_0 - A_1/A_0) \times 100$$

A_0 and A_1 are the absorbance of the control and sample.

2.11 GC-MS Analysis of Chloroform Extract of *S. mysorensis*

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to identify and characterize the bioactive constituents present in the chloroform extract of *S. mysorensis*. The analysis was performed using a Shimadzu GC-MS system (Model QP2010S) equipped with a capillary column ELITE-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness).

The GC-MS system was operated under the following conditions: the column oven temperature was initially maintained at 70° C for 2 minutes, then increased at a rate of 10° C/min to 200° C, held for 5 minutes, followed by a further increase at 5° C/min to 280° C, and maintained for 15 minutes. The total run time was 51 minutes.

The injection temperature was set at 260° C, and the injection was performed in split less mode with a split ratio of 50:1. Helium was used as the carrier gas at a constant flow rate of 1.00 mL/min in linear velocity mode (36.7 cm/s). Other flow parameters included a total flow of 54.0 mL/min, purge flow of 3.0 mL/min, and inlet pressure of 61.5 kPa. The sampling time was 2 minutes.

Identification of compounds in the GC-MS analysis was performed by comparing the obtained mass spectra with the NIST (National Institute of Standards and Technology) mass spectral library. A match factor cut-off value of 70% or higher was set as the threshold for compound confirmation, in line with established analytical practice, where compounds with match factors above 700 on the NIST scale are considered reliable identifications.

Mass spectrometric detection was carried out in Electron Ionization (EI) mode at 70 eV, with the ion source temperature set at 200° C and interface

temperature at 280° C. The mass spectra were recorded in scan mode over the m/z range of 50-500, with a scan speed of 1000 u/s and event time of 0.5 seconds. The solvent cut time was 6.50 minutes[22].

3. Results

3.1 Plant Description and Macromorphology

Sida mysorensis is a perennial herb or small shrub, thriving in tropical and subtropical regions, particularly in dry, open fields and roadsides. It has a deep taproot system and an erect, branched stem with fine stellate hairs. The ovate to cordate leaves, 2 - 8 cm long, are alternately arranged, with serrated margins and pubescent surfaces, especially underneath. The plant bears small, yellow flowers (1 - 2 cm in diameter) in axillary or terminal inflorescences, with five free petals, a lobed green calyx, and numerous stamens forming a column. The fruit is a schizocarp breaking into 8 - 12 mericarps, each containing a single dark brown,



Figure 1. Whole plant of *S. mysorensis.*

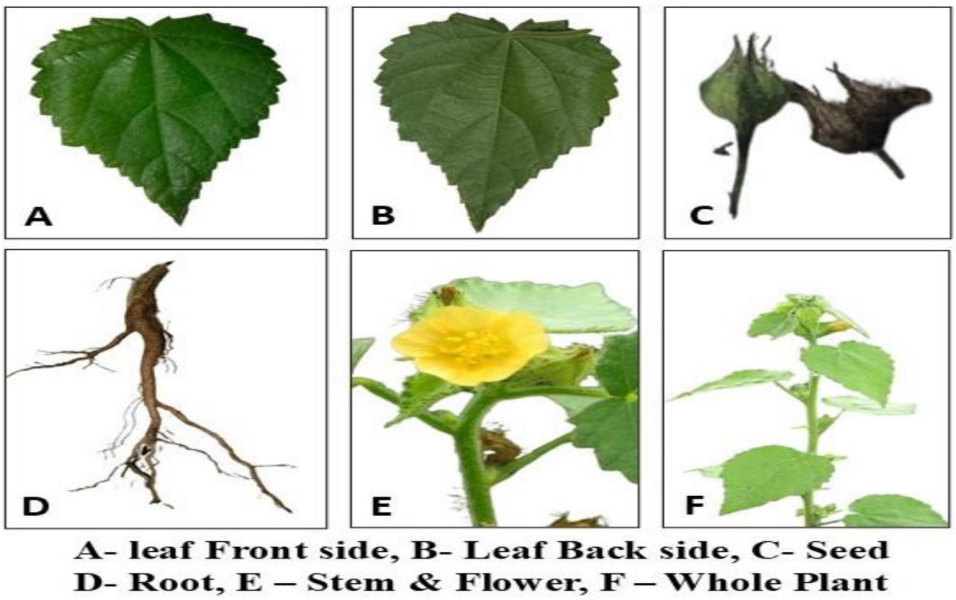


Figure 2. Plant description and macromorphology of *S. mysorensis.*

kidney-shaped seed. Additionally, the organoleptic properties of the fresh stem, leaves, and root were examined (Table 1). Figure 1 and 2 shows the plant parts of *S. mysorensis*.

3.2 Plant Anatomy and Quantitative Microscopy

3.2.1 Transverse Section Study of the Leaf

The microscopic characteristic of the leaves of *S. mysorensis* typically involve detailed observations under a microscope to identify various cellular and structural features. Epidermis: The epidermal cells are rectangular or polygonal in shape. Trichomes: Hair-like structures (Stellate trichomes) present on the surface of the leaf, though their density and type varied. Mesophyll: The mesophyll (the tissue between the upper and lower epidermis) is dorsiventral, common in dicot leaves. It is typically made up of palisade parenchyma on the upper side. Vascular bundles: Collateral (with xylem and phloem located next to each other), a common feature in many dicotyledonous plants. Vascular tissue was observed as a series of tubes transporting water (xylem) and nutrients (phloem). Figure 3 (A) shows the TS of the leaf of *S. mysorensis*.

3.2.2 Transverse Section Study of the Stem

The microscopical structure of the stem of *S. mysorensis* includes the following key features. Outer epidermis: The epidermis is a single layer of cells, consisting of thin-walled parenchyma cells. The epidermal cells have a smooth surface, or some may possess stomata (in the case of xerophytic or adapted plants). Cortex: Below the epidermis, the cortex comprises parenchymatous cells that may store water or starch. The cortex can also contain collenchyma or sclerenchyma cells in varying amounts, providing structural support to the stem.

Table 1. Organoleptic evaluation of fresh plant of *S. mysorensis*

Parameter	Leaves	Stem	Root
Colour	Light green	Light green	Light brownish
Odour	Aromatic	Aromatic	Non aromatic
Taste	Slightly bitter	Slightly bitter	Bitter
Shape	Ovate to cordate	Slender, erect	Taproot and fibrous
Size	2-5 cm	1-2 meter	15-30 cm

Vascular bundle: The vascular bundles in *S. mysorensis* are collateral or bicollateral (*i.e.*, phloem and xylem present in the same plane, with phloem located on the outer side of the xylem, or in cases of bicollateral bundles, phloem is present on both sides of the xylem). Pith: The central part of the stem, which contains large, thin-walled parenchyma cells. Figure 3 (B) shows the TS of the stem of *S. mysorensis*.

3.2.3 Transverse Section Study of the Root

The root of *S. mysorensis* has been examined for various microscopical characters to understand its structure. Epidermis: The epidermis in the root comprises a single layer of parenchymatous cells. These cells contain irregularly shaped stomata, though they are more common in aerial parts of plants. The outermost layer was covered with a cuticle, which reduces water loss. Cortex: The cortical region consists of several layers of parenchymatous cells, which may contain starch grains or other metabolic products. The cells of the cortex are loosely arranged and may contain tannins or other secondary metabolites that help in defense. Vascular bundles: The vascular bundles in the root are arranged in a radial pattern. They consist of xylem (water transport) and phloem (nutrient transport). The xylem was located in the center of the vascular bundle, while the phloem was arranged towards the periphery. The xylem contains vessels, tracheids, and fibers; the phloem contains sieve elements and companion cells. Pith: Some roots exhibited the pith, a central region composed of parenchyma cells. Figure 3 (C) shows the TS of the root of *S. mysorensis*.

3.3 Powder Microscopical Studies

Powder microscopic analysis of the whole plant powdered crude drugs showed the following characters such as calcium oxalate crystal, pitted vessels, fiber bundles, trichomes, vascular bundles, and epidermis with mesophylls. The found characters are presented in Figure 4 (a - f).

3.4 Physicochemical Parameters

The results of the total ash and other physicochemical parameters of the powdered whole plant were summarized in Table 2. The findings indicate that the values of total ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive, and moisture

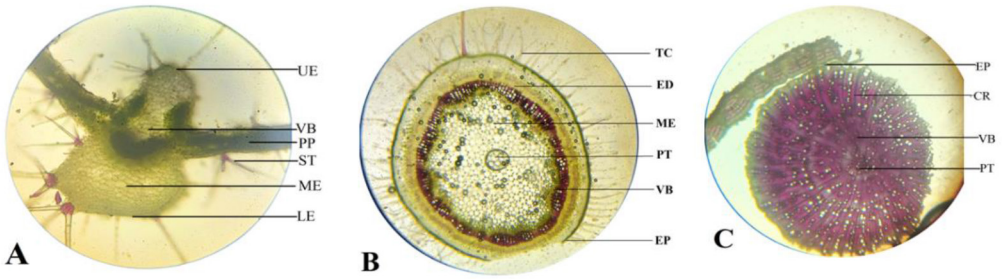


Figure 3. TS of *S. mysorensis*. **(A)** Leaf, **(B)** Stem, **(C)** Root.
UE - Upper Epidermis, VB - Vascular Bundle, PP - Palisade Parenchyma, ST - Stellate Trichomes, ME - Mesophyll, LE - Lower Epidermis, TC - Trichomes, ED - Endodermis, EP - Epidermis, CR - Cortex, PT - Pith.

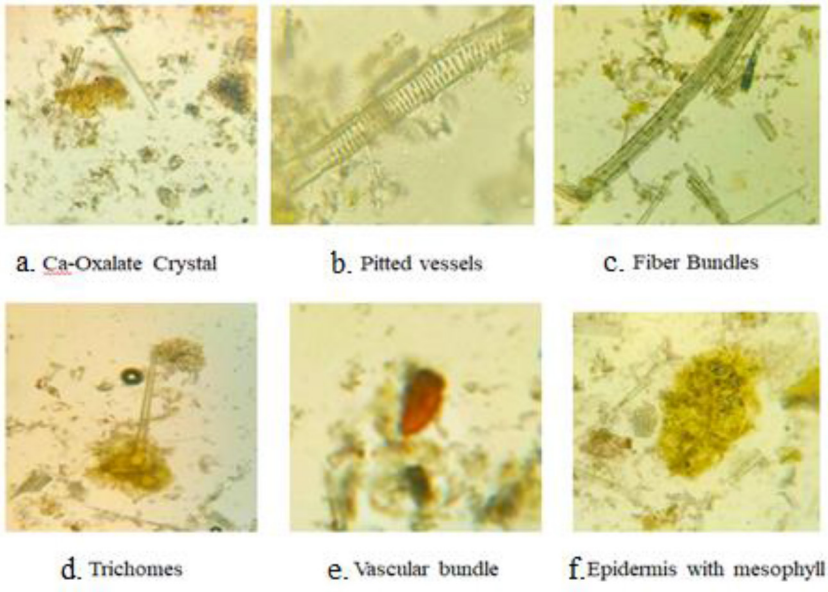


Figure 4. The whole plant powder character of *S. mysorensis.*

Table 2. Physicochemical parameters of the whole plant of *S. mysorensis*

Parameter	Values (%) w/w
Total ash	13.33 ± 0.02
Acid insoluble ash	1.01 ± 0.14
Water-soluble extractive	4.27 ± 0.24
Alcohol soluble extractive	4.01 ± 0.10
Moisture content	1.42 ± 0.18

content fall within the acceptable limits as defined by the Ayurvedic Pharmacopoeia of India.

3.5 Extraction

The whole plant of *S. mysorensis* was sequentially extracted using various organic solvents. The resulting extracts were concentrated by evaporation, and the percentage yield was calculated, as shown in Table 3. Among all the extracts, the yield from chloroform (6.8 ± 0.01% w/w) was notably higher compared to ethanol and Pet. ether.

The choice of chloroform and ethanol as extraction solvents is scientifically justified, both by their polarity range and effectiveness in isolating active phytoconstituents. Chloroform is a moderately non-polar solvent, especially effective for extracting nonpolar and moderately polar compounds such as phenolics, triterpenoids, certain alkaloids, and steroids, as shown in the current GC-MS analysis. Ethanol, being a polar protic solvent, efficiently extracts more polar constituents like flavonoid glycosides and polyphenols. The sequential extraction approach maximizes recovery of both polar and nonpolar compounds. Supporting literature validates

that chloroform and ethanol efficiently extract a wide phytochemical spectrum from related *Sida* species, capturing bioactive compounds with neuroprotective, antioxidant, and anti-inflammatory properties[15,14,23]. The superior antioxidant and acetylcholinesterase inhibitory activities observed in the chloroform extract in this study further rationalize this solvent choice.

3.5.1 Extraction: Yield Calculation and Reproducibility

The percentage yield of each extract was calculated according to the formula:

Yield (%)=Weight of dried extract (g)Weight of dry plant material (g)×100Yield (%)=Weight of dry plant material (g)Weight of dried extract (g)×100

To ensure reproducibility, the extraction process for each solvent was repeated three times, and the average yield is reported as mean ± standard deviation, following established extraction protocols in phytochemistry

Table 3. Extractive value and colour of the extract of *S. mysorensis*

Solvent	Extractive Value (%w/w)	Colour of the Extract
Pet. ether	1.23±0.2	Dark green
Chloroform	6.8±0.1	Green
Ethanol	3.8±.012	Brownish green

Table 4. Phytochemical analysis of *S. mysorensis* (key: + = Presence;– = Absence)

Phytochemicals	Chloroform Extract	Ethanol Extract
Alkaloids	+	+
Saponins	+	+
Glycosides	-	+
Carbohydrates	+	+
Tannins	+	+
Phenols	+	+
Flavonoids	+	+
Steroids	+	+
Proteins	-	-
Triterpenoids	+	+
Fixed oil and fat	+	+
Gum and mucilage	-	-

3.6 Phytochemical Analysis

Phytochemical analysis revealed that the CESM and EESM have bioactive compounds, alkaloids, phenols, flavonoids, saponins, terpenoids and tannins, *etc*. Because of the polarity of the solvents and the progressive extraction strategy, the phytochemicals were recognized as more or less the same. Table 4 displays the results of the phytochemical study of *S. mysorensis*.

3.7 Total Phenolic and Flavonoid Content

CESM had a larger amount of phenols (63.90 mg GAE/g) and flavonoids (37.68 mg QE/g) than EESM (33.50 mg GAE/g and 20.82 mg QE/g) (Table 5). The calibration curves used for determining total phenolic and flavonoid contents are shown in Figure 5 and Figure 6, respectively. This implies that plant extracts from *S. mysorensis* contain more phenols than flavonoids.

3.8 ABTS Radical Scavenging Ability of *S. mysorensis*

The ABTS scavenging activity of CESM and EESM extracts, along with their absorbance values, is presented in Table 6. As shown in Table 8, the percentage of inhibition increases with the concentration. Among all extracts, the better antioxidant scavenging activity was observed at a concentration of 320 µg/mL. The EESM extract exhibited weak ABTS+ scavenging activity, whereas the CESM demonstrated moderate activity in comparison to the standard. The IC_{50} values for the ABTS assay were determined to be 21.08 µg/mL for the CESM and 26.3 µg/mL for the EESM, while the standard with IC_{50} of 13.69 µg/mL.

3.9 Efficiency of *S. mysorensis* in Scavenging H_2O_2

Table 7 shows the H_2O_2 scavenging activity of *S. mysorensis* CESM and EESM extracts at different concentrations (5–320 µg/mL). The scavenging effect increased with concentration for both extracts. The

Table 5. Total phenols and flavonoids of extracts

Extract	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
CESM	63.90	37.68
EESM	33.50	20.82

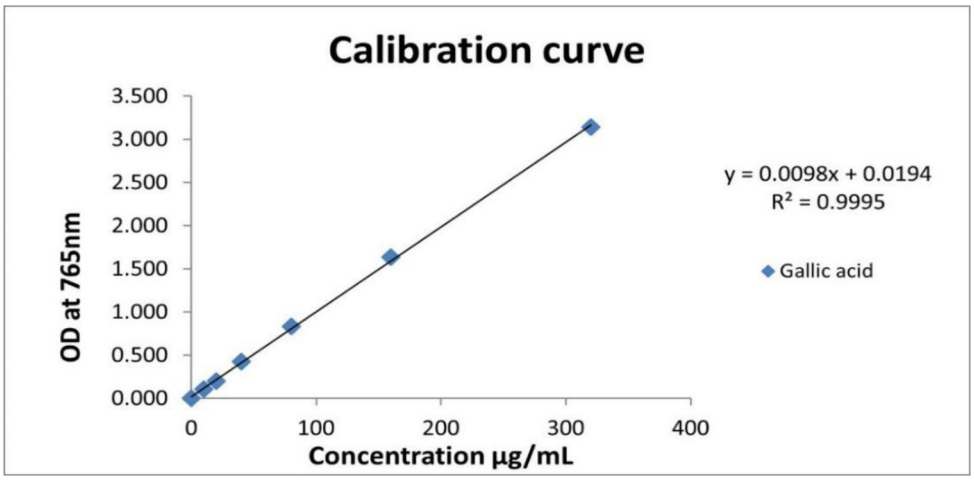


Figure 5. Calibration Curve for total phenolic content.

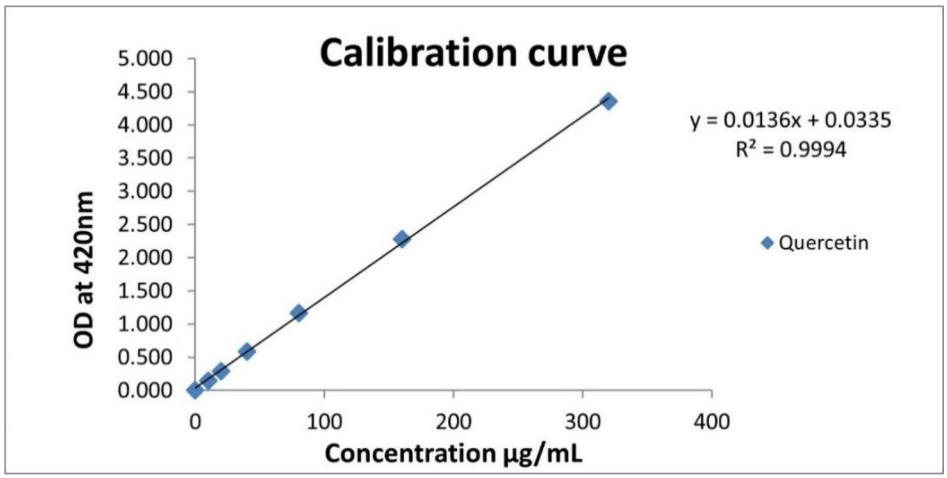


Figure 6. Calibration curve for flavonoid contents.

Table 6. IC_{50} (µg/mL) values of ABTS scavenging assay of *S. mysorensis* extracts

Conc. (µg/mL)	CESM	EESM	Ascorbic Acid
5	15.27	17.94	9.89
10	30.46	22.32	44.21
20	58.70	42.43	71.87
40	68.41	66.92	86.76
80	83.05	81.88	93.82
160	86.71	86.71	96.38
320	92.54	89.83	97.85
IC_{50} (µg/mL)	21.08	26.3	13.69

Table 7. IC_{50} (µg/mL) values of H_2O_2 scavenging assay of *S. mysorensis* extracts

Conc. (µg/mL)	CESM	EESM	α-Tocopherol
5	2.05	0.65	7.08
10	24.48	9.64	28.72
20	40.10	17.64	51.38
40	69.85	24.58	76.99
80	78.97	34.29	85.30
160	83.93	56.41	93.06
320	89.26	65.71	96.17
IC_{50} (µg/mL)	30.98	143.99	22.57

CESM had a strong scavenging ability, with an IC_{50} value of 30.98 µg/mL, and fairly close to the standard α-Tocopherol (IC_{50} = 22.57 µg/mL). However, the EESM had a much higher IC_{50} value of 143.99 µg/mL, indicating a weaker scavenging effect. These findings suggest that CESM was more effective at neutralizing H_2O_2 than EESM, making it a promising antioxidant source.

3.10 Efficiency of *S. mysorensis* in Scavenging ROS

Plant extracts from *S. mysorensis* have been studied for their antioxidant activity based on their ability to reduce DPPH (Table 8).

Table 8 shows the significant concentration-dependent radical scavenging activity of CESM and EESM. In the DPPH experiment, the CESM and EESM extracts had respective IC_{50} values of 35.06 µg/mL and 52.62 µg/mL. The standard has an IC_{50} of 14.23µg/mL. The CESM extract consequently demonstrated the highest antioxidant activity in contrast to the EESM, which had the lowest IC_{50} at increasing doses.

3.11 AChE Inhibition Assay

The AChE inhibitory potential of *S. mysorensis* was evaluated using the widely recognized modified Ellman's method, with the results presented in Table 9. Both the CESM and EESM extracts exhibited dose-dependent AChE inhibition. The findings indicated that the CESM extract displayed the highest inhibition against AChE. The IC_{50} values for the CESM and EESM were observed to be 132.46 µg/mL and 290.76 µg/mL. In comparison, the standard galantamine exhibited an IC_{50} value of 38.18 µg/mL. While CESM demonstrated stronger activity than the EESM, its potency was moderate when compared to the standard galantamine, which is currently used in AD treatment. Nonetheless, these findings confirm the AChE inhibitory activity of *S. mysorensis*.

3.11.1 Statistical Analysis

All experiments including antioxidant assays and AChE inhibition were performed in triplicate (n=3). Results are expressed as mean ± Standard Deviation (SD). IC_{50} values were calculated by nonlinear regression analysis with confidence intervals reported where applicable. Statistical comparisons among groups were conducted using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant. This statistical treatment ensures robustness and reproducibility of the findings.

3.11.2 Interpretation of Results

The discussion should be revised to include more critical comparisons with previous studies on other *Sida* species such as *S. rhombifolia* and *S. cordata*, especially regarding antioxidant potency and AChE inhibitory activity. For instance, the stronger AChE inhibition exhibited by *S. mysorensis* (IC_{50} 132.46 µg/mL) versus lower inhibition reported for *S. rhombifolia* highlights its superior pharmacological profile. Additionally, reference standard antioxidants (ascorbic acid, tocopherol) and inhibitors (galantamine) should be used as benchmarks for potency comparison.

3.11.3 Claims of Neuroprotective Potential

Conclusions should be moderated by emphasizing these are preliminary *in vitro* results. Statements should clarify that while antioxidant and AChE inhibitory activities suggest potential neuroprotective benefits, confirmation requires comprehensive *in vivo* studies and mechanistic investigations to validate efficacy and safety in biological systems relevant to neurodegenerative conditions.

These revisions will strengthen scientific rigour, provide balanced interpretation, and appropriately caution readers about the scope of the findings. For statistical analysis, include that all experiments were done in triplicate (n=3), presenting data as mean ±

Table 8. IC_{50} (µg/mL) values of DPPH scavenging assay of *S. mysorensis* extracts

Conc.(µg/mL)	CESM	EESM	Ascorbic Acid
5	7.42	6.19	12.85
10	16.89	11.50	47.56
20	34.20	21.93	64.66
40	56.22	32.12	79.13
80	76.20	68.48	93.38
160	88.12	79.17	95.42
320	91.73	88.25	97.75
IC_{50} (µg/mL)	35.06	52.62	14.23

Table 9. IC_{50} (µg/mL) values of AChE inhibition assay of *S. mysorensis* extracts

Conc.(µg/mL)	CESM	EESM	Galantamine
25	0.98	0.30	40.64
50	14.20	13.26	52.95
100	38.30	25.45	72.88
200	63.11	37.39	83.52
400	88.14	65.27	90.57
IC_{50} (µg/mL)	132.46	290.76	38.18

standard deviation. IC_{50} values were calculated using nonlinear regression, and significance was assessed by one-way ANOVA with $p < 0.05$ considered significant.

In the discussion, compare the antioxidant and AChE inhibition results critically with those from other *Sida* species and standard compounds like galantamine or ascorbic acid, highlighting differences in potency.

Temper neuroprotective claims by clarifying that findings are based on *in vitro* assays only, and that *in vivo* studies and mechanistic data are needed to substantiate therapeutic potential.

3.12 GC-MS Analysis of Chloroform Extract of *S. mysorensis*

The GC-MS chromatogram of the chloroform extract of *S. mysorensis* (Figure 7) revealed the presence of a diverse range of phytochemical compounds with varying retention times, molecular weights, and relative abundances (Table 10). A total of 38 bioactive compounds were identified based on their retention times, molecular formulae, and comparison with NIST library data.

The major constituent detected was 3-O-Methyl-D-glucose (32.41%), followed by Resorcinol (14.08%) and N-Dodecylurea (8.14%), indicating that carbohydrates, phenolic compounds, and nitrogenous metabolites are predominant in the chloroform fraction. Other notable components include n-Hexadecanoic acid (4.34%), Phenol, 4-propyl- (1.94%), 1,3-Benzenediol, 4-propyl- (1.87%), and (9E,11E)-Octadecadienoic acid (1.20%), all of which have been previously reported for their antimicrobial, antioxidant, and anti-inflammatory activities.

The chromatogram showed distinct peaks corresponding to retention times ranging from 3.96 to 17.62 minutes, confirming the complex phytochemical profile of the extract. The identification of phenolic acids (*e.g.*, salicylic acid), fatty acids (*e.g.*, dodecanoic acid, octadecanoic acid), and heterocyclic compounds suggests that *S. mysorensis* possesses significant pharmacological potential. These bioactive constituents collectively contribute to the observed biological properties of the extract, including its antioxidant and neuroprotective effects.

4. Discussion

As one of the most prevalent and devastating neurodegenerative disorders, AD affects millions of people worldwide, responsible for 60-70% of cases, and its incidence is projected to rise sharply with the aging global population. According to the WHO, more than 55 million people around the world suffer from dementia, and approximately 10 million new cases are reported every year. This highlights an urgent need for effective strategies for overall management of the condition[24].

Despite decades of research, AD remains incurable, with current therapies offering only temporary symptomatic relief. The complexity of AD, driven by Aβ plaque formation, tau protein hyperphosphorylation, oxidative stress, mitochondrial dysfunction,

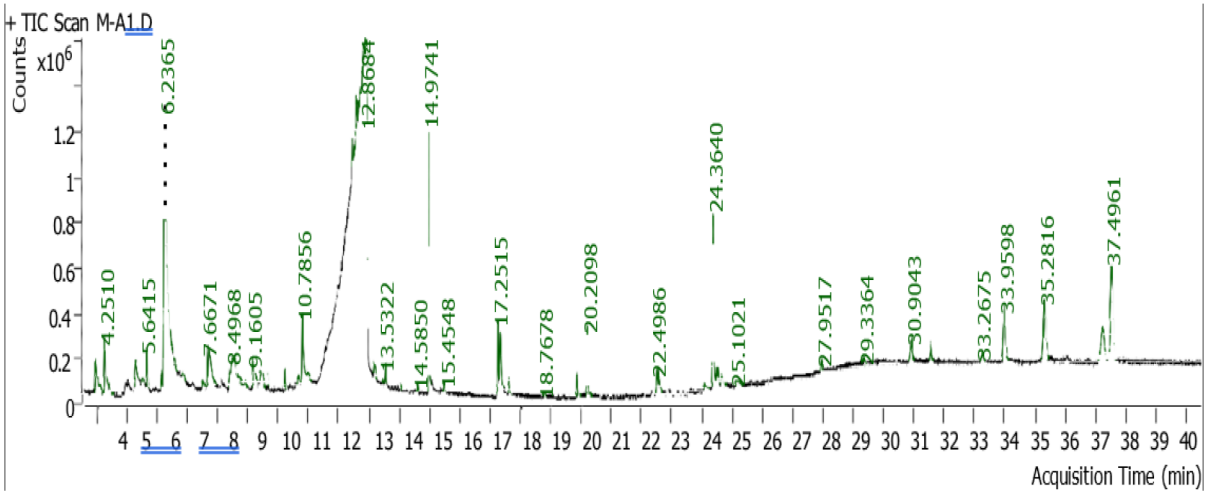


Figure 7. GC-MS chromatogram of chloroform extract of *S. mysorensis*.

Table 10. GC-MS chromatogram of chloroform extract of *S. mysorensis*

S. No	Retention Time	Compound Name	Molecular Formula	Molecular Weight	Area (%)	Match Factor	CAS ID
1	3.96	Dihydroxyacetone	$C_3H_6O_3$	90.03	1.08	71.6	96-26-4
2	4.09	l-Alanine, N-methoxycarbonyl-, heptyl ester	$C_{12}H_{23}NO_4$	245.2	0.26	58.2	1000313-37-7
3	4.25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.62	95.9	28564-83-2
4	4.39	Valproic acid	$C_8H_{16}O_2$	144.1	0.36	65	99-66-1
5	5.28	4-Vinylphenol	C_8H_8O	120.1	0.76	89.4	2628-17-3
6	5.49	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	0.17	76.9	67-47-0
7	5.64	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.1	0.74	74.7	106-61-6
8	6.15	1,3-Diazacyclooctane-2-thione	$C_6H_{12}N_2S$	144.1	0.3	71.6	5269-85-2
9	6.24	Resorcinol	$C_6H_6O_2$	110	14.08	98.2	108-46-3
10	6.50	Salicylic acid	$C_7H_6O_3$	138	0.48	83.9	69-72-7
11	6.58	Thiophene, 2-butyltetrahydro-	$C_8H_{16}S$	144.1	0.19	54.5	1613-49-6
12	6.83	1-Naphthyl n-propylcarbamate	$C_{14}H_{15}NO_2$	229.1	0.19	63.4	25216-27-7
13	7.51	Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	$C_{10}H_{12}O_2$	164.1	0.21	63.4	19784-98-6
14	7.67	Phenol, 4-propyl-	$C_9H_{12}O$	136.1	1.94	90.3	645-56-7
15	8.44	d-Glycero-d-tallo-heptose	$C_7H_{14}O_7$	210.1	1.49	74.8	1000130-14-7
16	8.50	Butanoic acid, 3-oxo-, 2-methylpropyl ester	$C_8H_{14}O_3$	158.1	1.33	55.3	7779-75-1
17	8.65	4-Vinylbenzene-1,2-diol	$C_8H_8O_2$	136.1	0.32	57.8	07-02-6053
18	8.86	o-Methoxy-.alpha.-phenethylamine	$C_9H_{13}NO$	151.1	0.17	63.5	1000334-51-1
19	9.16	β-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162.1	0.84	83.4	498-07-7
20	9.41	3-(Methoxymethoxy)butanoic acid	$C_6H_{12}O_4$	148.1	0.64	57.5	1086255-73-3
21	9.64	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.2	0.14	88.1	96-76-4
22	10.22	Dodecanoic acid	$C_{12}H_{24}O_2$	200.2	0.31	85.5	143-07-7
23	10.67	Levodopa	$C_9H_{11}NO_4$	197.1	0.26	64.9	59-92-7
24	10.79	1,3-Benzenediol, 4-propyl-	$C_9H_{12}O_2$	152.1	1.87	84.6	18979-60-7
25	10.98	5-Thio-D-glucose	$C_6H_{12}O_5S$	196	0.39	64.1	20408-97-3
26	12.43	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.1	0.27	77.7	32811-40-8
27	12.59	N-Dodecylurea	$C_{13}H_{28}N_2O$	228.2	8.14	64.8	2158-09-0
28	12.87	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.1	32.41	79.4	1000127-25-9
29	13.14	2-Hydroxy-5-methylisophthalaldehyde	$C_9H_8O_3$	164	0.35	77.9	7310-95-4
30	13.45	Acetamide, N-(4-ethoxy-3-hydroxyphenyl)-	$C_{10}H_{13}NO_3$	195.1	0.05	51.1	16060-48-3
31	13.53	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	$C_{22}H_{42}O_2$	338.3	0.25	83.2	10236-16-5
32	14.04	sTetrahydroionone	$C_{13}H_{24}O$	196.2	0.08	68.6	60761-23-1
33	14.59	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.2	0.15	84.8	82304-66-3
34	14.97	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.2	4.34	98.1	57-10-3
35	15.45	trans-Sinapyl alcohol	$C_{11}H_{14}O_4$	210.1	0.2	81.2	20675-96-1
36	17.25	(9E,11E)-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.2	1.2	97.2	544-71-8
37	17.33	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.3	1.39	92.2	506-17-2
38	17.62	Octadecanoic acid	$C_{18}H_{36}O_2$	284.3	0.23	87.3	57-11-4

neuroinflammation, and cholinergic deficits, presents a significant challenge[25]. Notably, oxidative stress and decreased acetylcholine levels are major contributors to the progression of AD. Thus, targeting oxidative damage and enhancing cholinergic function represent two crucial therapeutic strategies[26]. This underpins the importance of discovering novel antioxidant and AChE inhibitory agents, especially from natural sources with low toxicity profiles[27].

According to the anatomical examination, the leaves have a dorsiventral mesophyll, discrete vascular bundles, and stellate trichomes on both sides. These characteristics support the accurate identification and authentication of *S. mysorensis* as a valuable medicinal plant because they are consistent with those of other *Sida* species. The plant's potential therapeutic use was supported by its physicochemical characteristics, which included moisture levels and ash content that were within the permissible ranges specified in the Ayurvedic Pharmacopoeia of India. CESM and EESM extractions produced the most extractable material, indicating that these solvents are very good at separating active ingredients.

We investigated the phytochemical and pharmacological potential of *S. mysorensis*, a medicinal plant traditionally used in Indian folk medicine. Phytochemical screening verified the presence of diverse bioactive compounds, flavonoids, alkaloids, phenols, saponins, tannins, triterpenoids, and steroids. These constituents are known to be neuroprotective, particularly their antioxidant abilities and interaction with neurotransmitter systems.

Several studies have highlighted the neuroprotective potential of phenolic and flavonoid-rich plant extracts. Importantly, the CESM of *S. mysorensis* showed higher total phenols and flavonoids than the EESM, suggesting a higher potential for free radical scavenging and AChE inhibition. The abundance of phenols and flavonoids in CESM could be attributed to its non-polar to moderately polar extraction capacity, enhancing the yield of specific neuroprotective molecules.

A crucial role in AD pathology is the oxidative stress. ROS damage cellular components, disrupt mitochondrial function, and exacerbate amyloid and tau toxicity, leading to progressive neuronal degeneration[28].

CESM consistently demonstrated better antioxidant activity across all assays compared to EESM. The DPPH scavenging of CESM was significantly lower than that of EESM, indicating stronger hydrogen-donating ability. Similarly, CESM was more effective in ABTS and H_2O_2 scavenging assays, closely approaching the activity of α-tocopherol, a known antioxidant. For instance, Islam *et al* reported that *Clitoria ternatea* extract, rich in flavonoids, exhibited significant antioxidant activity, similar to our findings with CESM[5]. Likewise, Simpson *et al*. found that polyphenolic compounds from *Bacopa monnieri* improved memory and reduced oxidative stress in animal models of AD[19].

These findings affirm the capacity of CESM to neutralize ROS, potentially protecting neurons from oxidative damage. Given the well-established role of oxidative stress in the early stages of AD, natural antioxidants like those present in *S. mysorensis* could be valuable in preventive or adjunctive therapy.

Also, in the present study, CESM showed AChE inhibitory activity with an IC_{50} of 132.46 µg/mL. These findings are noteworthy when compared to previous studies on other *Sida* species. For instance, a study on *S. rhombifolia* reported AChE inhibition of 58.55% at 100 µg/mL concentration[17], indicating a less potent effect compared to *S. mysorensis*[3]. Furthermore, the AChE inhibitory activity of *S. mysorensis* suggests its potential to enhance cholinergic neurotransmission by preventing the breakdown of acetylcholine, thereby improving cognitive functions[29-31]. This dual action of antioxidant and AChE inhibition positions *S. mysorensis* as a promising therapeutic option for AD management[31].

To gain deeper insight into the active chemical constituents responsible for the observed bioactivities, GC-MS profiling of CESM was performed. The analysis identified 38 phytochemicals with retention times ranging from 3.96 to 37.5 minutes[23,32]. The diversity of metabolites revealed by GC-MS spanned various chemical classes, including phenolics, fatty acids, sugars, sterols, triterpenoids, alkaloids, and heterocyclic compounds, many of which are linked to significant pharmacological activities[23,31,32].

Among phenolic compounds, resorcinol (14.08%)[33], salicylic acid, and 4-vinylphenol were abundant, known for their potent antioxidant and anti-inflammatory properties[33]. The high concentration of resorcinol supports the strong antioxidant activity observed in bioassays and aligns with the plant's traditional use in managing inflammatory ailments[33].

The presence of bioactive fatty acids, such as n-hexadecanoic acid, cis-vaccenic acid, and (9E,11E)-octadecadienoic acid, underscores the plant's potential anti-inflammatory and lipid-modulating effects[34]. In addition, glycerol derivatives like glycerol 1-palmitate may contribute to skin healing and emollient properties, suggesting possible dermatological applications[34].

The detection of significant sugar content including 3-O-methyl-D-glucose and D-glycero-D-tallo-heptose indicates metabolic support through osmoprotection or energy provision under oxidative stress[23,32]. Sugar derivatives such as β-D-glucopyranose may also offer prebiotic or anti-diabetic effects, enhancing the multifunctionality of the extract[23,32].

Several phytosterols were also identified, including stigmasterol, γ-sitosterol, and campesterol, alongside triterpenoids like glutinol and lupeol[35,36], known for their anti-inflammatory, anticancer, and antidiabetic properties[35-37]. These findings add weight to the potential of *S. mysorensis* in the treatment of chronic inflammatory and metabolic diseases[35-37].

Furthermore, compounds typically associated with neurological activity such as valproic acid and levodopa[38] were detected, suggesting an additional neuroprotective layer through modulation of neurotransmitter pathways[38]. The identification of N-dodecylurea and heterocyclic structures like 1,3-diazacyclooctane-2-thione, thiophenes, and anthracene derivatives supports the presence of antimicrobial and anticancer properties[31], broadening the pharmacological scope of the extract[31].

Overall, the GC-MS profile of *S. mysorensis* supports its ethnopharmacological claims, particularly for its anti-inflammatory, antioxidant, anti-diabetic, and cholesterol-lowering effects. The presence of diverse and abundant secondary metabolites especially phenolics, sterols, triterpenoids, and fatty acids affirm the potential of this plant as a source of bioactive natural products for therapeutic applications.

5. Conclusion

Collectively, the findings validate *S. mysorensis* as a promising source of diverse bioactive compounds with strong neuroprotective, antioxidant, anti-inflammatory, and AChE inhibitory properties. GC-MS profiling revealed a complex phytochemical profile, including phenolics, flavonoids, fatty acids, sterols, and alkaloids many of which are known for their therapeutic relevance in neurodegenerative disorders. Notably, *S. mysorensis* demonstrated greater antioxidant and AChE inhibitory activity compared to other *Sida* species, underscoring its superior pharmacological potential. The plant's dual mechanism of action targeting both oxidative stress and cholinergic deficits highlights its suitability for developing natural therapeutics against Alzheimer's disease. These promising *in vitro* outcomes strongly support the need for future *in vivo* and clinical studies to further assess the efficacy and safety of *S. mysorensis* in managing Alzheimer's and related neurological conditions.

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7. Author Contribution

Kokila Kuppusamy carried out conceptualization, methodology, investigation, data curation, formal analysis, and writing of the original draft. Hemnath Elango worked on the supervision, validation, review and editing. All authors have read and agreed to the published version of the manuscript.

8. References

1. World Health Organization. A blueprint for dementia research. WHO. 2022.
2. Better MA. Alzheimer's disease facts and figures. Alzheimers Dement. 2023; 19(4):1598-1695. https://doi.org/10.1002/alz.13016
3. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener. 2019; 14(32). https://doi.org/10.1186/s13024-019-0333-5
4. Metaxas A, Kempf SJ. Neurofibrillary tangles in Alzheimer's disease: Elucidation of the molecular mechanism by immunohistochemistry and tau protein phospho-proteomics. Neural Regen Res. 2016; 11(10):1579-1581. https://doi.org/10.4103/1673-5374.193234
5. Marucci G, Buccioni M, Ben DD, Lambertucci C, Volpini R, Amenta F. Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. Neuropharm. 2021; 190:108352. https://doi.org/10.1016/j.neuropharm.2020.108352

6. Teleanu RI, Niculescu AG, Roza E, Vladacenco O, Grumezescu AM, Teleanu DM. Neurotransmitters-key factors in neurological and neurodegenerative disorders of the central nervous system. Int J Mol Sci. 2022; 23(11):5954. https://doi.org/10.3390/ijms23115954
7. Vieira THF, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: Targeting the cholinergic system. Curr Neuropharmacol. 2016; 14(1):101-115. https://doi.org/10.2174/1570159x13666150716165726
8. Moreira NCDS, Lima JEBDF, Marchiori MF, Carvalho I, Sakamoto-Hojo ET. Neuroprotective effects of cholinesterase inhibitors: Current scenario in therapies for Alzheimer's disease and future perspectives. J Alzheimer's Dis Rep. 2022; 6(1):177-193. https://doi.org/10.3233/ADR-210061
9. Dhapola R, Beura SK, Sharma P, Singh SK, Reddy DHK. Oxidative stress in Alzheimer's disease: Current knowledge of signaling pathways and therapeutics. Mol Biol Rep. 2024; 51(48). https://doi.org/10.1007/s11033-023-09021-z
10. Tamagno E, Guglielmotto M, Vasciaveo V, Tabaton M. Oxidative stress and beta amyloid in Alzheimer's disease. Which comes first: The chicken or the egg?. Antioxidants. 2021; 10(9):1479. https://doi.org/10.3390/antiox10091479
11. Kim H. Neuroprotective herbs for stroke therapy in traditional eastern medicine. Neurol Res. 2005; 27(3):287-301. https://doi.org/10.1179/016164105x25234
12. Safiri S, Jolfayi AG, Fazlollahi A, Morsali S, Sarkesh A, Daei Sorkhabi, A, Kolahi AA, *et al*. Alzheimer's disease: A comprehensive review of epidemiology, risk factors, symptoms diagnosis, management, caregiving, advanced treatments and associated challenges. Front Med. 2024; 11:1474043. https://doi.org/10.3389/fmed.2024.1474043
13. Kushwah S, Maurya NS, Kushwaha S, Scotti L, Chawade A, Mani A. Herbal therapeutics for alzheimer's disease: Ancient Indian medicine system from the modern viewpoint. Curr Neuropharmacol. 2023; 21(4):764-776. https://doi.org/10.2174/1570159x21666230216094353
14. Sulaiman C, Saji A, Anandan EM, Balachandran I. Phytochemical comparison of selected Sida species using spectrophotometric and multiple chromatographic analyses. Future J Pharm Sci. 2024; 10(118). https://doi.org/10.1186/s43094-024-00692-4
15. Subramanya MD, Pai SR, Upadhya V, Anand GM, Bhagwat SS, Hegde HV. Total polyphenolic contents and in vitro antioxidant properties of eight Sida species from Western Ghats, India. J Ayurveda Integr Med. 2015; 6(1):24-28. PMID: 25878460. PMCID: PMC4395924. https://doi.org/10.4103/0975-9476.146544
16. Kanakiya A, Padalia H, Pande J, Chanda S. Physicochemical, phytochemical and pharmacognostic study of *Limonium stocksii*, a halophyte from Gujarat. TJP. 2018; 7(3):312-318. http://dx.doi.org/10.31254/phyto.2018.7314
17. Karamian R, Ghasemlou F. Screening of total phenol and flavonoid content, antioxidant and antibacterial activities of the methanolic extracts of three *Silene* species from Iran. IJACS. 2013; 5(3):305-312.
18. Teleanu RI, Niculescu AG, Roza E, Vladacenco O, Grumezescu AM, Teleanu DM. Neurotransmitters-key factors in neurological and neurodegenerative disorders of the central nervous system. *International Journal of Molecular Sciences,* 2022; 23(11):5954. PMCID: PMC9180936. PMID: 35682631. https://doi.org/10.3390/ijms23115954
19. Chelliah R, MwineDaliri EB, Oh DH. Screening for antioxidant activity: Hydrogen peroxide scavenging assay. Methods in Actinobacteriology. Humana Press. 2022: p. 513-518. https://doi.org/10.1007/978-1-0716-1728-1_65
20. Williams WB, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology. 1995; 28(1):25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
21. Better MA. Alzheimer's disease facts and figures. Alzheimer's and Dementia. 2023; 19(4):1598-1695. https://doi.org/10.1002/alz.13016
22. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4th Edition. Allured Publishing Corporation. 2007.
23. Mani G, Mohankumar M. Extraction and identification of bioactive components in *Sida cordata* (Burm.f.) using gas chromatography-mass spectrometry. J Food Sci Technol. 2017; 54(11):3687-3696. https://doi.org/10.1007/s13197-017-2744-z
24. Sheppard O, Coleman M. Alzheimer's disease: Etiology, neuropathology and pathogenesis. Exon Publications. 2020: p. 1-21. PMID: 33400468. http://dx.doi.org/10.36255/exonpublications.alzheimersdisease.2020.ch1
25. Sharma A, Rudrawar S, Bharate SB, Jadhav HR. Recent advancements in the therapeutic approaches for Alzheimer's disease treatment: Current and future perspective. RSC Med Chem. 2025. http://dx.doi.org/10.1039/D4MD00630E
26. Zhao Y, Zhao B. Oxidative stress and the pathogenesis of Alzheimer's disease. Oxidative Medicine and Cellular Longevity. 2013. https://doi.org/10.1155/2013/316523
27. Rawat K, Tewari D, Bisht A, Chandra S, Tiruneh YK, Hassan HM, Al-Dies AAM. Identification of AChE targeted therapeutic compounds for Alzheimer's disease: An *in-silico* study with DFT integration. Scie Rep. 2024; 14(30356). https://doi.org/10.1038/s41598-024-81285-2
28. Kim S, Jung UJ, Kim SR. Role of oxidative stress in blood-brain barrier disruption and neurodegenerative diseases. Antioxidants. 2024; 13(12):1462. https://doi.org/10.3390/antiox13121462
29. Islam MA, Mondal SK, Islam S, Shorna MNA, Biswas S, Uddin MS, Saleh MA. Antioxidant, cytotoxicity,

antimicrobial activity, and *in silico* analysis of the methanolic leaf and flower extracts of *Clitoria ternatea*. Biochem Res Int. 2023. https://doi.org/10.1155/2023/8847876

30. Simpson T, Pase M, Stough C. *Bacopa monnieri* as an antioxidant therapy to reduce oxidative stress in the aging brain. Evidence-based Complementary and Alternative Medicine. 2015; 2015(1), 615384. https://doi.org/10.1155/2015/615384
31. Ahmed S, Khan ST, Zargaham MK, Ullah A, *et al*. Potential therapeutic natural products against Alzheimer's disease with reference to acetylcholinesterase. Biomed Pharmacother.2021; 139:111609. https://doi.org/10.1016/j.biopha.2021.111609
32. Xu Z, Gao P, Liu D, Song W, Zhu L, Liu X. Chemical composition and *in vitro* antioxidant activity of *Sida rhombifolia* L. volatile organic compounds. Molecules. 2022; 27(20):7067. https://doi.org/10.3390/molecules27207067
33. Lee SE, Kwon K, Oh SW, Kim MS, Choi HJ, Kim SY, *et al*. Mechanisms of resorcinol antagonism of benzo[a]pyrene-induced damage to human keratinocytes. Biomol Ther. 2021; 29(2):227-233. https://doi.org/10.4062/biomolther.2020.083
34. Gorniak A, Bartoszewski R, Kroliczewski J. Comprehensive review of antimicrobial activities of plant fatty acids. J Food Sci. 2021; 86(2):367-376.
35. Park JS, Rehman IU, Choe K, Ahmad R, Lee HJ, Kim MO. A triterpenoid lupeol as an antioxidant and anti-neuroinflammatory agent: Impacts on oxidative stress in Alzheimer's disease. Nutrients. 2023; 15(13):3059. https://doi.org/10.3390/nu15133059
36. Choe K, Park JS, Park HY, Tahir M, Park TJ, Kim MO. Lupeol protects against LPS-induced neuroinflammation and amyloid beta in adult mouse hippocampus. Front Nutr.2024; 11:1414696. https://doi.org/10.3389/fnut.2024.1414696
37. Bakrim S, Benkhaira N, Bourais I, Benali T. Health benefits and pharmacological properties of stigmasterol. Antioxidants. 2022; 11(10):1912. https://doi.org/10.3390/antiox11101912
38. Bhattacharyya KB. The story of levodopa: A long and arduous journey. Ann Indian Acad Neurol. 2022; 25(1):124-130. https://doi.org/10.4103/aian.aian_474_21