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Phytopharmacological studies of botanical sources of sahachara: *Barleria prionitis* L. *and Strobilanthes ciliate* Nees

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Ayurveda, the traditional Indian system of medicine, extensively utilises medicinal plants; however, region-specific availability and increasing global demand have led to the substitution of botanically distinct species under the same vernacular names, often without scientific validation. *Barleria prionitis* L. and *Strobilanthes ciliatus* Nees, both identified as Sahachara in classical Ayurvedic literature, are frequently used interchangeably in clinical practice for the management of Vata-related disorders, including neuromuscular and inflammatory conditions, with *S. ciliatus* Nees frequently replacing *B. prionitis* L. The present study was undertaken to critically evaluate the validity of this substitution by comparing the phytochemical characteristics, quality control parameters, and pharmacological activities of both species. Aqueous and hydroalcoholic extracts of different plant parts were prepared using the cold maceration method. Phytochemical profiling was performed using High Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography–Mass Spectrometry (GC-MS/MS). Acute oral toxicity was assessed according to OECD guideline 423 using a limit dose of 2000 mg/kg. Analgesic and anti-inflammatory activities were evaluated in experimental animal models at doses of 200 and 400 mg/kg body weight. The study established key quality control parameters and revealed distinct chromatographic and GC-MS/MS profiles, identifying species-specific phytochemical markers. Both extracts were found to be safe, with LD_{50} values exceeding 2000 mg/kg. In various analgesic models, aqueous and hydroalcoholic extracts of both plants produced significant analgesic effects compared to controls. A marked reduction in paw oedema volume confirmed their anti-inflammatory activity; however, no significant alterations were observed in pro-inflammatory cytokines such as TNF-α and IL-6. Overall, the findings suggest comparable anti-inflammatory and analgesic potential of both species.

Keywords: Analgesic activity, Anti-inflammatory activity, Ayurveda, GC-MS/MS, HPTLC, Pharmacological validation

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Introduction

In Ayurvedic and Sanskrit literature, plant names often denote therapeutic properties rather than precise botanical identity. As a result, multiple botanical species are used for a single Ayurvedic drug across different regions, influenced by variations in language, local availability, and traditional knowledge systems. In a geographically diverse country like India, the botanical source of a drug is frequently determined by regional accessibility and similarity in vernacular names or therapeutic actions, leading to the use of alternative species for the same classical formulation.

The preparation of traditional Ayurvedic formulations is increasingly challenged by the declining availability of authentic plant materials, driven by changing climatic conditions and resource constraints. Although the Ayurvedic Pharmacopoeia of India (API) provides standardised monographs for many medicinal plants, some listed species are not readily available, necessitating the exploration of region-specific alternatives. However, the lack of robust phytochemical and pharmacological validation for such substitutions often results in unintentional adulteration rather than evidence-based replacement[1]. Therefore, establishing scientific equivalence based on phytochemical composition and pharmacological activity is essential to ensure the quality, safety, and efficacy of substituted medicinal plants. For example, regional variations exist in which different botanical species are used for the same Ayurvedic drug, such as *Uraria picta* (Jacq.). DC. and *Desmodium gangeticum* (L.) DC., both of which are identified and used as Prishniparni in different parts of India[2]. Nair *et al.*

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highlighted the ambiguity surrounding the botanical identities of certain Ayurvedic plants, emphasising the importance of intertextual analysis. They reported that the number of constituents in formulations may vary and that multiple botanical species are sometimes associated with a single Sanskrit name[3].

Sahachara is a well-known Ayurvedic medicinal drug, widely used in various formulations for its anti-inflammatory, analgesic, and anti-arthritic properties, particularly in the management of musculoskeletal, respiratory, and gastrointestinal disorders. However, the term "Sahachara" does not correspond to a single botanical species, and multiple plants are identified under this name across different regions of India[4]. Among these, *B. prionitis* L. is traditionally regarded as the authentic source, predominantly distributed in northern India[5], whereas *Strobilanthes ciliatus* Nees, native to southern regions, is commonly used as a substitute due to its comparable therapeutic properties[6].

Identifying scientifically validated substitutes for medicinal plants is essential to address the scarcity and variability in Ayurvedic practice. The use of multiple plant species as substitutes without adequate scientific validation has led to inconsistencies and controversy[7]. Substitution, when evidence-based, can support the sustainable utilisation of medicinal resources by reducing pressure on limited or region-specific species, particularly those confined to high-altitude or restricted habitats[8]. Moreover, rational substitution helps conserve primary plant resources by preventing overexploitation and ensuring the long-term sustainability of medicinal flora.

In Kerala, a southern state of India with a strong Ayurvedic tradition, the use of regionally available plant species as substitutes is a well-established practice, primarily driven by the limited availability of original botanical sources. This knowledge has been transmitted through traditional practice and experiential learning[9,10]. Sahachara is an important Ayurvedic drug commonly used for the management of pain and inflammation. Although *B. prionitis L.* (whole plant) is recognised as the official source in the Ayurvedic Pharmacopoeia of India (API), its inconsistent availability across regions has led to the use of alternative species. In southern India, the root of *S. ciliata* Nees is widely employed as a substitute in various formulations[11,12].

S. ciliatus Nees (family Acanthaceae) possesses diverse pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, and antiproliferative effects, attributed to its rich phytochemical composition. It is extensively used in Kerala for the management of rheumatism and related disorders[4]. Similarly, *B. prionitis* L. possesses diverse pharmacological effects, such as anti-inflammatory, immunomodulatory, antioxidant, cardioprotective, and anticancer activities[13]. The comparable therapeutic potential of these species supports their traditional interchangeable use in Ayurvedic practice.

The present study aims to address the existing gap by undertaking a comprehensive pharmacognostical, physicochemical, and phytochemical comparison of *S. ciliatus* Nees and *B. prionitis* L., two botanicals traditionally associated with Sahachara. Detailed analytical approaches, including preliminary phytochemical screening, physicochemical evaluation, and chromatographic profiling using HPTLC[14], HPLC[15], and GC–MS/MS, were employed to assess their comparability. From a pharmacological perspective, *B. prionitis* is known for its anti-inflammatory, analgesic, immunomodulatory, and antioxidant properties[16], whereas *S. ciliata* exhibits free radical–scavenging, anti-arthritic, antiproliferative, and antidiabetic activities[17]. By correlating phytochemical profiles with pharmacological potential, the study aims to evaluate whether *S. ciliata* can serve as a reliable alternative to *B. prionitis* and to contribute toward the establishment of quality control standards for Sahachara-based Ayurvedic formulations, ensuring their safety, efficacy, and authenticity.

Materials and Methods

Plant material and botanical studies

The roots of *S. ciliata* Nees (root) were collected from the forest area near Mangalam Dam, Palakkad district, Kerala, India, in the month of May and supplied by AVP Kottakal Institute, Kerala. The whole plant of *B. prionitis* L. (Whole plant) was collected from Chikka Tattamangalam, Bengaluru region, India, in the month of September and supplied by CARI Bangalore institute. Both the obtained samples were authenticated by a botanist and deposited at National Ayurveda Research Institute for Panchakarma (NARIP), Cheruthuruthy, Kerala for future reference with RDR Number NARIP/IMR-PSP/003 for *B. prionitis* and NARIP/IMR-PSP/006 for *S. ciliate*. The micro-morphological character studies and close observation for the morphological investigations were conducted using the Unilab Zoom stereo microscope. The morphological features were evaluated by consulting previous literature. The root was examined using an

Olympus trinocular compound microscope after staining, and narrow free-hand slices were obtained using a stainless-steel blade. The powder of shade-dried root is examined using a compound microscope in accordance with API Part 1 Volume III[18].

Chemicals and reagents

HPLC-grade solvents, including methanol, acetonitrile, formic acid, and toluene, were sourced from Merck. Additionally, rectified alcohol and distilled water were employed to extract plants and their constituents. The aluminium 60 F_{254} TLC plates were supplied by Merck. Sigma-Aldrich, Bangalore, is the source of the reagents.

Extraction

Hydroalcoholic (HE) and aqueous (AE) extractions were carried out using 300 g of powdered plant material from the whole plant of *B. prionitis* L. (BP) and the root of *S. ciliata* Nees (SC). The samples were macerated separately in 2.5 L of a 1:1 hydroalcoholic solvent system (ethanol:water) and 2.5 L of double-distilled water in conical flasks for 24 h at controlled room temperature (25°C). The mixtures were then filtered under vacuum using a Buchner funnel. The extraction process was repeated three times with the same plant material, and the filtrates from the aqueous (AQS) and hydroalcoholic (HAS) extracts were pooled and stored at refrigerated conditions. The combined filtrates were subsequently concentrated using a rotary evaporator to remove the solvents.

The percentage yields obtained were 27% (w/w) for the hydroalcoholic extract of *B. prionitis* (HEBP) and 15% for its aqueous extract (AEBP). In contrast, *S. ciliata* yielded 11.57% (w/w) for the hydroalcoholic extract (HESC) and 14.57% for the aqueous extract (AESC).

Quality control analysis of the extracts

Preliminary phytochemical screening was carried out on the hydroalcoholic and aqueous extracts of *B. prionitis* L. (HEBP and AEBP) and *S. ciliatus* Nees (HESC and AESC) using standard qualitative methods to detect major classes of phytoconstituents such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics[19]. All extracts (HESC, AESC, HEBP, and AEBP) were further evaluated for organoleptic properties, including colour, odour, taste, and texture, according to standard pharmacognostical procedures. Physicochemical parameters such as loss on drying, total ash, acid-insoluble ash, water-soluble ash, and extractive values were determined in accordance with established pharmacopeial guidelines.

The extracts were also subjected to safety and quality evaluation. Pesticide residues were analysed using gas chromatography-mass spectrometry (GC-MS) methods, while aflatoxins (B1, B2, G1, and G2) were quantified using high-performance liquid chromatography (HPLC) with fluorescence detection after appropriate sample clean-up. Heavy metal content (Pb, Cd, As, and Hg) was determined using atomic absorption spectroscopy (AAS)/inductively coupled plasma–mass spectrometry (ICP–MS). Microbiological quality was assessed by total aerobic microbial count, total yeast and mould count, and tests for specified pathogens (e.g., *Escherichia coli*, Salmonella spp., *Staphylococcus aureus*) using standard plate count and selective media methods as per pharmacopoeial and WHO guidelines.

HPTLC analysis

Sample and standards preparation

Extracts of *B. prionitis* L. (HEBP and AEBP) and *S. ciliata* Nees (HESC and AESC) were individually dissolved in HPLC-grade methanol to obtain a final concentration of 100 mg/mL in volumetric flasks for HPTLC analysis. The solutions were sonicated at 45°C for 15 minutes to ensure complete dissolution, then filtered through a 0.22 µm PTFE membrane filter using a 5 mL syringe. The filtered samples were collected separately in 5 mL sample vials and stored for subsequent HPTLC fingerprint profiling. Reference standards of gallic acid (GA), β-sitosterol (BS), and quercetin (QE) were prepared individually at 1 mg/mL in HPLC-grade methanol.

Procedure

Samples of *S. ciliata* Nees extracts (AESC and HESC) were applied in volumes of 3, 5, and 10 µL in triplicate onto tracks 1–6. Similarly, extracts of *B. prionitis* L. (AEBP and HEBP) were applied at volumes of 5 and 10 µL in duplicate onto tracks 7–12. Reference standards—gallic acid (GA), β-sitosterol (BS), and quercetin (QE)—were applied individually at 1 µL onto tracks 13, 14, and 15, respectively. All applications were performed on pre-coated silica gel 60 F254 HPTLC plates (20 × 10 cm, Merck) using an automated applicator.

The plates were developed in a pre-saturated twin-trough chamber (20 × 10 cm) for 20 min using a mobile phase consisting of toluene:ethyl acetate:formic acid (5:4:1, v/v/v), allowing a migration distance of approximately 80 mm. Following development, the plates were air-dried and visualised under UV light at 254 and 366 nm. For derivatisation, the plates were dipped in p-anisaldehyde–sulphuric acid reagent and subsequently heated at 105°C for 3 minutes to visualise coloured bands at 545 nm. Chromatographic profiles were documented and analysed using VisionCATS software (version 3.1) for all extracts.

GC-MS/MS analysis

Sample preparation

A total of 0.45g of each extract was mixed with 5 mL of methanol, sonicated for 15 minutes, and then centrifuged at 5000 rpm for 5 minutes. After filtration using a 0.22-micron syringe filter, the supernatant was prepared for GC-MS analysis.

Instrumentation

Phytochemical profiling of the hydroalcoholic and aqueous extracts of *S. ciliata* Nees (HESC and AESC) and *B. prionitis* L. (HEBP and AEBP) was carried out using gas chromatography–tandem mass spectrometry (GC–MS/MS) on an Agilent 8890 gas chromatograph coupled to an Agilent 7000D triple quadrupole mass spectrometer. Chromatographic separation was performed on an Agilent HP-5MS UI fused silica capillary column (15 m × 250 µm internal diameter, 0.25 µm film thickness) coated with 5% phenyl methyl polysiloxane.

Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Samples (1 µL) were introduced through an autosampler (ALS) in split mode with a split ratio of 5:1. The oven temperature was initially maintained at 60°C and subsequently programmed with sequential heating rates of 5°C/min, 15°C/min, and 10°C/min until reaching 220°C, where it was held for 10 min.

Mass spectral detection was performed under electron impact (EI) ionisation at 70 eV. The ion source and transfer line temperatures were maintained at 230°C and 280°C, respectively. Data were acquired in positive ion mode with a scan time of 300 ms, and a solvent delay of 2 min was applied. Nitrogen gas served as the collision gas at a flow rate of 1.5 mL/min, whereas helium was employed as the quench gas at 2.25 mL/min. Tentative identification of the detected compounds was achieved by matching the mass spectra of major peaks with entries available in the NIST GC–MS spectral database.

Animals

About 8-10 weeks old Male and female Sprague Dawley (SD) rats (100–150 g) and Swiss albino mice (25–35 g) were sourced from a certified small animal breeding facility. The animals were kept under standard conditions (25±2°C, 60-70% relative humidity, and a 12-hour light/dark cycle) and were provided with a standard rodent pellet diet and access to water ad libitum. Before the start of the investigation, the rats were acclimated to the laboratory conditions for one week. The investigations were carried out in accordance with the principles of laboratory animal care guidelines, with prior approval (IAEC/NARIP/2023-24/01, dated 29/04/2023) from the Institute Animal Ethics Committee (IAEC).

Acute oral toxicity study

Acute oral toxicity studies were performed in accordance with Organisation for Economic Cooperation and Development (OECD) Guideline No. 423 using female Sprague–Dawley (SD) rats. The study was conducted in two phases, namely an initial exploratory step followed by a confirmatory step. In each phase, three animals were assigned to the control group and three animals to the treated group. Distilled water was used as the vehicle, and both the vehicle and test extracts were administered orally at a dose volume of 1.0 mL/100 g body weight. A single limit dose of 2000 mg/kg body weight was employed to determine the LD_{50} of the extracts. Feed intake and body weight changes were recorded weekly, whereas clinical signs and behavioural changes were monitored daily throughout the experimental period. No abnormal clinical signs or mortality were observed during the 14-day observation period. At the end of the study, the animals were euthanised using a CO_2 asphyxiation chamber and subjected to gross pathological examination. Major organs, including the liver, kidneys, heart, lungs, spleen, ovaries, stomach, adrenals, and brain, were examined for any visible morphological abnormalities[20].

Efficacy study (analgesic & anti-inflammatory activity)

The animals were divided into groups with six animals in each group. To test the pain-relieving and anti-inflammatory effects of the source and substitute

plants. The aqueous and hydroalcoholic extracts of the source and substitute plant were subjected to evaluate analgesic and anti-inflammatory activity through different models by using Swiss mice for analgesic activity and SD rats for anti-inflammatory activity at two dose levels, viz. 200 and 400 mg/kg (p.o). The standard drug Tramadol 10 mg/kg was used in the hot plate and tail flick methods to assess the central analgesic response[21], while Diclofenac 10 mg/kg was used in the paw pressure and formalin methods to evaluate the peripheral analgesic response[22]. All amounts were made up in normal saline (0.9% w/v) with 2% Tween 80. The control group only got a vehicle.

Thermal hyperalgesia

Eddy's Hot plate test

The animals were divided into groups with six animals in each group. The aqueous and hydroalcoholic extracts of the source and substitute plant were subjected to evaluate analgesic activity by using a hot plate method in Swiss mice at two dose levels, viz. 200 and 400 mg/kg (p.o). The standard drug Tramadol 10 mg/kg was used to assess the central analgesic response[21]. Swiss white mice were used for the Eddy's hot plate test. The mice were put on an Indosati hot plate that was kept at 55±2°C. The reaction time, or latency, was determined by measuring the time it took for the mice to respond to the heat stimulus by either licking their paws or jumping. The reaction time (latency) was measured before the extracts were given (0 min) and 30, 60, 90, 120, and 180 minutes after they were given[23]. The cut –off time was set at 15 seconds to minimise skin injury.

Tail flick method

The animals were divided into groups with six animals in each group. The aqueous and hydroalcoholic extracts of the source and substitute plant were subjected to evaluate analgesic activity by using the tail flick method in Swiss mice at two dose levels viz. 200 and 400 mg/kg (p.o). The standard drug Tramadol 10mg/kg was used to assess the central analgesic response[21]. A digital tail flick analgesiometer (Orchid Scientific) was used. The apparatus applies a heat stimulus through radiant heat to the mice tail. Before giving the drugs, the basal readings were recorded. The extracts were then given to different groups of animals, and the tail flick reaction (latency) was recorded 30, 60, 90, 120, and 180 minutes after the extracts were given[24].

Mechanical hyperalgesia

Randall Selitto test or Paw pressure method

The animals were divided into groups with six animals in each group. The aqueous and hydroalcoholic extracts of the source and substitute plant were subjected to evaluate analgesic activity by using paw pressure method in swiss mice at two doses levels viz. 200 and 400 mg/kg (p.o). The standard drug Diclofenac 10 mg/kg was used to assess the peripheral analgesic response[21]. The Randall-Selitto test, also known as the paw pressure test, was performed using the Orchid Scientific Paw Pressure Analgesiometer. The pressure applicator was placed on the hind paw of the mouse, and a gradually increasing pressure stimulus was applied to the dorsal surface of the paw until the animal either vocalised or withdrew its paw. The pain threshold force (gf) was measured before (0 min) and 30, 60, 90, 120, and 180 minutes after the extracts were taken by mouth[25].

Chemical hyperalgesia

Formalin-induced algesia

20 µL of 10% v/v formalin, prepared in normal saline from 37% formaldehyde, was injected under the skin of a mouse leg one hour after the extract was given. The effect of formalin was shown by licking and shaking of the hind legs, which mostly happened in two stages. The early phase, called Phase I, lasts for about 10 minutes. It is followed by a short time of relative calmness that lasts for about 5 minutes. Finally, the late phase, called Phase II, which includes shaking and licking the back legs, lasts for another 50 minutes or so. The first phase is thought to be caused by primary afferent sense neurons being directly activated. The second phase, on the other hand, is thought to be caused by afferent input and central sensitisation working together in the dorsal horn[26].

Acute anti-inflammatory study: Carrageenan induced rat paw oedema

The animals were divided into groups with six animals in each group. To test the anti-inflammatory effects of the source and substitute plants. The aqueous and hydroalcoholic extracts of the source and substitute plant were subjected to evaluate anti-inflammartory activity in SD rats at two dose levels, viz. 200 and 400 mg/kg (p.o). The standard drug Diclofenac 10 mg/kg was used in acute oedema model, which was caused by injecting 0.1 mL of newly made carrageenan suspension (Sigma Aldrich, USA) into the right hind paw of each rat by

intraplantar injection of 100 µL λ-carrageenan. Test extracts and Diclofenac (10 mg/kg) were orally administered one hour before the carrageenan injection. The paw volume of rats was measured by a digital plethysmometer (Orchid Science), before and after the injection of carrageenan at different time intervals (1, 2, 3, 4, and 5 hours)[27]. Blood samples of the animals were collected from the retro-orbital plexus under isoflurane anaesthesia. The blood samples were allowed to clot at room temperature for 30 min and centrifuged at 3000 rpm for 10 min to obtain serum. The separated serum samples were subjected to ELISA using an Erba ELISA reader to determine inflammatory cytokines such as TNF-α and IL-6[27].

Statistical analysis

Statistical analysis of the acute toxicity study was performed using the t-test (n=3), while the efficacy study data were analysed by one-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test (n=6). All analyses were conducted using GraphPad Prism 8.4.2 (Dotmatics, Boston, MA, USA). Data are expressed as mean ± standard error of the mean (SEM), and a p-value <0.05 was considered statistically significant.

Results

Plant material authentication and pharmacognosy

The identities of *B. prionitis* L. and *S. ciliata* Nees were authenticated, as shown in Fig. 1a and 1b. The botanical characteristics of the collected plant materials were examined as a preliminary investigation. The morphological, anatomical, and powder characteristics of *S. ciliatus* are presented in Fig. 2, while the morphological and powder characteristics of *B. prionitis* are shown in Fig. 3. A comparative summary of these features is provided in Table 1.

Quality control analysis

Preliminary phytochemical screening revealed distinct variations between the extracts of *B. prionitis* L. and *S. ciliata* Nees. The hydroalcoholic (HEBP) and aqueous (AEBP) extracts of *B. prionitis* showed the presence of glycosides, saponins, and flavonoids, with moderate to low intensity, while proteins, alkaloids, and tannins were absent. In contrast, the hydroalcoholic (HESC) and aqueous (AESC) root extracts of *S. ciliata* exhibited a comparatively richer phytochemical profile. Glycosides and saponins were strongly present (+++), and flavonoids and phenolic compounds were notably higher, particularly in the aqueous extract (AESC). Carbohydrates were absent in *B. prionitis* extracts but detected in *S. ciliata*, with higher intensity in AESC. Proteins were detected only

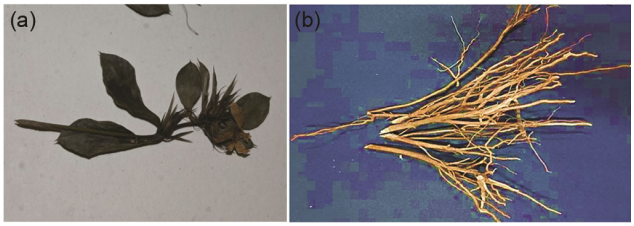

Fig. 1 — (a) Whole plant of *Barleria prionitis* L., and (b) Roots of *Strobilanthes ciliata*.

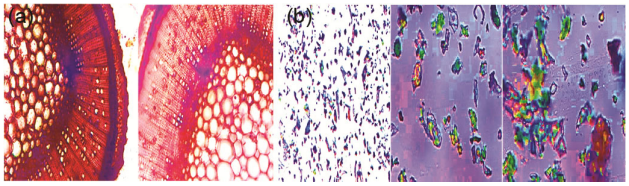

Fig. 2 — (a) Microscopy, and (b) Powder Microscopy whole plant of *Barleria prionitis*.

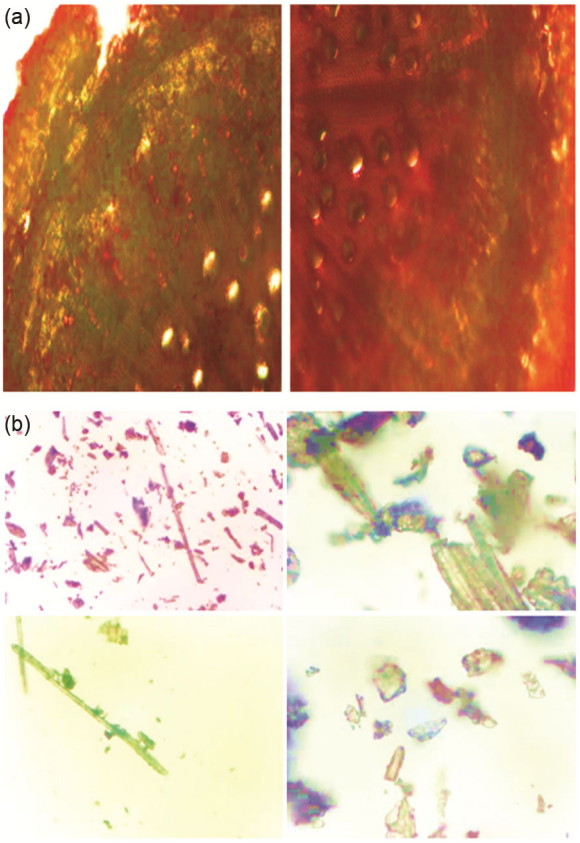

Fig. 3 — (a) Microscopy, and (b) Powder microscopy of *Strobilanthes ciliata* Root.

Table 1 — Comparative features of *Barleria prionitis* Linn. and *Strobilanthes ciliata* Nees

Name of the parameters	Characters	*Barleria prionitis* L. (whole plant)	*Strobilanthes ciliata* Nees (Root)
Morphological and Organoleptic characters	Shape	Well-developed, Cylindrical and tapering	Cylindrical
	Colour	External surface greyish-brown, wood cream	Pale brown, Blusih white colour
	Texture	The stem is erect, terete, hard, and glabrous, measuring approximately 1–8 mm in thickness. Nodes are distinctly swollen and give rise to branches. The stem is slightly quadrangular in outline, and the leaf axils typically bear three to four divaricate spines.	Cylindrical in outline, 2-10 mm thickness,
	Taste	Slightly bitter	Bitter
	Odour	Indistinct	Pleasant smell
Microscopic characters	The transverse section of the stem showed a cork region comprising 6–24 or more layers of rectangular cells arranged radially. The secondary cortex consisted of 8–15 layers of thin-walled, tangentially elongated parenchymatous cells containing cellular inclusions. The secondary phloem was comparatively narrow and composed of heterogeneous cell types. The secondary xylem was well developed and comprised vessels, tracheids, fibres, and xylem parenchyma traversed by distinct xylem rays. The central pith was made up of isodiametric parenchymatous cells.		C.S. of root showed cork, cork cambium and phelloderm. Lenticels are present. Secondary cortex has parenchyma cells in rows and some stone cells. Stelar region consists of secondary xylem and secondary phloem.
Powder characters	Colour	Green	White
	Crystals, Cells and cell inclusions	Powder microscopy revealed the presence of cork cell fragments, calcium oxalate crystals, and portions of leaf lamina exhibiting distinct palisade and mesophyll cells	Aciular silica crysal calcium oxalate, Starch grains, Parenchyma cells, stone cells.

Table 2 — Preliminary phytochemical analysis of *B. prionitis* and *S. ciliata* root extracts

S No	Tests for Phytochemicals		*B. prionitis* (whole plant) hydroalcoholicextract (HEBP)	*B. prionitis* (whole plant) Aqueous extract (AEBP)	*S. ciliata* Root hydroalcoholic extract (HESC)	*S. ciliata* Root Aqueous extract (AESC)
1	Glycosides	Salkowski	++	+	+++	+++
2	Protein	Ninhydrin	-	-	-	+
3	Alkaloid	*Wagner's*	-	-	-	-
4	Tannin	*Braymer's*	-	-	-	++
5	Saponins	Foam	+	++	+++	+++
6	Flavonoids	Lead acetate	+	++	+	+++
7		Shinoda	-	-	-	-
8	Phenols	Lead acetate	+	-	+	+++
9		Ferric chloride	-	-	-	++
10	Carbohydrates	*Fehling's* Test	-	-	+	+++
		Molish Test	-	-	+	++

in AESC, whereas alkaloids were absent in all extracts. Tannins were observed exclusively in AESC (Table 2). Overall, the aqueous extract of *S. ciliata* (AESC) showed higher phytochemical abundance and diversity than the other extracts.

All extracts, hydroalcoholic (HEBP, HESC) and aqueous (AEBP, AESC) of *B. prionitis* L. and *S. ciliata* Nees were evaluated for physicochemical parameters (Table 3) microbiological quality, pesticide residues, aflatoxins, and heavy metals. Total aerobic bacterial count, total yeast and mould count, and specified pathogens were assessed. All extracts complied within permissible limits, with pesticide residues below detection thresholds and aflatoxin and heavy metal levels within API standards. The results are presented in Table 4. No specified pathogens were detected in any of the extracts. A comparatively higher bacterial load was observed in HEBP than in AEBP of

Table 3 — Physicochemical parameters of *Barleria prionitis* (whole plant) and *Strobilanthes ciliate* (Rt.) raw drugs hydro alcoholic extract and aqueous extract

S No	Test parameters	Results (n=3)		Results (n=3)	
		Barleria prionitis (whole plant) hydroalcoholicextract (HEBP)	*Barleria prionitis* (whole plant) Aqueous extract (AEBP)	*Strobilanthes ciliata* Root hydroalcoholic extract (HESC)	*Strobilanthes ciliata* Root Aqueous extract (AESC)
1	Description	Sticky semi-solid	Sticky semi-solid	Sticky paste	Paste less denser
2	Colour	Dark brown	Brownish	Dark brownish	Brownish
3	Odour	Pleasant Smell	Pleasant Smell	Pleasant Smell	Pleasant Smell
4	Taste	Slight magnanimous	Slight magnanimous	Slight magnanimous	Slight bitter
5	Loss on Drying (w/w %)	12.336 ± 0.208	22.713 ± 0.299	14.77 ±0.26	30.42±0.29
6	Total Ash (w/w %)	37.900 ± 0.177	34.380 ± 0.126	18.18±0.14	24.87±0.17
7	Acid Insoluble ash (w/w %)	6.06 ± 0.05	4.623 ± 0.231	2.49±0.28	6.66±0.25
8	pH (10% Aq.)	5.993 ± 0.005	8.153 ± 0.011	6.32±0.005	8.52±0.005

Table 4 — *Barleria prionitis* (whole plant) and *Strobilanthes ciliata* extracts safety parameters

S No	Tests parameters	Results (n=2)		Results (n=2)	
		HEBP	AEBP	HESC	AESC
1	Total Bacterial Count	8.4×10^3 CFU/gram	6×10^2 CFU/gram	3.2×10^2 CFU/gram	1.3×10^5 CFU/gram
2	Total fungi/yeast count	<10 CFU/gram	2×10^1CFU/gram	<10 CFU/gram	<10 CFU/gram
3	Test for Specific microorganisms like *S. aureus, Pseudomonas aeruginosa, Salmonella* sp., and *E. coli*	Not detected	Not detected	Not detected	Not detected
4	Pesticide Residues	Below Detection Limit	Below Detection Limit	Below Detection Limit	Below Detection Limit
5	Test for Aflatoxins (ppm) (B1, G1, B2, G2)	Below Detection Limit	Below Detection Limit	Below Detection Limit	Below Detection Limit
6	Test for Heavy Metals (ppm) (Pb, Cd, Hg, As)	Complies as per API	Complies as per API	Complies as per API	Complies as per API

B. prionitis. Overall, extracts of both plants met quality and safety criteria; notably, *S. ciliatus* extracts demonstrated consistent compliance with purity and quality standards.

HPTLC analysis

Phytochemical profiling of *Barleria prionitis* L. and *Strobilanthes ciliata* Nees extracts was carried out using HPTLC fingerprint analysis. The aqueous extract of *B. prionitis* (AEBP) exhibited 9, 5, and 5 bands at 254, 366, and 545 nm, respectively, whereas the hydroalcoholic extract (HEBP) showed 7, 16, and 8 bands at the corresponding wavelengths, suggesting a comparatively richer phytochemical composition in the hydroalcoholic extract. Marker compound analysis revealed the presence of gallic acid and quercetin in AEBP, while β-sitosterol was detected exclusively in HEBP.

Similarly, the aqueous extract of *S. ciliatus* (AESC) displayed 4, 6, and 3 bands at 254, 366, and 545 nm, respectively, whereas the hydroalcoholic extract (HESC) exhibited 9, 12, and 6 bands, indicating a higher phytochemical diversity. Gallic acid and quercetin were identified in HESC, while β-sitosterol was predominantly detected in AESC. Overall, the HPTLC profiles suggest that hydroalcoholic extracts exhibit greater phytochemical diversity compared to aqueous extracts, as illustrated in Table 5 and Fig. 4.

GC-MS/MS analysis

GC–MS/MS analysis of the hydroalcoholic (HESC) and aqueous (AESC) extracts of *S. ciliata* Nees revealed a diverse chemical composition with clear differences between the solvent systems. The HESC extract exhibited a broader range of compounds distributed over a wide retention time (Rt) range, indicating greater phytochemical diversity (Fig. 5a). The major constituents identified in HESC included fatty acids such as nonanoic acid (Rt 21.223 min) and n-hexadecanoic acid (Rt 28.506 min), methyl esters of fatty acids (e.g., methyl tetradecadienoates at ~31

Table 5 — HPTLC Rf values of aqueous and hydroalcoholic extracts of *Barleria prionitis* L. and *Strobilanthes ciliatus* Nees

Track number	Name of the Sample	R_F values		
		Developed plate at 254 nm	Developed plate at 366 nm	Derivatized plate at 545 nm
1, 2 & 3	*Strobilanthes ciliata*-Aqueous Extract-AESC	Number of bands: 04 0.013, 0.158, 0.546, 0.676	Number of bands: 06 0.14 (Sky Blue), 0.23 (Sky Blue), 0.36 (Light Blue), 0.46 (Light Blue), 0.53 (Ash), 0.71 (Blue)	Number of bands: 03 0.003, 0.035, 0.758 (Violet)*
4, 5 & 6	*Strobilanthes ciliata*-Hydroalcoholic Extract-HESC	Number of bands: 07 0.008, 0.036, *0.347,* 0.447, 0.565, 0.679, 0.764	Number of bands: 12 0.07 (Light Blue), 0.13 (Light Blue), 0.24 (Light Blue), 0.38 (Light yellow), 0.42 (Light Red), 0.46 (Light Blue), 0.49 (Sky Blue), 0.56 (Ash), 0.59 (Light Red), 0.62 (Light Blue), 0.73 (Blue), 0.79 (Light Red)	Number of bands: 06 0.010, 0.260, 0.499, 0.542, 0.765 (Violet) *, 0.840
7 & 8	*Barleria prionitis*-Aqueous Extract-AEBP	Number of bands: 09 0.008, 0.035, 0.072, 0.160, 0.253, 0.333, 0.396, 0.550, 0.671	Number of bands: 05 0.02 (Light Blue), 0.07 (Light Blue), 0.10 (Light Blue), 0.54 (Light Blue), 0.83 (Light Red)	Number of bands: 05 0.004, 0.028, 0.097 (light green), 0.203 (Blue), 0.450 (Light Blue)
9 & 10	*Barleria prionitis*-Hydro Alcoholic Extract-HEBP	Number of bands: 07 0.011, 0.079, 0.171, 0.504, 0.558, 0.651, 0.804	Number of bands: 16 0.03 (Light Blue), 0.08 (Light Red), 0.15 (Light Red), 0.20 (Light Blue), 0.24 (Light Blue), 0.34 (Light Red), 0.42 (Red), 0.49 (Red), 0.54 (Red), 0.60 (Red), 0.64 (Red), 0.70 (Light Red), 0.73 (White shade), 0.79 (Dark Red), 0.85 (Red), 0.90 (Light Red)	Number of bands: 08 0.026, 0.128, 0.243 (Cyan Blue), 0.383, 0.496 (Light Blue), 0.658 (Blue), 0.765 (Dark Violet), 0.860
11 & 12	*Barleria prionitis*-Alcoholic Extract-BP	Number of bands: 05 0.10, 0.55, 0.76, 0.80, 0.84	Number of bands: 12 0.07 (Light Blue), 0.10 (Light Green), 0.35 (Light Red), 0.50 (Light Red), 0.54 (Red), 0.60 (Yellow), 0.64 (Light Red), 0.70 (Light Red), 0.73 (White shade), 0.79 (Dark Red), 0.85 (Red), 0.90 (Light Red)	Number of bands: 05 0.10, 0.26, *0.72 (Light Blue)*,* 0.77 (Light Blue), 0.85
13	Gallic acid-GA	Number of bands: 01 0.346	Number of bands: 01 0.346	Number of bands: 01 0.346
14	Beta-Sitosterol-BS	Number of bands: ND	Number of bands: ND	Number of bands: 01 0.713 (Violet)
15	Quercetin-QE	Number of bands: 01 0.535	Number of bands: 01 0.535	Number of bands: 01 0.535

Note: *: Similar moiety of Chemical group

min), phenolic derivatives such as 7-hydroxycadalene (Rt 28.969 min), and several heterocyclic compounds including benzotriazole, quinoline, and pyridazine derivatives. These compounds collectively reflect the chemical complexity and potential bioactivity of the hydroalcoholic extract.

In contrast, the AESC extract demonstrated a comparatively simpler chemical profile (Fig. 5b), with fewer identified compounds and a narrower Rt distribution. The principal constituents included hydrazinecarbothioamide (Rt 2.175 min), pentanoic acid (Rt 2.696 min), cyclododecyne (Rt 9.878 min), and 1-isothiocyanato-3-methyl-adamantane (Rt 26.735 min). The reduced number and diversity of compounds in AESC suggest limited extraction efficiency of aqueous solvents, primarily favouring polar constituents.

Notably, commonly reported analytical artifacts such as siloxane derivatives and phthalate esters were

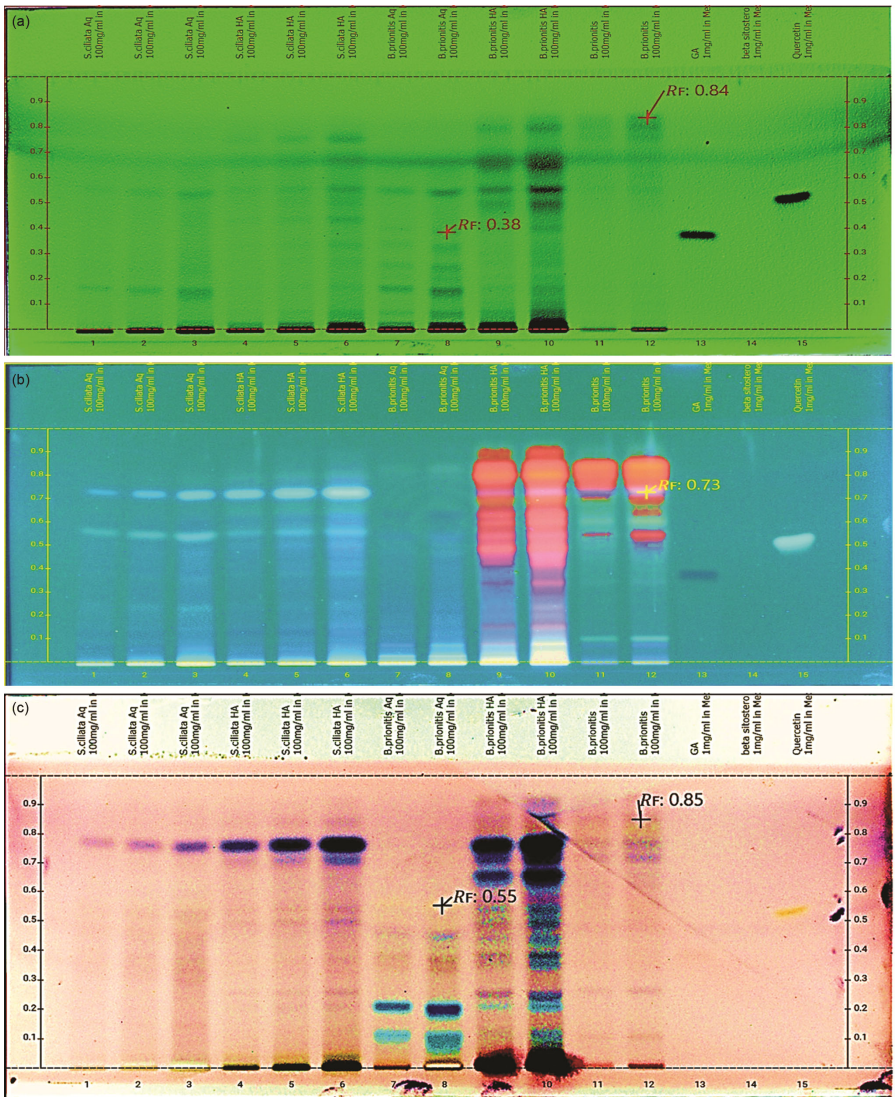

Fig. 4 — HPTLC Chromatogram {*Strobilanthes ciliata*-Aqueous Extract (tracks-1,2,3), *Strobilanthes ciliata*-Hydroalcoholic Extract (tracks-4,5,6), *Barleria prionitis*- Aqueous Extract (tracks-7,8), *Barleria prionitis*-Hydro Alcoholic Extract (tracks-9,10), *Barleria prionitis*-Alcoholic Extract (tracks-11,12), Gallic acid-GA (track-13), Beta-Sitsoterol-BS (track-14), and Quercetin-QE (track-15) at (a) 254 nm, (b) 366 nm, and (c) 545 nm}.

excluded from interpretation, as they are associated with instrumental contamination rather than true plant-derived constituents. Overall, the hydroalcoholic extract (HESC) exhibited a more complex and chemically diverse profile compared to the aqueous extract (AESC), highlighting the effectiveness of hydroalcoholic solvents in extracting a broader spectrum of phytochemicals. These compositional differences may contribute to variations in the pharmacological potential of the extracts. The detailed compound profiles are presented in Table 6 and Fig. 5.

GC–MS analysis of the hydroalcoholic (HEBP) and aqueous (AEBP) extracts of *B. prionitis* L. revealed a diverse array of phytochemicals with notable differences in composition and abundance between the two solvent systems. The HEBP extract exhibited a greater number of compounds distributed over a wider retention time (Rt) range, indicating a more complex phytochemical profile (Fig. 6a). The major constituents identified in HEBP included fatty acids such as n-hexadecanoic acid (Rt 28.507 min), fatty acid methyl esters such as methyl 10,11-tetradecadienoate (Rt 31.810 min), and several heterocyclic and oxygenated compounds, including benzopyran derivatives, phthalazine derivatives, thiadiazepane derivatives, and oxacyclic compounds. The presence of β-D-glycosyloxyazoxymethane (Rt 21.880 min) and other structurally diverse compounds

Table 6 — GC–MS/MS-based identification of chemical constituents in aqueous and hydroalcoholic extracts of *Strobilanthes ciliata* Nees

S No	Retention Time (Rt, min)	Compound Name	Extract
1	2.513	Benzyl (1,2,3-thiadiazol-4-yl) carbamate	HESC / AESC
2	4.609	Trans-2,4-Dimethylthiane, S,S-dioxide	HESC
3	5.589	Boronic acid, ethyl-bis (2-mercaptoethyl ester)	HESC
4	9.897	4-(1-benzotriazolyl)-6-(2-furoyl)-2-phenyl-pyridazin-3(2H)-one	HESC
5	11.998	1,2,3,5-Tetra-O-acetyl-1-deuterio-4-O-methyl-D-arabinitol	HESC
6	14.007	3-Diazo-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	HESC
7	16.712	3-(9-borabicyclo[3.3.1]nonane-9-yl)-4-iodo-(Z)-3-hexene	HESC
8	17.455	(1-Phenyl-1H-1,2,3-triazol-4-yl)methanamine	HESC
9	17.890	2-Butoxy-4-methyl-[1,3,2]dioxaneborinane	HESC
10	21.223	Nonanoic acid	HESC
11	21.425	β-D-Glycosyloxyazoxymethane	HESC
12	26.506	2,3-Diazabicyclo[2.2.1]hept-2-ene, 7,7-dimethyl-5-phenyl-	HESC
13	26.685	2-Ethoxyquinoline 1-oxide	HESC
14	27.342	1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-1,4-methanocycloocta[d]pyridazine	HESC
15	28.173	Oxacyclotetradeca-4,11-diyne	HESC
16	28.506	n-Hexadecanoic acid	HESC
17	28.969	7-Hydroxycadalene	HESC
18	31.027	Polycyclic hydrocarbon derivative	HESC
19	31.148	Methyl 11,12-tetradecadienoate	HESC
20	31.259	3-Amino-7-nitro-1,2,4-benzotriazine-1-oxide	HESC
21	31.693	Methyl 10,11-tetradecadienoate	HESC
22	31.800	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	HESC
23	32.809	4,4-Bis(dichlorofluoromethyl)-1,2-oxathietane-2,2-dioxide	HESC
24	2.175	Hydrazinecarbothioamide	AESC
25	2.696	Pentanoic acid	AESC
26	9.878	Cyclododecyne	AESC
27	26.735	1-Isothiocyanato-3-methyl-adamantane	AESC

further reflects the chemical complexity and potential bioactivity of the hydroalcoholic extract.

In contrast, the AEBP extract demonstrated a comparatively simpler chemical profile with fewer compounds and a narrower Rt distribution (Fig. 6b). The principal constituents identified in AEBP included benzyl (1,2,3-thiadiazol-4-yl) carbamate (Rt 2.503 min), diazabicyclic compounds (Rt 13.114 min), and several nitrogen-containing heterocyclic derivatives. The relatively lower number and diversity of compounds in the aqueous extract indicate its limited extraction efficiency, primarily favouring polar constituents.

Notably, siloxane derivatives, phthalate esters, and quaternary ammonium compounds were excluded from interpretation, as these are commonly associated with analytical or environmental contamination rather than true plant-derived constituents. Overall, the hydroalcoholic extract (HEBP) demonstrated a more diverse and chemically rich phytochemical profile compared to the aqueous extract (AEBP), highlighting the superior efficiency of hydroalcoholic solvents in extracting a broader spectrum of bioactive compounds. These variations in chemical composition may contribute to differences in the pharmacological potential of the extracts. The detailed compound profiles are presented in Table 7 and Fig. 6.

Acute oral toxicity

Acute oral toxicity evaluation of the aqueous (AEBP, AESC) and hydroalcoholic (HEBP, HESC) extracts of *B. prionitis* L. and *S. ciliatus* Nees at a limit dose of 2000 mg/kg demonstrated no mortality, behavioural abnormalities such as locomotor activity, alertness, aggressiveness, grooming, and tremors or signs of toxicity in experimental animals during both the exploratory and confirmatory phases. Body weight gain and feed intake remained comparable to those of the vehicle control group throughout the study period, with no statistically significant differences ($p > 0.05$), as

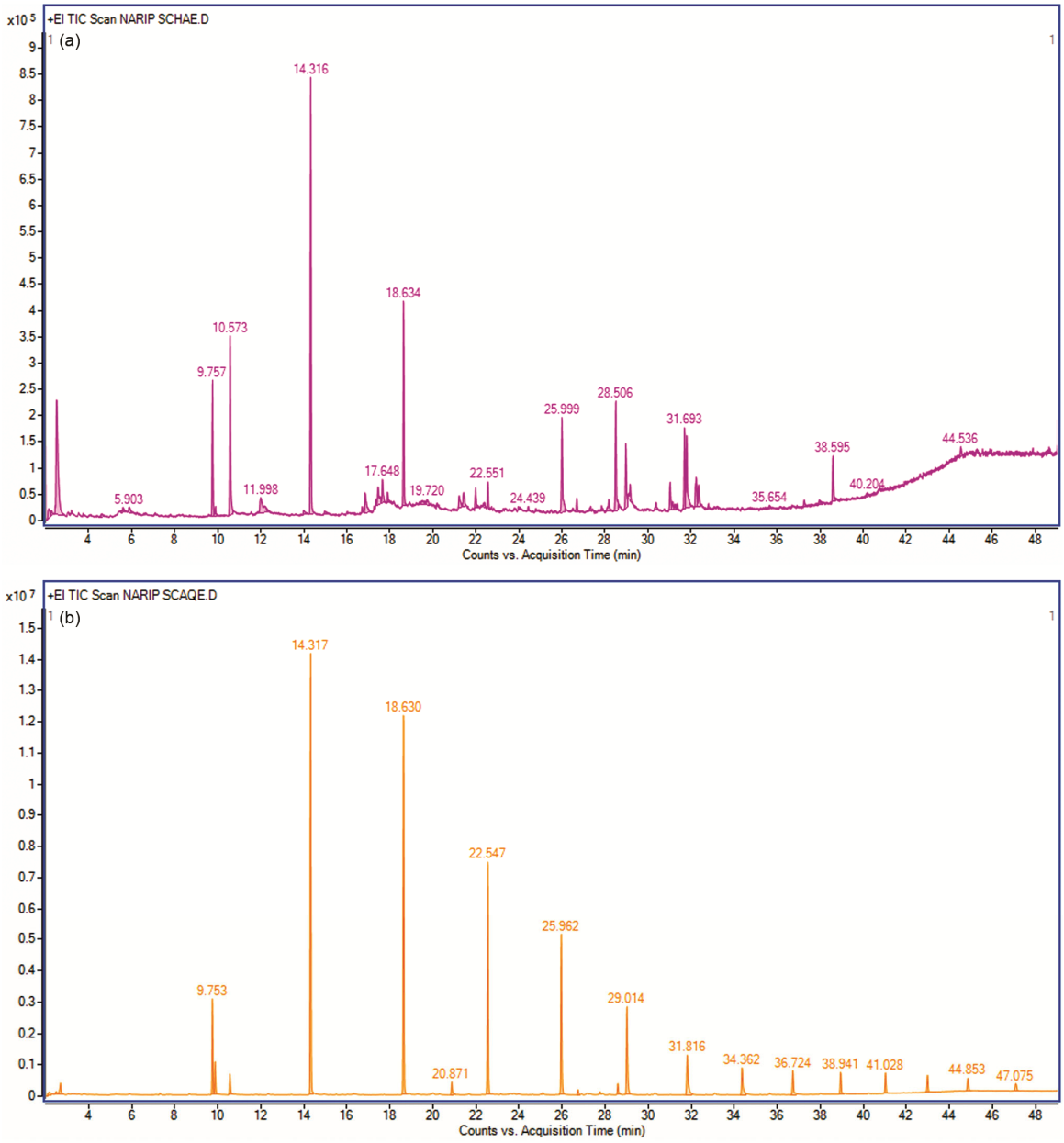

Fig. 5 — GC-MS Chromatogram of (a) HESC, and (b) AESC.

presented in Tables 8A and 8B. Gross necropsy findings were normal, and relative organ weights did not show any significant variation compared to the control group ($p > 0.05$), as shown in Table 8C. These findings indicate that the tested extracts are well tolerated at the administered dose and suggest a favourable acute safety profile under the experimental conditions.

Analgesic and anti-inflammatory activity

Hot plate test

The aqueous and hydroalcoholic extracts of *B. prionitis* L. and *S. ciliata* Nees were administered at doses of 200 and 400 mg/kg body weight. Both extracts produced a significant ($p < 0.001$) increase in pain latency in the hot plate model compared to the normal control group, indicating marked analgesic activity. No statistically significant ($p > 0.05$) difference was observed between S. ciliata and B. prionitis in the onset of analgesic action, as illustrated in Fig. 7a.

Tail flick test

The aqueous and hydroalcoholic extracts of *B. prionitis* L. and *S. ciliata*, administered at doses of 200 and 400 mg/kg body weight. The aqueous extract with the dose of 400 mg/kg exhibited a maximum

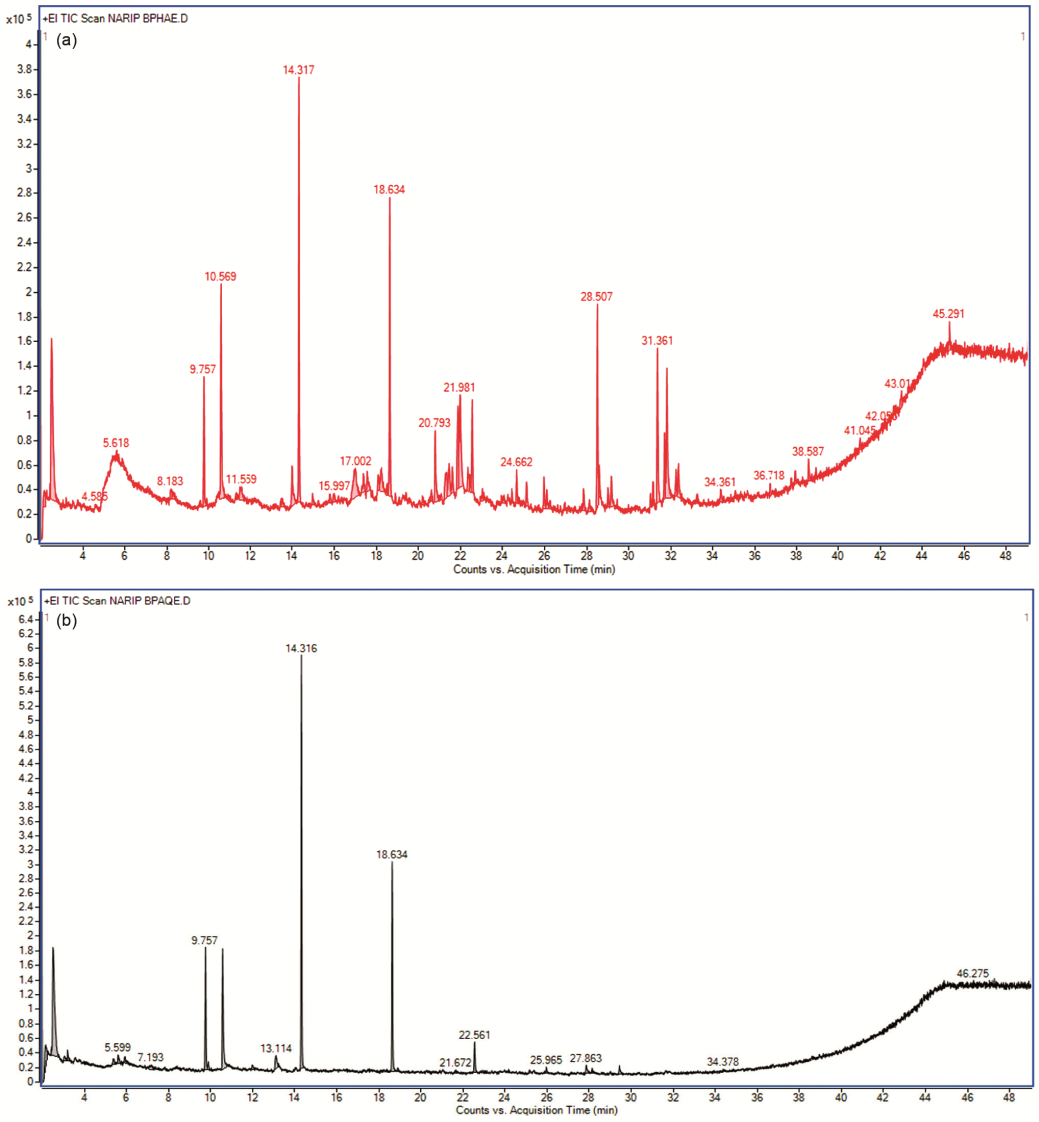

Fig. 6 — GC -MS Chromatogram of (a) HEBP, and (b) AEBP.

significant ($p < 0.0001$) increase in tail withdrawal latency in the tail flick model after 60 minutes of administration compared to the normal control group. No significant ($p > 0.05$) difference was observed between the two plant extracts in terms of onset of analgesic action, as shown in Fig. 7b.

Paw pressure or Randall–Selitto test

The aqueous and hydroalcoholic extracts of *B. prionitis* L. and *S. ciliata* Nees, administered at doses of 200 and 400 mg/kg *body weight,* produced a significant ($p < 0.01$) increase in pain threshold in the paw pressure (Randall–Selitto) test compared to the normal control group. The observed analgesic effect was comparable between the two plant extracts, with no statistically significant ($p > 0.05$) difference in pain threshold force gram-force (gf) between S. ciliata and B. prionitis, as illustrated in Fig. 8a.

Formalin test

The aqueous and hydroalcoholic extracts of *B. prionitis* and *S. ciliata* treated animals at tested doses of 200 mg/kg and 400 mg/kg body weight showed significant ($p < 0.01$) analgesic effect with extract of *B. prionitis* in the Formalin test model when compared with the normal control. There was no significant ($p > 0.05$) difference in paw licking time (seconds) between *B. prionitis* and *S. ciliata* plants in the early (neurogenic) and late (inflammatory) stages following formalin treatment, as shown in Fig. 8(b).

Table 7 — GC–MS/MS-based identification of chemical constituents in aqueous and hydroalcoholic extracts of *Barleria prioritis* L.

S No	Retention Time (Rt, min)	Compound name	Extract
1	2.170	Methyl S-2-dimethylaminoethylpropylphosphonothiolate	HEBP
2	2.513	Benzyl (1,2,3-thiadiazol-4-yl) carbamate	HEBP / AEBP
3	11.559	Cyclonona-1,2,6-triene	HEBP
4	13.988	3-Diazo-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	HEBP
5	17.002	7-Methylene-9-oxabicyclo[6.1.0]non-2-ene	HEBP
6	17.379	Methyl 2-benzyl-5-cyclopentyl-1,2,5-thiadiazepane-3-carboxylate 1,1-dioxide	HEBP
7	17.562	1,4,4a,5,6,7,8,8a-octahydro-1,4,9,9-tetramethyl-1,4-methanophthalazine	HEBP
8	18.243	5β,8β-Epoxy-3,5,8,8a-tetrahydro-1H-2-benzopyran	HEBP
9	18.514	Trimethyl (2-methyl-1-propenylidene) cyclopropane	HEBP
10	19.088	Oxacyclotetradeca-4,11-diyne	HEBP
11	20.793	Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester	HEBP
12	21.610	Oxacyclotetradeca-4,11-diyne	HEBP
13	21.880	β-D-Glycosyloxyazoxymethane	HEBP
14	22.349	3-Methylene-4-(1,2-propadienyl)cyclohexane	HEBP
15	24.662	Methanophthalazine derivative	HEBP
16	25.135	4-Methyl-3-(1H-tetrazol-5-yl)-1-oxa-spiro[4.4]non-3-en-2-one	HEBP
17	27.845	1-Hexadecanaminium, N,N,N-trimethyl-octadecanoate	HEBP
18	28.507	n-Hexadecanoic acid	HEBP
19	28.584	1-Isothiocyanato-3-methyladamantane	HEBP
20	29.164	3-n-Butylthiane-S-oxide	HEBP
21	31.810	Methyl 10,11-tetradecadienoate	HEBP
22	2.503	Benzyl (1,2,3-thiadiazol-4-yl) carbamate	AEBP
23	3.064	2-tert-Butyl-5-(dimethoxyphosphoryl)-3-methyl-4-oxoimidazolidine -1-carboxylic acid, tert-butyl ester	AEBP
24	5.362	1-Propanamine, 3-dibenzo[b,e]thiepin-11(6H)-ylidene-N,N-dimethyl-S-oxide	AEBP
25	5.599	4-Ethylmethcathinone	AEBP
26	5.927	(S)-9-[(S)-2-(Hydroxymethyl)pyrrolidin-1-yl]-3-methyl-3,4-dihydro-2H-benzo[b][1,4,5]oxathiazepine 1,1-dioxide	AEBP
27	13.114	1,4-Dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene	AEBP
28	27.863	4-(Ethenyl)-1,2-dimethyl-trans-decahydroquinolin-4-ol N-oxide	AEBP

Table 8A — Effect on body weight during acute toxicity study

Day	Control	AEBP (2000 mg/kg)	HEBP (2000 mg/kg)	AESC (2000 mg/kg)	HESC (2000 mg/kg)
			Initial Exploratory Step		
0	149±1	148±3.5@-ns	154±3.3@-ns	148±1.5@-ns	149±2@-ns
7	161±1.2	156.7±1.9@-ns	163±2.1@-ns	158±2.4@-ns	160±0.9@-ns
14	172±1.5	163.7±1.9@-ns	171.7±2.4@-ns	170±2.2@-ns	170±0.9@-ns
			Confirmatory Step		
0	146.7±2.2	152±2.6@-ns	146±3.0@-ns	149±2.4@-ns	149±5.2@-ns
7	157±1.5	161.3±3.8@-ns	155.3±2.4@-ns	159±2@-ns	159±5@-ns
14	167±2.2	169±2.1@-ns	164±0.6@-ns	167±3.1@-ns	170±3.2@-ns

Table 8B — Effect on feed intake during acute toxicity study

Day	Control	AEBP (2000 mg/kg)	HEBP (2000 mg/kg)	AESC (2000 mg/kg)	HESC (2000 mg/kg)
			Initial Exploratory Step		
7	75±5.5	69.7±3.7@-ns	70.3±4.5@-ns	77±3.1@-ns	81±2.8@-ns
14	75±4.3	75.7±2.9@-ns	72±4.0@-ns	69±1.5@-ns	71±2.3@-ns
			Confirmatory Step		
7	78±2	71.3±1.8@-ns	68.7±2.2@-ns	74±5.9@-ns	72±6.4@-ns
14	65±3.7	71.7±3.8@-ns	71.3±1.2@-ns	73±3.2@-ns	69±2@-ns

Table 8C — Effect on relative organ weight following acute toxicity study

Organ	Control	AEBP	HEBP	AESC	HESC
			Initial Exploratory Step		
Brain	0.9±0.02	1±0.02@-ns	1±0.03@-ns	1±0.02@-ns	1±0.02@-ns
Heart	0.4±0.01	0.4±0.01@-ns	0.4±0.01@-ns	0.3±0.01@-ns	0.4±0.01@-ns
Lungs	0.6±0.01	1.0±0.04@-ns	1.1±0.11@-ns	0.8±0.04@-ns	0.7±0.05@-ns
Liver	3±0.14	3.7±0.3@-ns	4.0±0.03@-ns	3±0.3@-ns	3±0.1@-ns
Kidney	0.7±0.03	0.7±0.02@-ns	0.7±0.04@-ns	0.7±0.03@-ns	0.7±0.02@-ns
Adrenal	0.03±0.002	0.03±0.004@-ns	0.04±0.03@-ns	0.03±0.002@-ns	0.03±0.003@-ns
Spleen	0.2±0.004	0.3±0.02@-ns	0.3±0.02@-ns	0.2 ± 0.02@-ns	0.2±0.02@-ns
Ovaries	0.1±0.001	0.1±0.003@-ns	0.1±0.001@-ns	0.1 ± 0.004@-ns	0.1±0.01@-ns
Stomach	0.7±0.05	0.8±0.02@-ns	0.9±0.02@-ns	0.7 ± 0.1@-ns	0.7±0.03@-ns
			Confirmatory Step		
Brain	1±0.04	1±0.04@-ns	1±0.07@-ns	1.1±0.02@-ns	1.1±0.07@-ns
Heart	0.4±0.02	0.4±0.04@-ns	0.4±0.02@-ns	0.5±0.02@-ns	0.5±0.03@-ns
Lungs	0.8±0.04	1.0±0.08@-ns	1.1±0.04@-ns	0.7±0.06@-ns	0.8±0.02@-ns
Liver	3.4±0.3	3.8±0.2@-ns	4.1±0.1@-ns	3.5±0.15@-ns	3.4±0.1@-ns
Kidney	0.7±0.01	0.7±0.04@-ns	0.8±0.05@-ns	0.9±0.1@-ns	0.9±0.03@-ns
Adrenal	0.03±0.001	0.04±0.0004@-ns	0.04±0.006@-ns	0.1±0.0004@-ns	0.04±0.008@-ns
Spleen	0.2±0.005	0.3±0.05@-ns	0.4±0.02@-ns	0.3±0.05@-ns	0.4±0.05@-ns
Ovaries	0.1±0.004	0.1±0.01@-ns	0.1±0.01@-ns	0.1±0.01@-ns	0.1±0.01@-ns
Stomach	1±0.01	0.9±0.06@-ns	0.9±0.02@-ns	1.1±0.09@-ns	1.1±0.03@-ns

N = 3; data expressed as mean ± SEM. Statistical analysis was performed using the t-test. ns = non-significant ($p > 0.05$). @ = comparison with vehicle control. AEBP: aqueous extract of *Barleria prionitis* L.; HEBP: hydroalcoholic extract of *Barleria prionitis* L.; AESC: aqueous extract of *Strobilanthes ciliatus* Nees; HESC: hydroalcoholic extract of *Strobilanthes ciliatus* Nees.

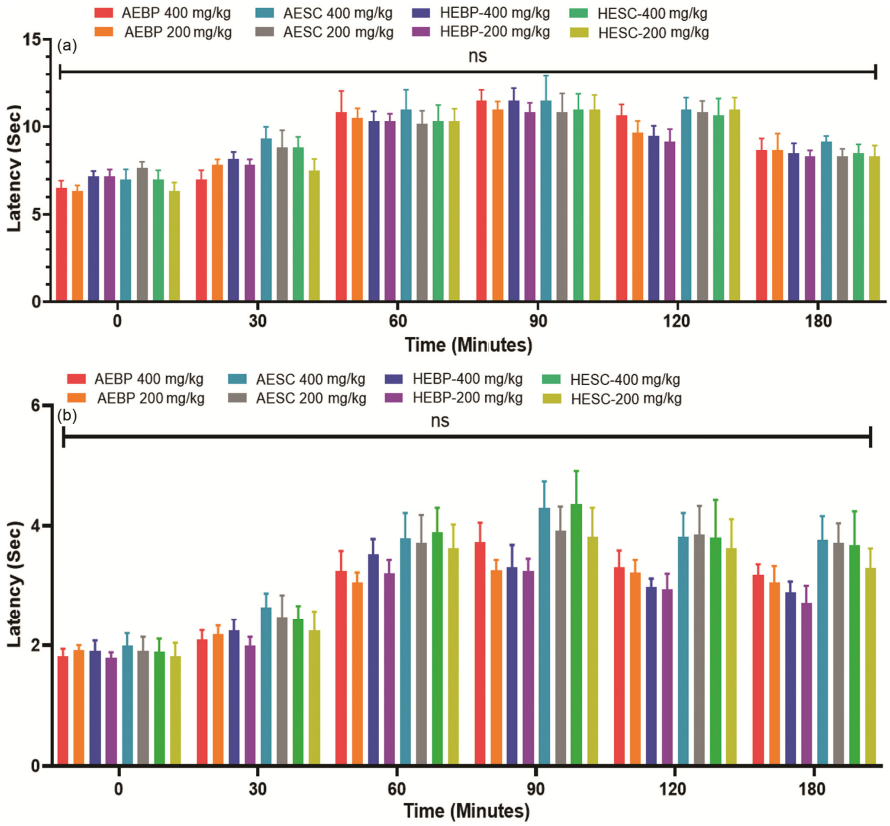

Fig. 7 — (a) Hot plate, and (b) Tail flick tests- Comparison of analgesic activity of *Barleria prionitis* vs *Strobilanthes ciliata* extracts. Data are expressed as mean ±SEM (n = 6), comparison of latency of AESC with AEBP and HESC with HEBP, No significant difference was observed ns (not significant, p >0.05).

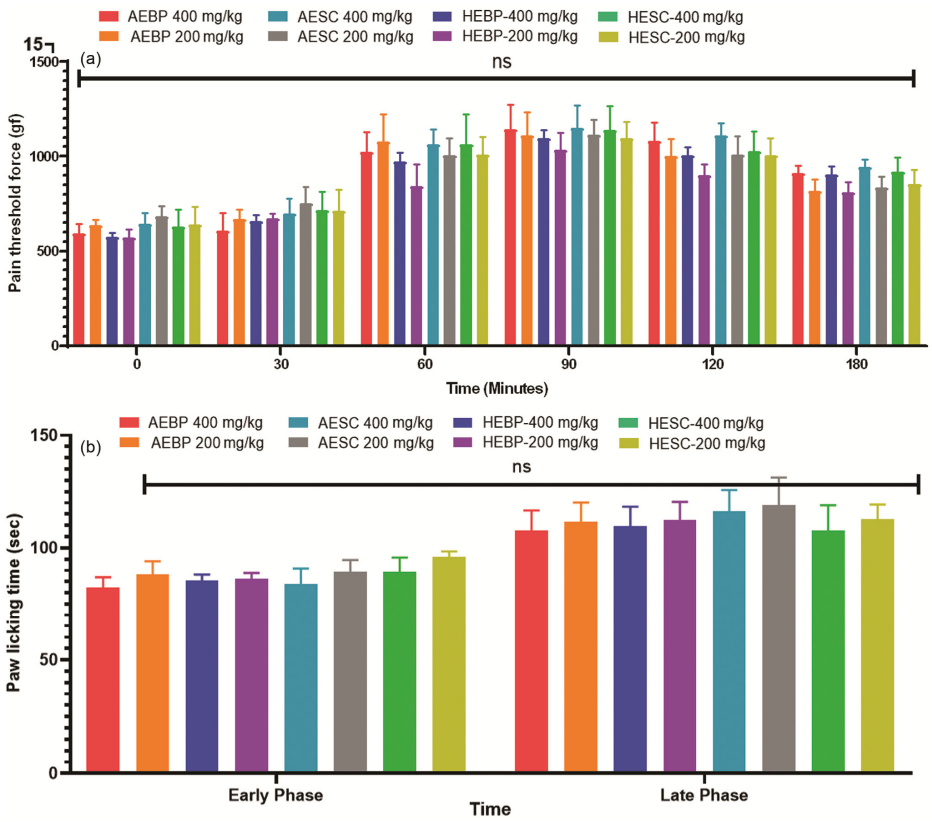

Fig. 8 — (a) Formalin Paw pressure, and (b) Formalin test- Comparison of analgesic activity of *Barleria prionitis* vs *Strobilanthes ciliata* extracts. Data are expressed as mean ±SEM (n = 6), comparison of analgesic effect of AESC with AEBP and HESC with HEBP, No significant difference was observed (ns).

Anti-inflammatory study (Carrageenan-induced acute inflammation)

The aqueous and hydroalcoholic extracts of *B. prionitis* and *S. ciliata,* administered at doses of 200 and 400 mg/kg body weight, demonstrated mild to moderate anti-inflammatory activity in the current model when compared to the disease control group. As illustrated in Fig. 9, the serum levels of the inflammatory markers such as TNF-α and IL-6 were significantly elevated in the disease control (DC) group compared to the normal control (NC) group ($p < 0.01$). Animals treated with Diclofenac at a dose of 10 mg/kg showed statistically significant reduction in IL-6 and TNF-α levels ($p < 0.05$). Animals treated with the aqueous and hydroalcoholic extracts did not show a significant reduction in TNF-α and IL-6 levels compared to the disease control (DC) group ($p > 0.05$). As shown in Fig. 10 and Fig. 11, no significant difference was observed in paw oedema volume between both aqueous and hydroalcoholic extracts of *S. ciliata* and *B. prionitis* treatments at doses of 200 and 400 mg/kg body weight ($p > 0.05$).

Data are expressed as mean ± SEM, with n = 6 per group. Values of DC were compared with those of NC, and all other groups were compared with the DC group. Statistical significance was considered at *$p < 0.05$; ns indicates non-significant differences.

Both extracts, administered at doses of 200 and 400 mg/kg body weight, demonstrated a significant analgesic effect across all analgesic models compared with the disease control group. The studies indicate that aqueous and hydroalcoholic extracts of both plants exhibited notable peripheral analgesic effects. The analgesic response between the source plants and their substitute plants was not statistically significant ($p > 0.05$).

The anti-inflammatory activity was evident through a significant reduction in paw volume in all extract-treated animals compared to the carrageenan-induced control group. Despite the marked anti-inflammatory effect, as evidenced by the reduction in paw oedema volume, animals treated with the test drug did not exhibit any statistically significant changes in TNF-α and IL-6 levels. The preliminary findings indicate the anti-inflammatory responses of both substitute plants and source plants.

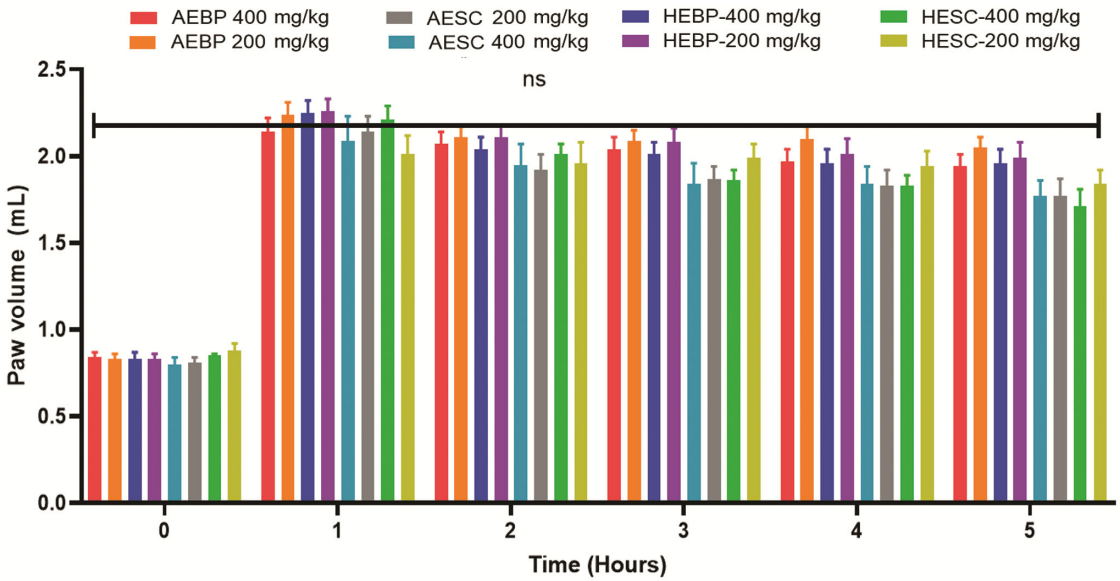

Fig. 9 — Anti-inflammatory activity (Paw volume of Rats) - Comparison of *Barleria prionitis* (AEBP, HEBP) vs *Strobilanthes ciliata* (AESC, HESC) extracts. Data are expressed as mean ±SEM (n = 6), Paw volume of AEBP with AESC and HEBP with HESC, No significant difference was observed (ns)

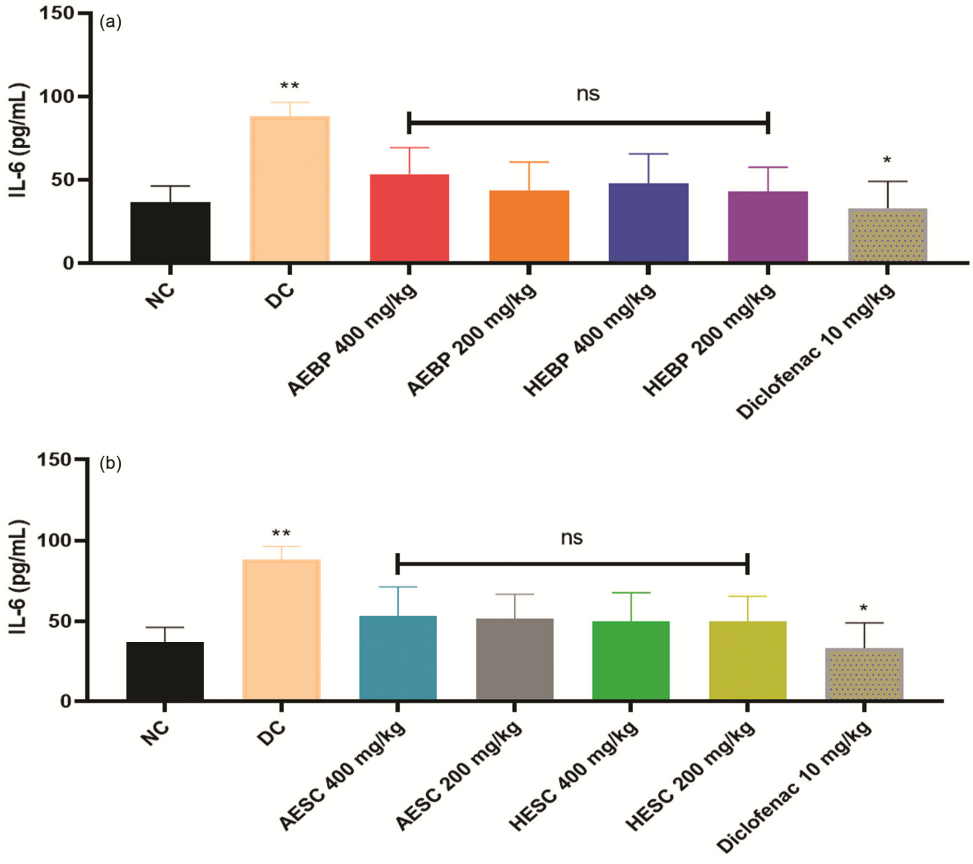

Fig. 10 — (a) IL-6 levels-Source Plants, and (b) IL-6 levels-Substitute Plants.
Data are expressed as mean ±SEM (n = 6), value of DC was compared with NC and all other groups were compared with the DC group. Level of significance expressed as *p <0.05, **p <0.01, ns-non significant

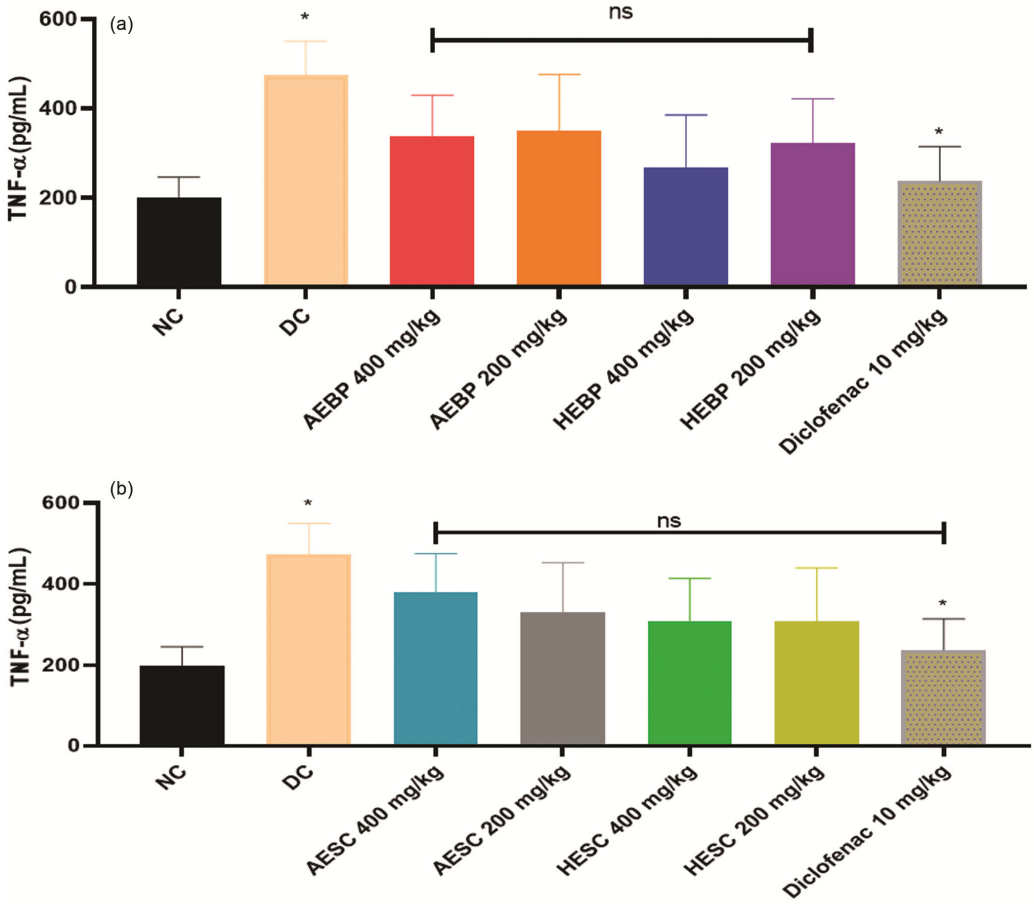

Fig. 11 — (a) TNF-α levels-Source plants, and (b) TNF-α levels-Substitute plants.

Discussion

Substitution rationale and importance

Substitution in Ayurveda plays a critical role in ensuring the continuity of therapeutic practices by identifying botanically and pharmacologically comparable alternatives when the primary drug is scarce. The limited availability of *B. prionitis* in certain regions has necessitated the exploration of alternative sources of Sahachara. *S. ciliata*, widely used in southern India, has been traditionally recognised as a substitute.

Quality and safety assurance

The extracts of *B. prionitis* and *S. ciliatus* complied within permissible limits for pesticide residues, aflatoxins, heavy metals, and microbial contamination, indicating their suitability for use in medicinal formulations. Physicochemical evaluation further demonstrated that the extracts of *B. prionitis* and *S. ciliata* exhibited comparable parameters, suggesting similarity in quality attributes between the source and substitute plants.

HPTLC Phytochemical Profiling Insights

HPTLC fingerprint profiling revealed both similarities and variations in the phytochemical composition of aqueous and hydroalcoholic extracts of *B. prionitis* and *S. ciliatus*. The presence of several common bands across AEBP, HEBP, AESC, and HESC indicates a degree of phytochemical similarity between the source and substitute plants. Marker compound analysis further supports this observation, as gallic acid and quercetin were detected in AEBP and HESC, while β-sitosterol was identified in HEBP and AESC. These findings suggest that both plants share key bioactive constituents, which may contribute to their comparable pharmacological effects. At the same time, variations in band number and intensity between aqueous and hydroalcoholic extracts reflect differences in solvent extraction efficiency, with hydroalcoholic extracts demonstrating greater phytochemical diversity. Overall, the HPTLC profiles support the concept of *Strobilanthes ciliatus* as a suitable substitute for *B. prionitis*, based on the presence of common marker compounds and comparable phytochemical patterns.

Table 9 — Comparison of common phytoconstituents based on retention time (Rt) in GC–MS/MS analysis of *S. ciliata* and *B. prionitis*

	Strobilanthes ciliata	
S No	Hydroalcoholic Extract (HESC)	Aqueous Extract (AESC)
	Rt (min) – Compound	Rt (min) – Compound
1	2.513 – Benzyl (1,2,3-thiadiazol-4-yl) carbamate	2.494 – Benzyl (1,2,3-thiadiazol-4-yl) carbamate
2	28.173 – Oxacyclotetradeca-4,11-diyne	Not detected
3	28.506 – n-Hexadecanoic acid	Not detected
4	14.007 – 3-Diazo-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	Not detected
	Barleria prionitis	
S No	Hydroalcoholic Extract (HEBP)	Aqueous Extract (AEBP)
	Rt (min) – Compound	Rt (min) – Compound
1	2.513 – Benzyl (1,2,3-thiadiazol-4-yl) carbamate	2.503 – Benzyl (1,2,3-thiadiazol-4-yl) carbamate
2	13.988 – 3-Diazo-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	13.114 – 1,4-Dimethyl-2,3-diazabicyclo[2.2.1]hept-2-ene
3	19.088 / 21.610 – Oxacyclotetradeca-4,11-diyne	Not detected
4	28.507 – n-Hexadecanoic acid	Not detected
5	31.810 – Methyl 10,11-tetradecadienoate	Not detected
	Common Compounds Between Both Species	
S No	Common Phytoconstituent	Approx. Rt (min)
1	Benzyl (1,2,3-thiadiazol-4-yl) carbamate	~2.5

GC-MS/MS Analysis of both plant extracts

The GC–MS/MS findings clearly demonstrate that solvent selection plays a crucial role in determining the efficiency and spectrum of phytochemical extraction from *B. prionitis* L. and *S. ciliata* Nees. The hydroalcoholic extracts (HEBP and HESC) exhibited a greater number of chromatographic peaks than their aqueous counterparts (AEBP and AESC), indicating enhanced extraction of chemically diverse phytoconstituents. Specifically, HESC showed a greater number of identifiable compounds than AESC, and HEBP similarly exhibited a richer profile than AEBP. This observation supports the established principle that hydroalcoholic solvents, owing to their intermediate polarity, are more effective in extracting both polar and moderately non-polar constituents.

Further analysis revealed the presence of certain common compounds at similar retention times within and between the extracts, suggesting partial phytochemical consistency. In *S. ciliata*, compounds such as benzyl (1,2,3-thiadiazol-4-yl) carbamate (Rt ~2.5 min), oxacyclic compounds (Rt ~28.1–28.2 min), and fatty acids such as n-hexadecanoic acid (Rt ~28.5 min) were identified in the hydroalcoholic extract, whereas fewer overlapping compounds were observed in the aqueous extract. In *B. prionitis*, benzyl (1,2,3-thiadiazol-4-yl) carbamate (Rt ~2.5 min) was consistently detected in both HEBP and AEBP, along with selected heterocyclic compounds and oxygenated derivatives.

Importantly, benzyl (1,2,3-thiadiazol-4-yl) carbamate was identified as a common constituent across both plant species, indicating a degree of phytochemical overlap between the source and substitute. Such shared constituents may contribute to similarities in their pharmacological activities.

It is noteworthy that siloxane derivatives, phthalate esters, and other probable analytical contaminants were excluded from interpretation, as these compounds are commonly associated with column bleed, solvents, or sample handling artifacts rather than true plant-derived metabolites.

Overall, the hydroalcoholic extracts demonstrated superior extraction efficiency and a more complex phytochemical composition, which may underlie their enhanced biological activity. These findings highlight the importance of solvent selection in phytochemical investigations and support the use of hydroalcoholic systems for comprehensive profiling of bioactive constituents in both *B. prionitis* and *S. ciliata*. The comparative retention time analysis is presented in Table 9.

Analgesic and Anti-inflammatory activities

The acute toxicity evaluation of AEBP, HEBP, AESC, and HESC as per OECD 423 revealed that no toxicity was observed up to 2000 mg/kg body weight. Hence, the approximate LD_{50} is more than 2000 mg/kg.

The present study demonstrated that oral administration of aqueous and hydroalcoholic extracts

of source plant (AEBP and HEBP) and substitute plant (AESC and HESC) at 200 mg/kg and 400 mg/kg body weight exhibited analgesic effect in hotplate and tail flick as well as formalin-induced pain models, and anti-inflammatory activity in carrageenan-induced paw oedema model in rats.

Pain, an unpleasant experience both physically and emotionally, arises from various sources, such as physical and chemical stimuli, nervous system damage, and tissue damage[28]. The hot plate method is one of the most widely used thermal nociception models for assessing the central analgesic efficacy of drugs or compounds. This method is selective for the centrally acting analgesics and evaluates the antinociceptive effect of test drugs through central mechanisms[29,30]. Tail flicking is predominantly a spinal reflex and is considered to be selectively sensitive to centrally acting analgesic compounds[24]. The test extracts showed an analgesic effect compared to the vehicle control[31,32]. The non-significant analgesic effect between source plant (AEBP and HEBP) and substitute plant (AESC and HESC) indicates that both plant extracts have similar antinociceptive effects comparable to the standard drug, tramadol. The central analgesic action is mediated via inhibition of central pain receptors[33,34]. Therefore, it is possible that the extracts may exhibit analgesic effects through these mechanisms.

The persistent-pain model of formalin-induced hind-paw licking/flinching was used to evaluate the analgesic effects of plant extracts in the present study. The sub-plantar injection of formalin (2.5%) in rats produces pain, as indicated by paw licking and flinching, as a distinct biphasic response: an early phase (neurogenic) and a late phase (inflammatory). The early phase is attributed to the direct activation of nociceptors and primary afferent fibres, particularly C-fibres, leading to the release of bradykinin and tachykinins[35,36]. This phase is inhibited by opioid analgesics. The late phase is due to an inflammatory reaction caused by tissue injury, leading to the release of histamine, serotonin, prostaglandins, and excitatory amino acids[37,38]. This phase is inhibited by both non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics[39]. Oral administration of aqueous and hydroalcoholic extracts at 200 and 400 mg/kg body weight significantly decreased the number of hind paw lickings and flinching in both phases. This indicates the antinociceptive (pain-relieving) effect of the extracts and suggests that they influence both neurogenic and inflammatory pain responses. The inhibition of neurogenic pain indicates that extracts may be affecting the stimulation of peripheral nociceptors and the activity of transduction molecules, viz., substance P and bradykinins, involved in central nociception. The inhibition of inflammatory pain by the extracts also indicates their influence on inflammatory mediators, such as serotonin and prostaglandin production, released in the late phase. The above antinociceptive effect of the extracts in formalin-induced pain substantiates their therapeutic use in persistent and recurrent tonic-painful inflammatory conditions.

The Randall-Selitto test, also known as the paw pressure test, is used to assess the intensity of the reaction to mechanical pressure and is commonly regarded as a measure of acute mechanical pain[40]. The test assesses pain-related behaviours, such as paw withdrawal, vocalisation, or writhing, as indicators of the pain threshold[41]. Increased pain threshold in aqueous and hydroalcoholic extracts (200 and 400 mg/kg body weight) treated rats indicates an analgesic effect and the ability to tolerate pain.

Carrageenan-induced paw oedema in rats is an acute model of inflammation and development of inflammation is a biphasic event, with early hyperemia due to the release of histamine and serotonin and the delayed oedema due to the release of bradykinins and prostaglandins which result in the increased vascular permeability and migration of leucocytes to the inflamed site causing vasodilation, which is one of the most common symptoms of inflammation[42]. NO, TNF-α, and interleukins (IL-1 β and IL-6) are reported to cause vasodilation[43]. Oral administration of AEBP and HEBP, as well as AESC and HESC, at 200 and 400 mg/kg body weight significantly decreased carrageenan-induced paw oedema. The inhibition of paw oedema was higher from the 3rd hour of treatment, without much significant inhibition at the 1st hour. Similarly, the standard drug, diclofenac sodium, also inhibited the paw oedema at the 3rd to 5th hour. The maximum inhibition observed between the 3rd and 5th hour indicates that the extracts may influence the release of bradykinins and prostaglandins at the site of inflammation. The test extracts reduced TNF-α and IL-6 as compared to disease control, similar to diclofenac sodium. There was no significant change in TNF-α and IL-6 levels among test extract groups, which indicates a similar ability of the test extracts to decrease inflammatory mediators.

The exact mechanisms of analgesic and anti-inflammatory actions of AEBP, HEBP, AESC, and HESC are difficult to elucidate; however, it is possible that they may have an influence on the arachidonic acid pathway and inflammatory mediators like prostaglandins, mainly by inhibiting the cyclooxygenase enzyme, as does diclofenac sodium. Extracts have an inhibitory influence on pro-inflammatory cytokines, thereby reducing both neurogenic and inflammatory pain and inflammation.

Test extracts contain various anti-inflammatory constituents like terpenoids (lupeol), flavonoids (apigenin and luteolin), phytosterols, and various phenolic acids[44]. The triterpenoids[45,46] and flavonoids[47,48] showed analgesic and anti-inflammatory activity and have inhibitory effects on COX2 and inflammatory markers[49]. Hence, the observed analgesic and anti-inflammatory activity may be attributed to these ingredients.

Conclusion

The present study provides a comprehensive comparative evaluation of *B. prionitis* L. and *S. ciliata* Nees with respect to phytochemical composition, safety, and pharmacological activity. HPTLC and GC–MS/MS analyses revealed considerable overlap in key phytoconstituents, along with greater chemical diversity in hydroalcoholic extracts, indicating improved extraction efficiency. Both plants demonstrated comparable safety profiles in acute toxicity studies, with no significant adverse effects observed at the tested dose. Furthermore, the aqueous and hydroalcoholic extracts of both species exhibited significant analgesic activity across multiple experimental models.

Overall, the findings support the pharmacological similarity between the two species and suggest that *S. ciliata* may serve as a suitable substitute for *B. prionitis* in Ayurvedic formulations. However, considering the conservation status of *S. ciliata*, its utilisation should be guided by sustainable harvesting and cultivation practices. The study highlights the importance of evidence-based substitution while ensuring conservation of medicinal plant resources.

Conflict of interest

The authors declare that no conflicts of interest exist with respect to this study and its publication.

AI use disclosure

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) as an AI-assisted language tool to improve grammar, sentence structure, and overall readability of the text. The AI tool was not used for experimental design, data generation, data analysis, interpretation of results, preparation of figures, or formulation of scientific conclusions. All AI-assisted content was carefully reviewed and verified by the authors, who take full responsibility for the originality, accuracy, and integrity of the manuscript.

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