

**PHYTOCHEMICAL PROFILING, PHYSICOCHEMICAL
EVALUATION AND LC-MS CHARACTERIZATION OF THE
HYDROETHANOLIC EXTRACT OF *STROBILANTHES PAVALA*
(ROXB.) J.R.I. WOOD**

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ABSTRACT

The present study investigated the phytochemical composition and physicochemical properties of *Strobilanthes pavala* using qualitative and quantitative analyses, alongside liquid chromatography-mass spectrometry (LC-MS) characterization. Preliminary phytochemical screening of the hydroethanolic extract (SHE) revealed the presence of phenols, flavonoids, tannins, terpenoids, proteins, and carbohydrates. Quantitative analysis demonstrated appreciable levels of these secondary metabolites, with the extract exhibiting a total phenolic content of $125.05 \pm 4.62 \mu\text{g GAE/mg}$ and a total flavonoid content of $370.50 \pm 16.90 \mu\text{g QE/mg}$. Physicochemical evaluation of the raw plant powder yielded a total ash value of 11.20% and a moisture content of 4.55%, indicating acceptable quality and purity parameters. Furthermore, LC-MS analysis of the extract identified a diverse array of bioactive metabolites encompassing flavonoids, terpenoids, glycosides, triterpenoids,

alkaloids, and phenolic derivatives. Notable compounds identified include genistein, hydrangenoside C, neoandrographolide, ganolactone B, and dehydrolucidenic acid A. These findings provide a comprehensive phytochemical profile that supports the therapeutic

potential of *S. paval* and establishes a foundation for future pharmacological investigations.

KEYWORDS: *Strobilanthes paval*; Phytochemical screening; LC-MS; Total flavonoid content; Total phenolic content; Secondary metabolites.

INTRODUCTION

Traditional medicinal systems, particularly Ayurveda, remain a foundational healthcare resource, relied upon by approximately 70% of rural populations in India. While modern allopathic medicine maintains a dominant presence with roughly 700,000 practitioners across the country, the traditional Ayurvedic system continues to operate robustly alongside it, supported by an estimated 250,000 registered medical practitioners.

The efficacy of these traditional remedies is largely attributed to plant-derived secondary metabolites. Bioactive constituents such as flavonoids, alkaloids, tannins, phenolics, saponins, steroids, and terpenoids exhibit significant therapeutic potential and possess substantial commercial value across various industries (Biju *et al.*, 2023). Consequently, comprehensive phytochemical evaluations are essential for the accurate characterization and isolation of these valuable compounds. Within this context, the genus *Strobilanthes* holds considerable ecological and economic prominence, particularly for its integration into the Indian system of medicine (Venu *et al.*, 2006). Etymologically derived from the Greek terms “strobilos” (cone) and “anthos” (flower or shoot), *Strobilanthes* Blume represents the second most diverse genus within the Acanthaceae family. The taxa are indigenous to tropical Asia, with India serving as a primary center of diversity (Fernandes *et al.*, 2025). To date, roughly 150 species have been documented across the Indian subcontinent, with a heavy concentration of over 60 species localized specifically in the southern regions (Pradeep *et al.*, 2020).

Among these, *Strobilanthes paval* is highly valued in indigenous medical practices. The vegetative structures of *S. paval* are utilized extensively due to their diverse pharmacological profile, which demonstrates notable anti-diabetic, antimicrobial, antifungal, analgesic, and anti-inflammatory activities (Biju *et al.*, 2023). A prominent example of its application is in “Kurinji kuzhambu,” a traditional therapeutic formulation prescribed for pregnancy-related complications. Preliminary phytochemical profiling of the stem and leaf extracts of *S. paval* corroborates this medicinal efficacy, confirming the accumulation of key bioactive classes, including glycosides, sterols, carbohydrates, saponins, tannins, terpenoids, flavonoids, steroids, and alkaloids.

MATERIALS AND METHODS

Collection of the plant material: The fresh plant specimens of *Strobilanthes paval* utilized in this study (Figure 1a) were collected in December 2023 from the Joladalu region, located within the Shivamogga district of Karnataka, India (13.94992° N, 75.847157° E).

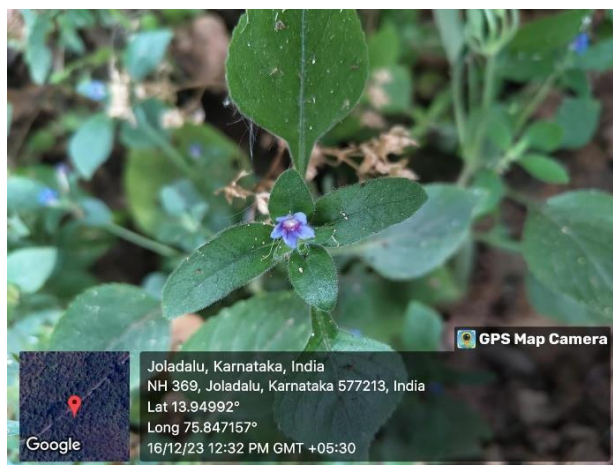


Fig. 1a: *Strobilanthes paval* Plant.



Fig. 1b: Filtration of the solvent extract.

Preparation of plant extract: The shade-dried whole plant material was pulverized into a coarse powder. For the extraction process, 20 g of the plant powder was subjected to cold maceration using 200 mL of 70% ethanol. The mixture was maintained at room temperature for 48 h with occasional shaking to facilitate optimal extraction of the phytoconstituents. The mixture was then filtered through Whatman No. 1 filter paper (Figure 1b). A small portion of the resulting liquid filtrate was reserved for preliminary qualitative phytochemical screening. The remaining filtrate was concentrated under reduced pressure to evaporate the solvent, yielding the dried crude hydroethanolic extract. The dried extract was stored at 4 °C in an airtight container until further use for quantitative estimations and LC-MS analysis.

Qualitative phytochemical analysis

The extract was subjected to preliminary qualitative phytochemical screening using standard protocols to detect the presence of various diverse bioactive compounds (Harborne *et al.*, 1998; Kokate *et al.*, 2007; Sofowora *et al.*, 1993).

Test for Phenols (Lead Acetate Test): One milliliter (1 mL) of the sample was treated with a few drops of 10% lead acetate solution. The formation of a bulky white precipitate indicated the presence of phenolic compounds.

Test for Alkaloids

Wagner's Test: Wagner's reagent was added to 1 mL of the sample. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Dragendorff's Test: Dragendorff's reagent was carefully added to the sample. The formation of a prominent orange-red precipitate confirmed the presence of alkaloids.

Test for Flavonoids

Alkaline Reagent Test: The sample was treated with a few drops of 20% sodium hydroxide (NaOH) solution. The appearance of an intense yellow color, which became colorless upon the addition of dilute hydrochloric acid (HCl), indicated the presence of flavonoids.

Lead Acetate Test: A few drops of lead acetate solution were added to the sample. The formation of a yellow precipitate indicated the presence of flavonoids.

Test for Tannins (Ferric Chloride Test): One milliliter (1 mL) of the sample was treated with a few drops of 5% neutral ferric chloride (FeCl_3) solution. The appearance of a dark blue or blue-green color indicated the presence of tannins.

Test for Terpenoids (Salkowski Test): Five milliliters (5 mL) of the sample were mixed with 2 mL of chloroform, followed by the careful addition of 3 mL of concentrated sulfuric acid (H_2SO_4) along the sides of the test tube. The development of a reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Saponins (Foam Test): A small quantity of the extract (approx. 1 mL) was vigorously diluted and shaken with 5 mL of distilled water in a graduated cylinder. The formation of a persistent, stable froth indicated the presence of saponins.

Test for Glycosides (Keller-Killiani Test): The sample was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride (FeCl_3) solution, followed by the careful addition of 1 mL of concentrated sulfuric acid (H_2SO_4) along the walls of the tube. The formation of a brown ring at the junction of the two liquids indicated the presence of cardiac glycosides.

Test for Steroids (Liebermann-Burchard Test): The sample was dissolved in 2 mL of chloroform and treated with 2 mL of acetic anhydride, followed by the addition of a few drops of concentrated sulfuric acid (H_2SO_4). A color change from blue to green indicated the

presence of steroids.

Test for Proteins (Biuret Test): Two milliliters (2 mL) of the sample were treated with 2 mL of 40% sodium hydroxide (NaOH) solution and a few drops of 1% copper sulphate (CuSO_4) solution. The appearance of a violet or purple color indicated the presence of proteins.

Test for Carbohydrates (Benedict's Test): Two milliliters (2 mL) of the sample were treated with 5 drops of Benedict's reagent and heated in a boiling water bath for 5 minutes. The appearance of a light green, yellow, or reddish-brown precipitate indicated the presence of reducing sugars.

Quantitative phytochemical estimation

Total Phenolic estimation

The total phenolic content of the extract was determined following the method described by Singleton et al. (1999) using the Folin-Ciocalteu (FC) reagent, with gallic acid used as the standard. Briefly, to 100 μg of the appropriately diluted extract, 0.5 mL of FC reagent was added. The mixture was incubated at room temperature for 10 min, followed by the addition of 2 mL of 7% Na_2CO_3 solution. The reaction mixture was then boiled for 1 min, and the absorbance was recorded at 750 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The results were expressed as micrograms of gallic acid equivalents per milligram of extract ($\mu\text{g GAE/mg}$).

Total flavonoid estimation

The total flavonoid content of the extract was determined following the method described by Delcour and de Varebeke (1985). Briefly, to 200 μg of the sample, 5 mL of chromogen reagent (0.1% cinnamaldehyde solution in a chilled mixture of 75 mL methanol and 25 mL concentrated HCl) was added. After a 10 min incubation period, the absorbance was recorded at 640 nm using a UV-Vis spectrophotometer. The total flavonoid content was expressed as micrograms of quercetin equivalents per milligram of extract ($\mu\text{g QE/mg}$).

Physicochemical Evaluation of Plant Material

The physicochemical parameters of the plant powder, specifically moisture content and total ash value, were determined following standard guidelines to assess the quality and purity of the sample (World Health Organization, 1998).

Determination of Moisture Content

The moisture content was evaluated using the standard loss-on-drying method. A precisely weighed amount of the initial plant biomass was placed in a pre-weighed crucible and dried in a hot air oven at 100 °C for 10 h. Following the drying period, the sample was allowed to cool, and the final constant weight was recorded. The moisture content was calculated based on the weight loss and expressed as a percentage of the initial sample weight using the following formula:

$$\text{Moisture Content (\%)} = [(\text{Initial weight} - \text{Final weight}) / \text{Initial weight}] \times 100$$

Determination of Total Ash Content

The total ash content was determined to evaluate the amount of residual inorganic matter. Briefly, 5 g of the accurately weighed plant powder was placed in a previously tared crucible. The sample was then incinerated in a muffle furnace at 550 °C for 10 min. After incineration, the crucible was cooled, and the final ash weight was recorded. The total ash value was calculated based on the weight of the residual ash and expressed as a percentage of the total initial sample weight using the following formula:

$$\text{Total Ash (\%)} = (\text{Weight of ash} / \text{Weight of sample taken}) \times 100$$

LCMS Analysis

The LC-MS analysis was performed using an Acquity UPLC system coupled with a Synapt G2 mass spectrometer (Waters, USA). For sample preparation, 10 mg of the extract was dissolved in methanol, made up to a final volume of 10 mL (yielding a concentration of 1 mg/mL), and filtered prior to injection.

Chromatographic separation was achieved on an Acquity UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 µm particle size). The mobile phase consisted of 10 mM ammonium acetate in water (solvent A) and acetonitrile (solvent B) delivered at a flow rate of 0.40 mL/min. The gradient elution program was set as follows: 0.0–2.0 min, 2% B; 2.0–6.0 min, linear gradient from 2% to 50% B; 6.0–10.0 min, 50% to 98% B; 10.0–15.0 min, isocratic at 98% B; 15.0–17.0 min, return to 2% B; and 17.0–20.0 min, column equilibration at 2% B. The injection volume was 10 µL.

Mass spectrometry data were acquired with the following electrospray ionization (ESI) source parameters: capillary voltage of 2.5 kV, cone voltage of 30 V, source temperature of 130 °C, and a desolvation temperature of 400 °C. The desolvation gas flow rate was maintained at 600 L/h. Data acquisition and subsequent processing were carried out using MassLynx V4.1

software.

RESULTS

Qualitative phytochemical screening

The extraction yield of 9.32% demonstrates the efficiency of the cold maceration method using a hydroethanolic solvent, which is highly effective at drawing out both polar and moderately non-polar compounds. As summarized in Table 1, the qualitative analysis confirms that the *S. pavala* extract is rich in diverse therapeutic secondary metabolites. The distinct presence of phenols, flavonoids, and tannins strongly suggests that the plant possesses substantial antioxidant properties, as these compounds are well-known free radical scavengers. Furthermore, the detection of terpenoids points toward potential anti-inflammatory and antimicrobial activities. Together, these preliminary findings establish a strong phytochemical foundation for the plant's traditional medicinal use and justify the subsequent quantitative profiling of its bioactive components.

Preliminary qualitative phytochemical analysis

Table 1: Qualitative phytochemical analysis of the *Strobilanthes paval* ethanolic extract.

Sl No	Phytochemical constituents	SHE
1	Phenols	+
2	Alkaloids	-
3	Flavonoids	+
4	Tannins	+
5	Terpenoids	+
6	Saponins	-
7	Glycosides	-
8	Steroids	-
9	Proteins	+
10	Carbohydrates	+

SHE= *Strobilanthes paval* Hydroethanolic extract

Quantitative Phytochemical Analysis

The quantitative estimation of secondary metabolites, specifically total phenolic and flavonoid contents, for the SHE extracts is presented in Table 2 (and Figure 2). Phenolic compounds and flavonoids are widely recognized as highly significant plant metabolites due to their potent antioxidant, anti-inflammatory, and overall therapeutic properties.

The total phenolic content of the SHE extracts, evaluated using the Folin-Ciocalteu method, was found to be 125.05 ± 4.62 μg GAE/mg extract. Additionally, the total flavonoid content

was recorded at a notably high concentration of 370.50 ± 16.90 μg QE/mg extract. The substantial accumulation of these polyphenolic compounds suggests that the SHE extract possesses significant antioxidant potential. Because phenolics and flavonoids primarily exert their biological effects by neutralizing free radicals and chelating metals, these appreciable quantities strongly support the pharmacological value of the plant extract.

Table 2: Quantitative Estimation of Total Phenolic and Flavonoid Contents.

Sl. No.	Samples	Polyphenols ($\mu\text{g}/\text{mg}$ GAE)	Flavonoids ($\mu\text{g}/\text{mg}$ QE)
1	SHE	125.05 ± 4.62	370.50 ± 16.90

SHE= *Strobilanthes paval* Hydroethanolic extract

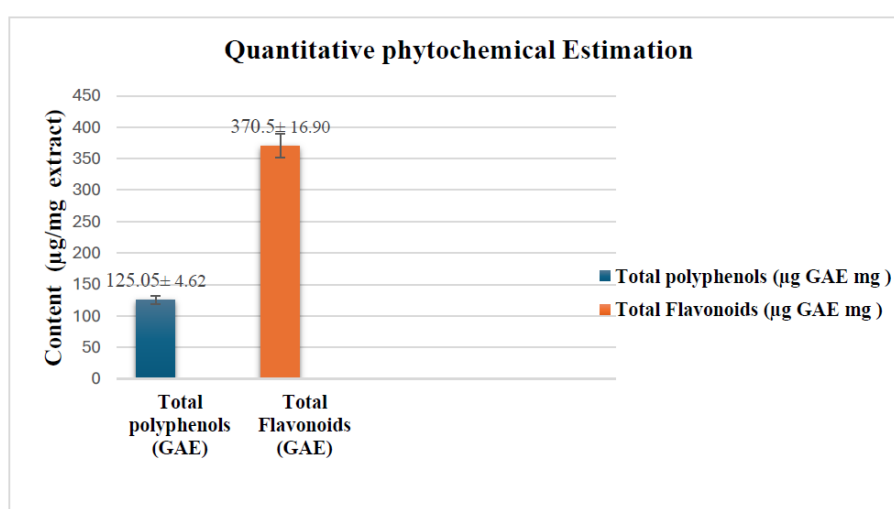


Fig. 2: Graphical representation of the total phenolic and flavonoid contents.

Moisture and Ash content

As detailed in Table 3, the plant powder exhibited a moisture content of 4.55% and a total ash content of 11.20%. The low moisture level falls well within acceptable limits (<10%), indicating optimal drying that protects the sample from microbial growth and phytochemical degradation during storage. The total ash value of 11.20% reflects the inorganic mineral content and overall purity of the sample. Together, these parameters confirm the acceptable quality and stability of the plant material for further extraction and biological evaluation.

Table 3: Moisture and total ash contents of the plant powder sample (%).

Sl. No.	Samples	Total Ash	Moisture
1	Plant powder	11.20	4.55

LCMS Analysis

LC–MS analysis identified phytochemical compounds in the ethanolic extract (Table 4), with retention times ranging from 0.406 to 8.119 min. The detected metabolites belonged to various phytochemical classes, including flavonoids, isoflavones, terpenoids, glycosides, triterpenoids, alkaloids, phenolic compounds, fatty acids, and benzoxazines. Major compounds identified included genistein (an isoflavone), hydrangenoside C (a terpene glycoside), neoandrographolide (a diterpene glycoside), ganolactone B (a triterpenoid), dehydrolucidenic acid A, and palmitic acid (a long-chain fatty acid). The diverse presence of flavonoids, phenolic compounds, and terpenoid derivatives confirms that the extract is highly rich in bioactive metabolites, which may contribute to its broad pharmacological and therapeutic potential.

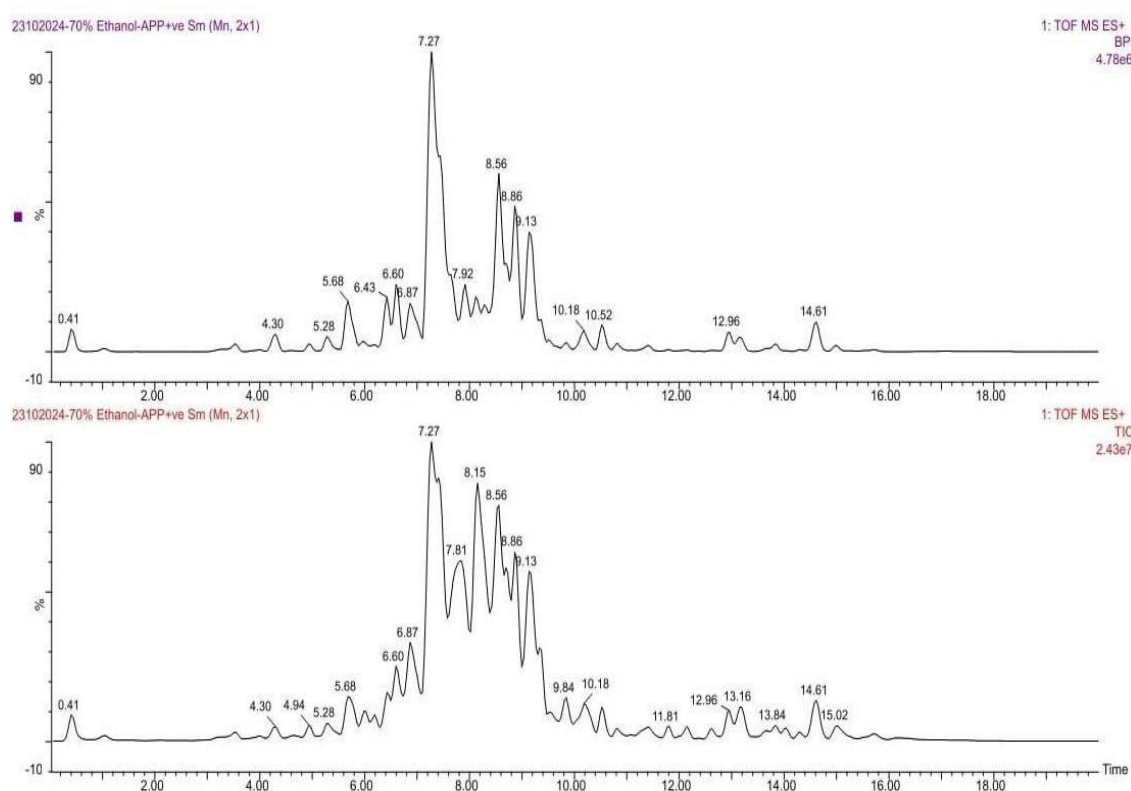


Fig. 3: Liquid Chromatography-mass spectrometry (LC-MS) Analysis of the Ethanolic extract.

Table 4: Phytochemical compounds identified in the *Strobilanthes paval* ethanolic

extract by LC-MS analysis.

Sl No	Proposed Compounds	Retention Time(min)	Precursor	Adduct	Formula	Ontology
1	8-methoxy-1',3'-dimethyl-2-phenyl-1'H-spiro[chroman-3,5'-pyrimidine]-2',4',6'(3'H)-trione	0.406	381.1501	[M+H] +	C ₂₁ H ₂₀ N ₂ O ₅	8-O-methylated flavonoids
2	Azacyclonol	1.049	268.1646	[M+H] +	C ₁₈ H ₂₁ NO	Diphenylmethanes
3	14-hydroxy-14-(hydroxymethyl)-5,9-dimethyltetracyclohexadecane-5-carboxylic acid	3.18	375.192	[M+K] +	C ₂₀ H ₃₂ O ₄	Kaurane diterpenoids
4	3-Amino-Beta-Pinene	3.552	188.1179	[M+H] +	C ₁₀ H ₁₈ ClN	Bicyclic monoterpenoids
5	HYDRANGENOSIDE C	3.992	579.2379	[M+H] +	C ₂₉ H ₃₈ O ₁₂	Terpene glycosides
6	3-Hydroxy-3-methylglutarate	4.296	163.0847	[M+H] +	C ₆ H ₁₀ O ₅	Hydroxy fatty acids
7	(1S,2R,9R,10S,12S)-12-hydroxy-7,15-diazatetracyclo[7.7.1.0 ^{2,7} .0 ^{10,15}]heptadecan-6-one	4.669	265.2034	[M+H] +	C ₁₅ H ₂₄ N ₂ O ₂	Sparteine, lupanine, and related alkaloids
8	4-Hydroxyphenylpyruvic acid	4.939	181.1352	[M+H] +	C ₉ H ₈ O ₄	Phenylpyruvic acid derivatives
9	2-ethyl-4-[(Z)-2-[(3-formamido-2-hydroxybenzoyl)amino]but-2-enoyl]oxy-3-(2-methylbutanoyloxy)pentanoic acid	5.278	493.2167	[M+H] +	C ₂₄ H ₃₂ N ₂ O ₉	N-acyl-alpha amino acids and derivatives
10	(1S,9S,E)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	5.717	227.1812	[M+Na] +	C ₁₅ H ₂₄	Sesquiterpenoids
11	Ganolactone B	5.751	481.2496	[M+Na] +	C ₂₇ H ₃₈ O ₆	Triterpenoids
12	Genistein	5.785	271.122	[M+H] +	C ₁₅ H ₁₀ O ₅	Isoflavones
13	Adrenosterone	6.428	301.1729	[M+H] +	C ₁₉ H ₂₄ O ₃	Androgens and derivatives
14	2-propyl-4H-benzo[d][1,3]oxazin-4-one	6.597	212.0726	[M+Na] +	C ₁₁ H ₁₁ NO ₂	Benzoxazines
15	Neoandrographolide	7.273	519.2367	[M+K] +	C ₂₆ H ₄₀ O ₈	Diterpene glycosides
16	Dehydrolucidenic acid A	7.476	457.2661	[M+H] +	C ₂₇ H ₃₆ O ₆	11-oxosteroids
17	(2R,3S,4S,5R,6R)-2-[(2R,3R,4R)-3,4-dihydroxy-4-(hydroxymethyl)oxolan-2-yl]oxymethyl-6-[(2E)-3,7-dimethylocta-2,6-dienoxy]oxane-	7.646	471.2849	[M+Na] +	C ₂₁ H ₃₆ O ₁₀	Terpene glycosides

	3,4,5-triol					
18	Palmitic Acid	7.916	274.3364	[M+H] +	C ₁₆ H ₃₂ O ₂	Long-chain fatty acids
19	5-[(Z)-14-(3,5-dihydroxyphenyl)tetradec-10-enyl]benzene-1,3-diol	8.119	413.2739	[M+H] +	C ₂₆ H ₃₆ O ₄	Resorcinols

DISCUSSION

The present findings reinforce the pharmacological relevance of *Strobilanthes pavala*, a species whose vegetative parts are traditionally valued in Indian folk medicine for conditions ranging from inflammation to pregnancy-related ailments (Biju *et al.*, 2023). The qualitative screening results, which confirmed the presence of phenols, flavonoids, tannins, terpenoids, proteins, and carbohydrates in the ethanolic extract, are broadly consistent with earlier phytochemical work on the genus, where alkaloids, saponins, glycosides, flavonoids, steroids, and tannins were similarly reported across *Strobilanthes* vegetative tissues (Biju *et al.*, 2023). The absence of alkaloids, saponins, glycosides, and steroids in the current hydroethanolic extract (SHE), however, suggests that phytoconstituent distribution in *S. pavala* may be extraction-solvent and tissue dependent. This is a pattern also noted for related species, such as *S. glutinosus*, where solvent polarity strongly influenced the yield of individual chemical classes (Aziz *et al.*, 2022).

Quantitatively, the extract's total phenolic (125.05 ± 4.62 µg GAE/mg) and flavonoid (370.50 ± 16.90 µg QE/mg) contents are appreciable relative to values reported for other medicinally important *Strobilanthes* species, such as *S. kunthianus*, where phenolic and flavonoid richness has similarly been linked to strong *in vitro* antioxidant performance (Singh & Hurkadale, 2020). Given that phenolics and flavonoids are well-established free-radical scavengers, the relatively high flavonoid content observed here is consistent with the antioxidant activity profile reported for this extract and supports the rationale for further isolation and bioassay-guided fractionation.

The physicochemical parameters (11.20% total ash, 4.55% moisture) fall within ranges generally considered acceptable for crude plant drug quality control, in line with World Health Organization benchmarks for medicinal plant materials (World Health Organization [WHO], 1998). This suggests that the raw material meets basic purity and stability criteria for further pharmaceutical standardization.

The LC–MS/MS profile adds considerable weight to the extract's therapeutic promise. Genistein, identified in the ethanolic extract, is among the most extensively studied isoflavones,

with documented antioxidant, anti-inflammatory, antibacterial, and anticancer activities operating through the modulation of NF- κ B and related inflammatory signaling pathways (Sharifi-Rad *et al.*, 2021). Similarly, neoandrographolide, a diterpenoid glycoside also detected in the extract, has been reported to exert antioxidant, anti-inflammatory, hepatoprotective, and cardioprotective effects (Kundu *et al.*, 2025). The co-occurrence of these two well-characterized bioactive metabolites, alongside triterpenoids (ganolactone B, dehydrolucidinic acid A), indicates that *S. pavala* extracts contain a chemically diverse and pharmacologically credible metabolite pool, rather than relying on activity attributable to a single compound class.

Collectively, these results support the traditional therapeutic use of *S. pavala* and position it as a candidate for further targeted pharmacological investigation, particularly the isolation and *in vivo* validation of its flavonoid- and diterpenoid-rich fractions, before its constituents can be considered for standardized nutraceutical or pharmaceutical development.

CONCLUSION

The present study demonstrates that *Strobilanthes pavala* is a rich source of bioactive phytoconstituents, with qualitative screening confirming the presence of phenols, flavonoids, tannins, terpenoids, proteins, and carbohydrates. Quantitative analysis revealed appreciable total phenolic (125.05 ± 4.62 μ g GAE/mg) and flavonoid (370.50 ± 16.90 μ g QE/mg) contents in the extract. Physicochemical parameters, including total ash (11.20%) and moisture content (4.55%), fell within acceptable quality limits, supporting the plant's suitability for standardized herbal use. Furthermore, LC-MS/MS analysis identified a diverse array of metabolites, including flavonoids, terpenoids, glycosides, and phenolic derivatives, such as genistein, neoandrographolide, and ganolactone B. These findings validate the traditional medicinal use of *S. pavala* and highlight its potential as a promising source of natural bioactive compounds for future pharmacological and nutraceutical applications. Further in-depth studies focusing on the isolation, purification, and mechanistic evaluation of these compounds are warranted to fully harness their therapeutic potential.

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