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Comprehensive Study on Phytochemical Constituents and Bioactive Potential of Aerial Part Extracts of *Strobilanthes attenuata*: An Underexplored Medicinal Plant

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Abstract

Strobilanthes attenuata (Acanthaceae), an underexplored plant from Uttarakhand, was evaluated for its phytochemical composition and biological potential. Four solvent extracts (SAH, SAE, SAA, and SAM) were prepared and assessed for qualitative phytochemical analysis, total phenolic content, total flavonoid content, antioxidant and antimicrobial activities. Preliminary phytochemical screening revealed diverse secondary metabolites in SAA and SAM. The highest TPC ($112.2 \pm 1.79 \mu\text{g GAE/mg}$) was exhibited by SAM, while SAA showed the highest TFC ($191.8 \pm 2.29 \mu\text{g QE/mg}$). Antioxidant assays revealed that SAA demonstrated 63.47% inhibition in DPPH and 90.84% inhibition in ABTS assays at $100 \mu\text{g/mL}$, whereas SAM showed the strongest reducing power in the FRAP assay (0.530 nm at $100 \mu\text{g/mL}$). In the phosphomolybdenum assay, SAA exhibited a high reducing potential ($204.50 \mu\text{g AAE/mg}$ at $500 \mu\text{g/mL}$). Antimicrobial evaluation indicated weak bacterial inhibition at lower concentrations, with moderate activity by SAM against *Staphylococcus aureus* and high inhibition by SAE against *Escherichia coli* at $1000 \mu\text{g/mL}$. SAA showed significant antifungal activity (19.6 mm zone of inhibition against *Candida albicans*). Both SAA and SAM were further evaluated for cytotoxicity against MCF-7 cells, where SAA was more potent ($\text{IC}_{50} = 61.23 \mu\text{g/mL}$) than SAM ($\text{IC}_{50} = 131.89 \mu\text{g/mL}$). GC-MS analysis of SAA identified 130 compounds dominated by triterpenoids (lupeol, 24.52%), sterols (γ -sitosterol, 10.70%), squalene (6.50%), phytol (4.96%), and γ -tocotrienol (3.10%). This preliminary investigation provides the first comprehensive insight into *S. attenuata* crude extracts, establishing SAA as the most bioactive fraction and a promising source of natural antioxidant, antifungal, and cytotoxic agent.

Keywords: phytochemical, antioxidant, antimicrobial, anticancer, MCF-7.

Introduction

Medicinal plants are a rich source of therapeutic agents and continue to play an essential role in modern drug discovery. Their bioactive compounds, particularly secondary metabolites such as flavonoids, phenols, and terpenoids, are known for diverse pharmacological activities including antioxidant, antimicrobial, and anticancer effects (Upadhyay et al., 2023). The family Acanthaceae comprises numerous medicinally important genera, among which *Strobilanthes* stands out for its chemical diversity and biological potential. Globally, the genus includes about 400–422 species, with nearly 150 recorded in India, especially across the Western Ghats and Himalayan regions (Wang et al., 2022; Suresh and Xavier, 2023; Balasubramaniam et al., 2020).

Several *Strobilanthes* species have demonstrated remarkable pharmacological properties. For instance, *S. crispa* is traditionally used in Malaysia and Indonesia for the treatment of breast and uterine cancers (Chen et al., 2023). *S. heyneanus* roots are used in Ayurvedic drug preparation and in rheumatic complaints (Sundaram et al., 2021), *S. barbatus* in curing inflammatory disorders (Sreelakshmi & Jayanthi, 2022), while *S. ciliatus* is widely used in ayurvedic medicine ‘Sahachara’ (Zhu et al., 2022). Despite many traditional applications, only a few species within the genus have been chemically or pharmacologically characterized in detail.

Strobilanthes attenuata (Wall. ex Nees) Jacq. ex Nees, originally described as *Ruellia attenuata* Nees (Wood, 2014), distributed from Afghanistan to the Indian subcontinent, is a perennial shrub that thrives in temperate habitats (POWO, 2024). It has received limited scientific attention despite belonging to a pharmacologically rich genus. Essential oil from its aerial parts have shown antioxidant, anti-inflammatory and antidiabetic activities (Upadhyay et al. 2023), while our recent study on its stem extract reported significant antioxidant and cytotoxic effects, with fatty acids and triterpenoids as dominant constituents (Kaur et al., 2025). However, there has been no comprehensive study on the phytochemical composition and biological potential of the crude extracts from the aerial parts of this plant.

Therefore, the present investigation aimed to provide a detailed phytochemical and biological evaluation of different solvent extracts of *S. attenuata* aerial parts. The study involved qualitative phytochemical screening, estimation of total phenolic and flavonoid contents, and assessment of antioxidant, antimicrobial, and anticancer activities. Furthermore, GC-MS profiling was conducted to identify the major bioactive compounds. This work seeks to bridge the existing

research gap and offer a holistic understanding of the chemical diversity and therapeutic potential of *S. attenuata*, contributing to its recognition as a promising Himalayan medicinal plant.

Morphological characters

S. attenuata is a perennial plant with an average height of about 0.5 meters. The leaves are simple, entire, and arranged alternately along the stem. They possess an ovate shape with a pointed apex and a cordate base. The leaf margins are serrated and the venation is unicostate reticulate with a noticeable midrib. These leaves exhibit a thin layer of pubescence on both surfaces with more prominent hairs along the margins and veins beneath. The petioles are winged. The inflorescence of *S. attenuata* is a terminal panicle of spikes. The flowers are arranged in distant opposite pairs. The branches of the inflorescence are glandular-pilose and the bracts especially at lower inflorescence branching points are unequal in each pair, ovate, acuminate, serrate, and resemble small sessile leaves. The calyx is 5-lobed to the base and becomes sticky with glandular-pilose hairs. The corolla is typically dark blue, occasionally pale blue, pinkish, or white. It has a curved shape and is pubescent on the lobes. The androecium is four and didynamous, has glabrous filaments and the anthers are erect and elongated (Wood, 2014). The characteristic features of *S. attenuata* are isophyllous leaves, elongated inflorescence, caducous bracts, and bluish corolla.

Materials and Methods

Plant collection and identification

Fresh samples of *S. attenuata* were collected in October, 2022 from Chamoli District, Uttarakhand, India (30.4025° N, 79.4364° E) (Fig. 1, Fig. 2). The plant material was authenticated at the Botanical Survey of India, Dehradun, and a voucher specimen was deposited under accession number BSI Acc. No. 1435.

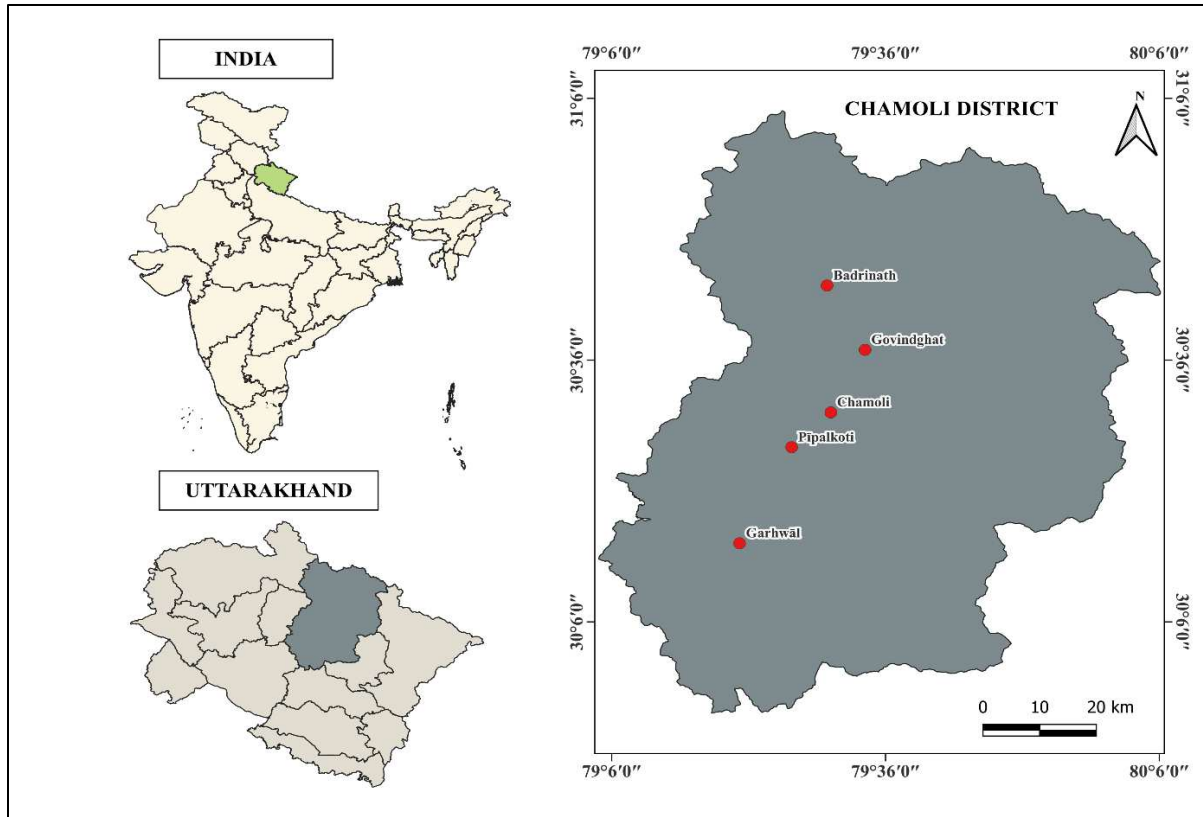


Figure 1. Showing the map of collected area with geographical co-ordinates (Created using QGIS Desktop 3.34.4).



Figure 2. Habit of *Strobilanthes attenuata*.

Preparation of plant extracts

The collected aerial parts of *S. attenuata* were thoroughly washed with tap water followed by rinsing with distilled water. The material was shade-dried for two weeks and then pulverized into a fine powder using a mechanical blender. For bioassays, 5 g of the powdered sample was macerated with 150 mL of different solvents for 24 h at room temperature. The solvents used were hexane (SAH), ethyl acetate (SAE), acetone (SAA), and methanol (SAM). The extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C until further use. The extraction yield of each solvent was calculated based on dry weight percentage.

Qualitative phytochemical screening

Preliminary phytochemical screening of all four extracts was performed using standard protocols (Bhandary *et al.*, 2012; Iqbal *et al.*, 2015; Ali *et al.*, 2018; Ramya *et al.*, 2019). The presence of

major phytoconstituents such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, terpenoids, saponins, tannins, steroids, phytosteroids, proteins and free amino acids were assessed through appropriate colorimetric and precipitation tests. The results are summarized in Table 2.

Estimation of total phenolic content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method with slight modifications (Chavan *et al.*, 2013). A volume of 100 μ L of plant extract (1 mg/mL) was mixed with 400 μ L of distilled water and 150 μ L of Folin-Ciocalteu reagent (1:1 v/v). After 5 min, 500 μ L of sodium carbonate solution (20% w/v) was added. The mixture was incubated at room temperature for 60 minutes, and absorbance was measured at 650 nm using an ELISA reader (Biotech Synergy HT). Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalents (GAE) per gram of extract.

Estimation of total flavonoid content

Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method (Bag *et al.* 2015). To 100 μ L of extract (1 mg/mL), 400 μ L methanol, 100 μ L of 10% aluminum chloride, and 100 μ L of 1 M sodium acetate were added. After incubation in the dark for 45 minutes at room temperature, absorbance was measured at 415 nm using an ELISA reader (Biotech Synergy HT). Quercetin served as the standard, and results were expressed as mg quercetin equivalents (QE) per g of extract.

***In vitro* antioxidant assays**

DPPH radical scavenging activity

The free radical scavenging capacity was evaluated using the DPPH assay (Khatua *et al.* 2017). A volume of 40 μ L of extract with different concentrations 20–100 μ g/mL was mixed with 160 μ L of 0.004% DPPH methanolic solution in a 96-well plate. After 30 minutes of incubation in the dark at room temperature, absorbance was read at 517 nm using an ELISA reader (Biotech Synergy HT). Butylated hydroxyanisole (BHA) was used as the positive control. The percentage of antioxidant activity was determined using the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

ABTS radical scavenging assay

ABTS [2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid] assay was carried out as described by Re *et al.* (1999). ABTS radical cations were generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating in the dark for 12-16 hours. The working solution was adjusted to an absorbance of 0.7 ± 0.02 at 734 nm. Various concentrations 20–100 µg/mL of the extract (20 µL) was mixed with 180 µL of the reagent, and absorbance was measured after 5 minutes using an ELISA reader (Biotech Synergy HT). Rutin was used as a standard. The percentage of radical scavenging activity was calculated using the formula:

$$\% \text{ Scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Phosphomolybdenum assay

The total antioxidant capacity was assessed using the phosphomolybdenum assay (Umamaheswari and Chatterjee, 2008). A reaction mixture of 0.1 mL of extract at different concentrations 100–500 µg/mL and 1 mL phosphomolybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) was incubated at 90°C for 90 minutes. After cooling, absorbance was measured at 695 nm using an ELISA reader (Biotech Synergy HT). Ascorbic acid was used as the reference standard.

Ferric ion reducing antioxidant power assay

FRAP assay was performed following Oyaizu (1986). Different concentrations of the plant extracts 20–100 µg/mL (200 µL) were mixed with 500 µL of phosphate buffer (0.2 M, pH 6.6) and 500 µL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 500 µL of 10% trichloroacetic acid. The supernatant was mixed with 0.1% FeCl₃ and absorbance was measured at 700 nm using an ELISA reader (Biotech Synergy HT). BHA was used as the standard.

Antimicrobial screening

Organisms

The Gram-positive organism such as *Staphylococcus aureus* (MTCC 96) as well as the Gram-negative organism such as *Escherichia coli* (MTCC 687) were selected for antibacterial study, while *Candida albicans* (MTCC 183) was chosen for antifungal study.

Standard

Ciprofloxacin and Itraconazole were used as the standard compounds for all bacterial cultures and fungal cultures, respectively.

Mueller-Hinton agar and Sabouraud Dextrose agar

MHA and SDA agar were procured from HiMedia, India to make up the medium for bacteria and fungi respectively. The MHA media was prepared by dissolving 38 g of MHA in 1 L of water, while SDA media was prepared by dissolving 65 g of SDA in 1 L of water. Both the media were autoclaved at 121°C and 15 psi for 15 minutes using autoclave. After sterilization, media was poured in sterile petri dishes in the laminar airflow chamber and each plate was poured with 30 mL of the culture media. The plates were then allowed to solidify in laminar airflow chamber.

Agar well diffusion assay

Antibacterial and antifungal activity of aerial part extracts of *S. attenuata* was carried out using the agar well diffusion method. The bacterial inoculum was uniformly spread on MHA petri plates, whereas fungal inoculum was uniformly spread on SDA petri plates using sterilized cotton swabs. Ten minutes after spreading, wells were punctured using sterile tips and filled with 50 µL plant extracts. The extracts were allowed to diffuse into the media and then the MHA as well as SDA plates were sealed with paraffin and incubated at 37°C for 24 hours. After incubation, the plates were observed for the clear zone around the well called as the zone of inhibition, and the diameter of these zones were measured (Wiegand *et al.*, 2008). Ciprofloxacin (100 ppm) and Itraconazole (350 ppm) were used as the positive control (standard). 8–13 mm: low inhibition; 14–19 mm: moderate inhibition; ≥ 20 mm: high inhibition (Ali *et al.*, 2018).

Cytotoxicity

Cell lines and maintenance

MCF-7 cell lines were procured from National Centre for Cell Science (NCCS), Pune, India. The cell line was maintained and cultivated as a monolayer in DMEM media with 10% FBS and

antibiotic-antimycotic solution. For cell growth, the humidified environment is required with 37°C in a CO₂ incubator (5%).

MTT assay

Cell viability is measured using the MTT reduction assay as described by Attri *et al.*, 2022. Human breast cancer cells (MCF-7) were seeded at a density of 5×10^3 cells per well in 96-well microtiter plates and incubated for 24 hours to allow cell attachment. After 24 hours, cells were treated with different concentrations of plant extract ranging from 62.5–1000 µg/mL. Following 24 hours of treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated at 37°C for 4 hours. The medium was then carefully removed, and 100 µL of DMSO was added to each well to dissolve the purple-colored formazan crystals formed. The plates were shaken gently at 150 rpm for 5 min to ensure complete solubilization. The absorbance was measured at 570 nm using an ELISA reader (Biotech Synergy HT). The cell viability was calculated as:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Morphological study

Cells were seeded in 24-well plates and treated with DMSO and the extract (at IC₅₀ concentration) for 24 h and after treatment period the image was captured by phase contrast microscope.

Statistical Analysis

All experiments were conducted in triplicate. Data were expressed as mean $\pm$ standard error. One-way ANOVA followed by multiple comparison test was conducted using GraphPad Prism 10.3.0, with significance set at $p \leq 0.05$.

Results

Extraction yields

The extraction yields of the aerial parts of *S. attenuata* varied depending on the solvent used (Table 1). Among the four solvents, SAM produced the highest yield, followed by SAH, SAA, and SAE.

These differences may be attributed to the polarity of solvents, with SAM extracting a broader range of polar phytoconstituents.

Table 1. Extraction yields (%) of different solvent extracts of *Strobilanthes attenuata* aerial parts.

Extract	% Yield
SAH	5.6
SAE	2.4
SAA	4.0
SAM	6.0

Qualitative phytochemical screening

The phytochemical screening of the aerial part extracts of *S. attenuata* revealed solvent-dependent variation in metabolite profiles (Table 2). SAA and SAM extracts demonstrated the broadest chemical diversity, containing abundant alkaloids, flavonoids, glycosides, phenols, tannins, saponins, and terpenoids. SAM extract additionally tested positive for proteins and showed a low presence of steroids, whereas the SAA extract was particularly rich in alkaloids, phenols, flavonoids, glycosides, hydrolysable tannins, and phytosteroids.

The SAH extract exhibited limited phytochemical content, with only trace amounts of alkaloids, flavonoids, and glycosides detected. The SAE extract contained low levels of flavonoids, glycosides, terpenoids, saponins, and phytosteroids. None of the solvent extracts tested positive for carbohydrates, free amino acids, or condensed tannins.

Overall, SAA and SAM extracts exhibited the most diverse phytochemical profile, indicating that these solvents are more effective for extracting bioactive secondary metabolites from the aerial parts of *S. attenuata*.

Table 2. Qualitative phytochemical analysis of *Strobilanthes attenuata* in different solvent system.

Phytochemical	Tests	SAH	SAE	SAA	SAM
Alkaloids	Wagner's test	+	-	++	++
Carbohydrates	Fehling's test	-	-	-	-

	Molisch test	-	-	-	-
Flavonoids	Alkaline reagent test	+	+	++	+
Glycosides	Keller Killani test	+	+	++	+
Phenols	Ferric chloride test	-	-	++	++
	Ellagic acid test	-	-	++	++
Proteins	Xanthoproteic test	-	-	-	++
Free amino acids	Ninhydrin test	-	-	-	-
Saponins	Foam test	-	+	+	+
Steroids	Ring formation test	-	-	-	+
Phytosteroids	Ring formation test	-	+	+	-
Tannins	Ferric chloride test	-	-	++	++
	Vanillin hydrochloride test	-	-	-	-
Terpenoids	Salkowski test	-	+	++	++

Legend: (-) absent, (+) low, (++) abundant

Total Phenolic and Flavonoid Content

The total phenolic content of *S. attenuata* extracts varied significantly. SAM exhibited the highest phenolic content (112.42 mg/g GAE), followed by SAA (61.72 mg/g GAE), while SAH and SAE showed comparatively lower levels. For total flavonoid content, SAA recorded the highest value (191.8 mg/g QE), significantly exceeding SAE (115.5 mg/g QE). Both SAH and SAM showed the lowest flavonoid contents.

Table 3. Total phenolic (expressed as gallic acid equivalents) and flavonoid (expressed as quercetin equivalents) content in aerial part extracts of *Strobilanthes attenuata*.

Plant extracts	Total phenolic content (mg/g GAE)	Total flavonoid content (mg/g QE)
SAH	32.77 ± 1.88 c	82.37 ± 2.19 c
SAE	30.75 ± 2.02 c	115.5 ± 2.99 b
SAA	61.72 ± 2.47 b	191.8 ± 2.29 a
SAM	112.2 ± 1.79 a	81.45 ± 2.34 c

Values are means of triplicates ± SE. Means followed by the same letter within columns are not significantly different ($p \leq 0.05$) using Tukey's multiple comparison test.

DPPH radical scavenging activity

All extracts displayed a concentration-dependent increase in radical scavenging activity (Fig. 3, Table 4). At 100 $\mu\text{g/mL}$, SAA and SAM were the most effective, showing 63.47% and 57.02% inhibition, respectively. In contrast, SAH and SAE fractions remained below 25% inhibition even at the highest concentration tested. The synthetic antioxidant BHA achieved nearly 76% inhibition at 100 $\mu\text{g/mL}$, confirming its superior activity. The potency trend was consistent with IC_{50} values, where SAA (67.94 $\mu\text{g/mL}$) and SAM (84.59 $\mu\text{g/mL}$) required much lower concentrations to achieve 50% inhibition compared to SAE (379.24 $\mu\text{g/mL}$) and SAH (439.59 $\mu\text{g/mL}$). Thus, the relative order of activity was $\text{BHA} > \text{SAA} > \text{SAM} > \text{SAE} > \text{SAH}$.

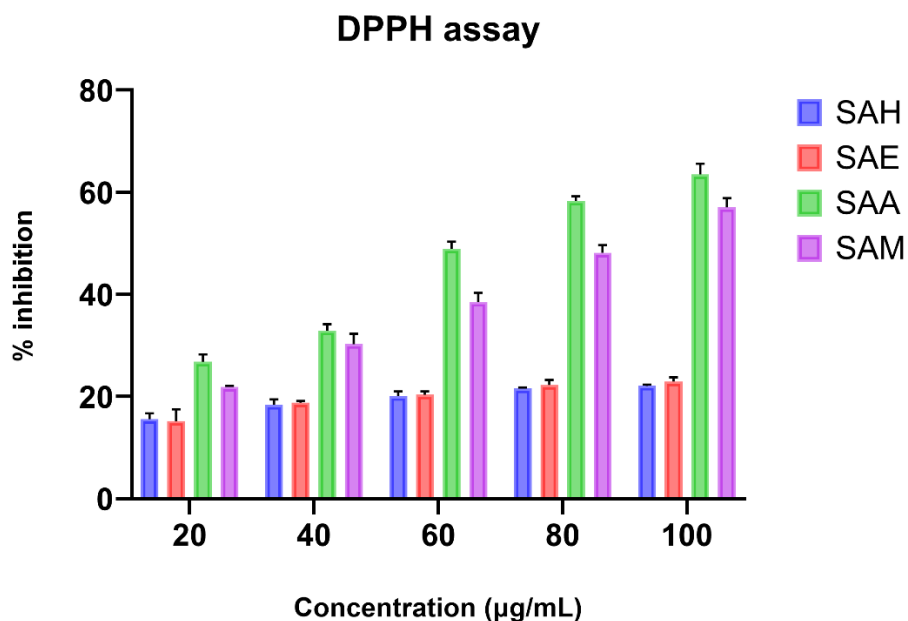


Figure 3. DPPH radical scavenging activity of aerial part extracts of *Strobilanthes attenuata*. Statistical Significance: $F(3, 16) = 7.908, p = 0.0019$.

ABTS assay

In the ABTS assay, a stronger antioxidant effect was evident, especially for SAA (Fig. 4, Table 4). At 100 $\mu\text{g/mL}$, SAA reached almost 91% inhibition, comparable to rutin (92%) used as a reference. Among the remaining extracts, SAE achieved 48% inhibition, while SAM and SAH showed relatively lower activities (38% and 27%, respectively). IC_{50} analysis supported these observations, with SAA (44.71 $\mu\text{g/mL}$) showing the greatest potency among extracts, while SAE

(107.70 $\mu\text{g/mL}$), SAM (153.50 $\mu\text{g/mL}$), and SAH (175.28 $\mu\text{g/mL}$) were less active. Rutin, however, remained the most effective ($\text{IC}_{50} = 29.50 \mu\text{g/mL}$). The potency order was Rutin > SAA > SAE > SAM > SAH.

Table 4. Percentage of antioxidant activity by DPPH and ABTS assay of aerial part extracts of *Strobilanthes attenuata*.

Extract/Standard	Concentration ($\mu\text{g/mL}$)					IC_{50} ($\mu\text{g/mL}$)
	20	40	60	80	100	
SAH	15.59 ± 1.11	18.36 ± 1.08	20.08 ± 0.93	21.52 ± 0.24	22.05 ± 0.29	439.59
SAE	15.59 ± 2.31 b	18.81 ± 0.33 ab	20.33 ± 0.68 ab	22.24 ± 0.99 a	22.91 ± 0.82 a	379.24
SAA	26.79 ± 1.44 c	32.88 ± 1.27 c	48.93 ± 1.39 b	58.32 ± 0.90 a	63.47 ± 2.09 a	67.94
SAM	21.88 ± 0.22 e	30.23 ± 2.04 d	38.50 ± 1.81 c	48.15 ± 1.50 b	57.02 ± 1.82 a	84.59
BHA (standard)	47.20 ± 2.41 c	57.28 ± 2.41 bc	61.76 ± 2.68 b	63.65 ± 1.84 b	75.97 ± 2.97 a	25.04
ABTS Assay						
SAH	5.12 ± 1.74 c	11.57 ± 0.45 b	14.90 ± 0.58 b	24.73 ± 0.98 a	27.39 ± 1.97 a	175.28
SAE	9.73 ± 0.48 d	12.95 ± 0.33 d	20.64 ± 1.56 c	24.58 ± 0.57 b	48.49 ± 0.35 a	107.70
SAA	27.04 ± 1.97 e	51.22 ± 2.30 d	64.74 ± 2.18 c	73.46 ± 0.67 b	90.84 ± 0.21 a	44.71
SAM	18.43 ± 1.25 c	25.86 ± 0.82 b	29.50 ± 2.64 b	31.95 ± 1.46 ab	38.10 ± 0.44 a	153.50
Rutin (standard)	42.65 ± 1.37 e	54.38 ± 0.55 d	74.40 ± 1.69 c	86.28 ± 1.09 b	92.22 ± 0.20 a	29.50

Values are means of triplicates $\pm$ SE. Means followed by the same letter within columns are not significantly different ($p \leq 0.05$) using Tukey's multiple comparison test.

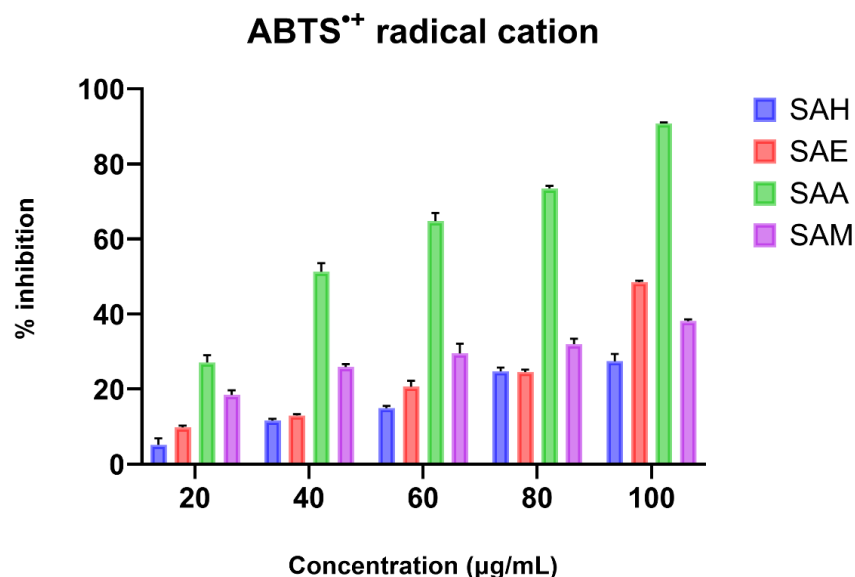


Figure 4. ABTS^{•+} radical scavenging activity of aerial part extracts of *Strobilanthes attenuata*. Statistical Significance: $F(3, 16) = 8.327, p = 0.0015$.

Phosphomolybdenum assay

In the phosphomolybdenum assay, all extracts of *S. attenuata* showed a concentration-dependent increase in total antioxidant capacity within the tested range (100–500 µg/mL), expressed as µg ascorbic acid equivalents per mg extract. SAA demonstrated the strongest activity, reaching 204.50 µg AAE/mg at 500 µg/mL, indicating a high reducing potential. SAM also exhibited considerable activity (150.01 µg AAE/mg), while SAE (125.27 µg AAE/mg) and SAH (102.71 µg AAE/mg) displayed comparatively lower values. These results indicate that all extracts possess measurable antioxidant potential, with SAA and SAM showing better activity than other fractions (Fig. 5).

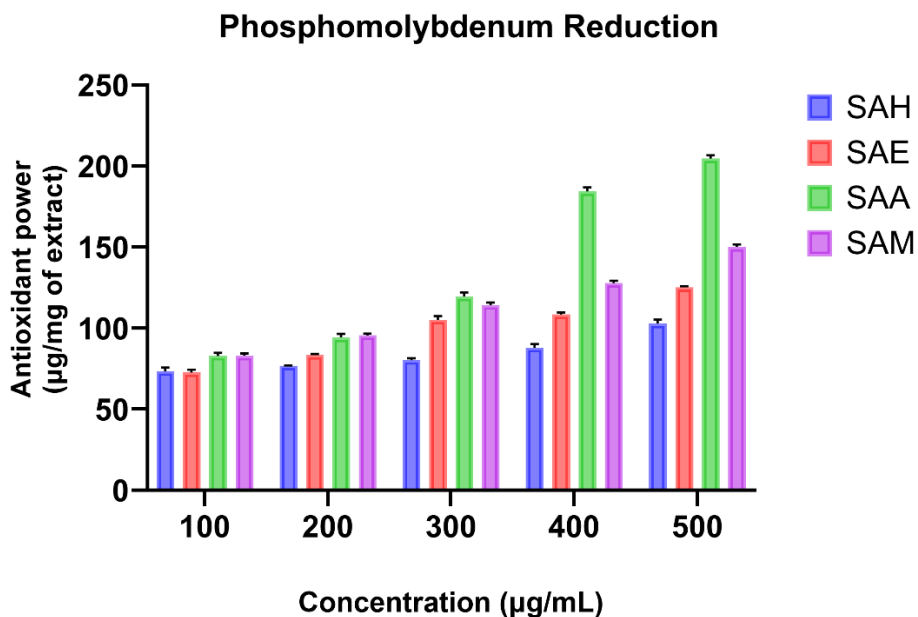


Figure 5. Total antioxidant capacity of aerial part extracts of *Strobilanthes attenuata* (SAH: hexane, SAE: ethyl acetate, SAA: acetone, SAM: methanol) measured by the phosphomolybdenum assay and expressed as ascorbic acid equivalents (µg/mg of extract). Statistical significance: $F(3, 16) = 2.403$, $p = 0.106$.

Ferric reducing power assay

The ferric reducing power assay (FRAP) revealed a concentration-dependent increase in reducing power for all extracts of *S. attenuata*. Among the solvent fractions, SAM showed the highest antioxidant activity, followed by SAA. At 100 µg/mL, SAM reached an absorbance of 0.530, which was higher than SAA (0.333), SAE (0.186), and SAH (0.204). The standard antioxidant BHA exhibited the strongest reducing potential, with an absorbance of 0.740 at the same concentration. The overall trend indicated that polar extracts, particularly methanol, possessed greater ferric reducing power compared to non-polar extracts (Fig. 6).

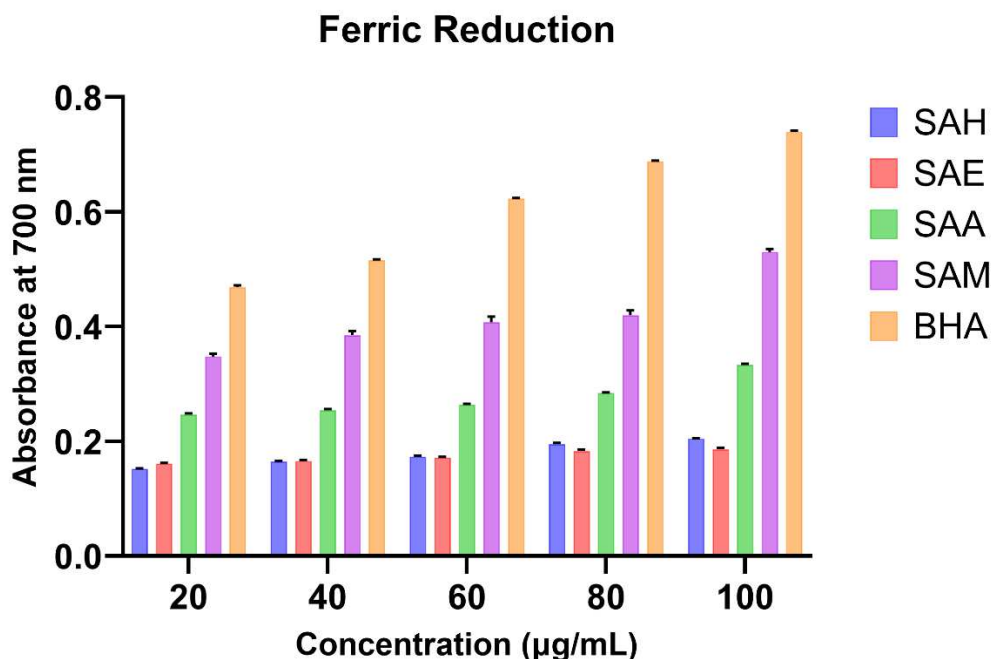


Figure 6. Ferric reducing antioxidant power activity of different aerial part extracts of *Strobilanthes attenuata*. Extracts include SAH: hexane, SAE: ethyl acetate, SAA: acetone, and SAM: methanol, with BHA (butylated hydroxyanisole) as the standard. Statistical significance: $F(4, 20) = 43.41, p < 0.0001$.

Antibacterial screening

The antibacterial potential of aerial part extracts of *S. attenuata* was evaluated against *Staphylococcus aureus* (MTCC 96) and *Escherichia coli* (MTCC 687) using the agar well diffusion method. Against *S. aureus*, SAH, SAE, and SAM extracts exhibited low inhibition only at 1000 µg/mL (9, 10, and 13.4 mm, respectively), while no activity was observed at 250 or 500 µg/mL. SAA was inactive at all tested concentrations. Ciprofloxacin used as the positive control showed high inhibition (23–24 mm), whereas the negative control showed no activity (Table 5, Fig. 7).

In the case of *E. coli*, all extracts demonstrated concentration-dependent inhibition. SAH produced low activity (9 mm at 1000 µg/mL). SAE showed low to moderate inhibition (10.4 mm at 500 µg/mL; 19.3 mm at 1000 µg/mL), while SAA exhibited a similar pattern (9mm at 500 µg/mL; 17 mm at 1000 µg/mL). SAM was inactive against this bacteria. The positive control ciprofloxacin exhibited high inhibition (23–26 mm) (Table 6, Fig. 8). Overall, all the four extracts were largely

ineffective against *S. aureus* and exhibited moderate to high activity against *E. coli*, indicating a selective antibacterial potential.

Table 5. Antibacterial activity of aerial part extracts of *Strobilanthes attenuata* against *Staphylococcus aureus* (MTCC 96) using agar well diffusion method.

Plant Extracts	Volume dispensed (μL)	Zone of inhibition (mm)				
		Concentration (μg/mL)			Positive control (15 μL)	Negative control (50 μL)
		C ₁ (250 μg/mL)	C ₂ (500 μg/mL)	C ₃ (1000 μg/mL)		
SAH	50 μL	0	0	9	23	0
SAE		0	0	10	24	0
SAA		0	0	0	24	0
SAM		0	0	13.4	23	0

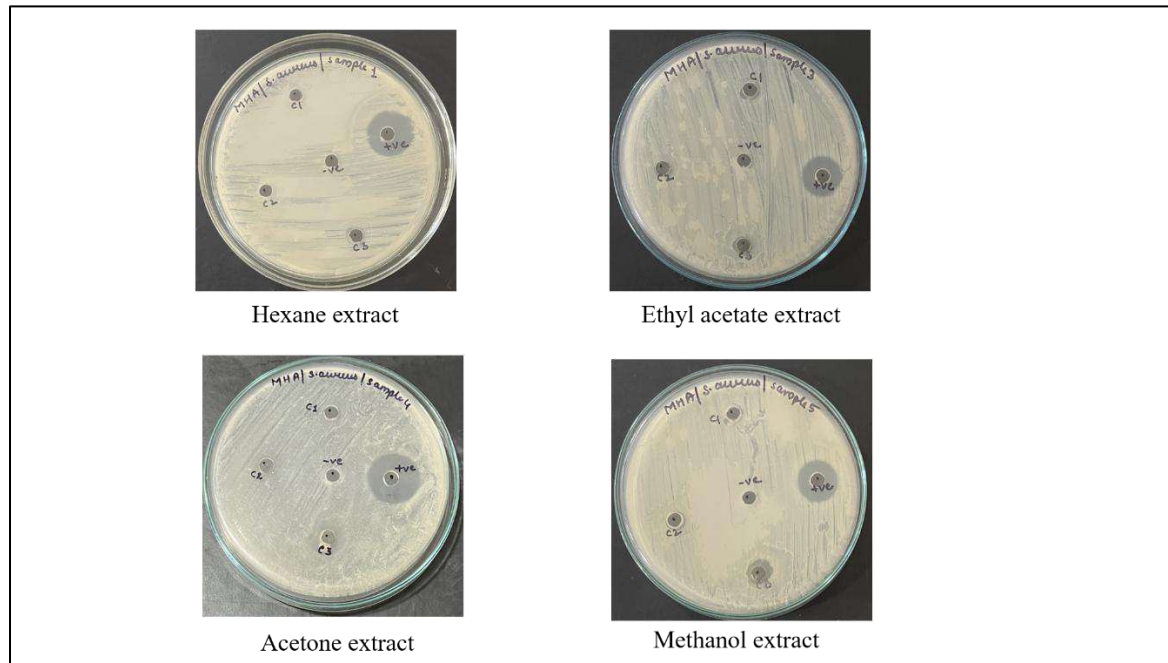


Figure 7. Antibacterial activity of various extracts of *Strobilanthes attenuata* against *Staphylococcus aureus* using the agar well diffusion method (C₁: 250 μg/mL; C₂: 500 μg/mL; C₃: 1000 μg/mL; +ve control: Ciprofloxacin; -ve control: Methanol).

Table 6. Antibacterial activity of aerial part extracts of *Strobilanthes attenuata* against *Escherichia coli* (MTCC 687) using agar well diffusion method.

Plant extracts	Volume dispensed (μL)	Zone of inhibition (mm)				
		Concentration (μg/mL)			Positive control (15 μL)	Negative control (50 μL)
		C1 (250 μg/mL)	C2 (500 μg/mL)	C3 (1000 μg/mL)		
SAH	50 μL	0	0	9	26	0
SAE		0	10.4	19.3	24	0
SAA		0	9	17	24	0
SAM		0	0	0	23	0

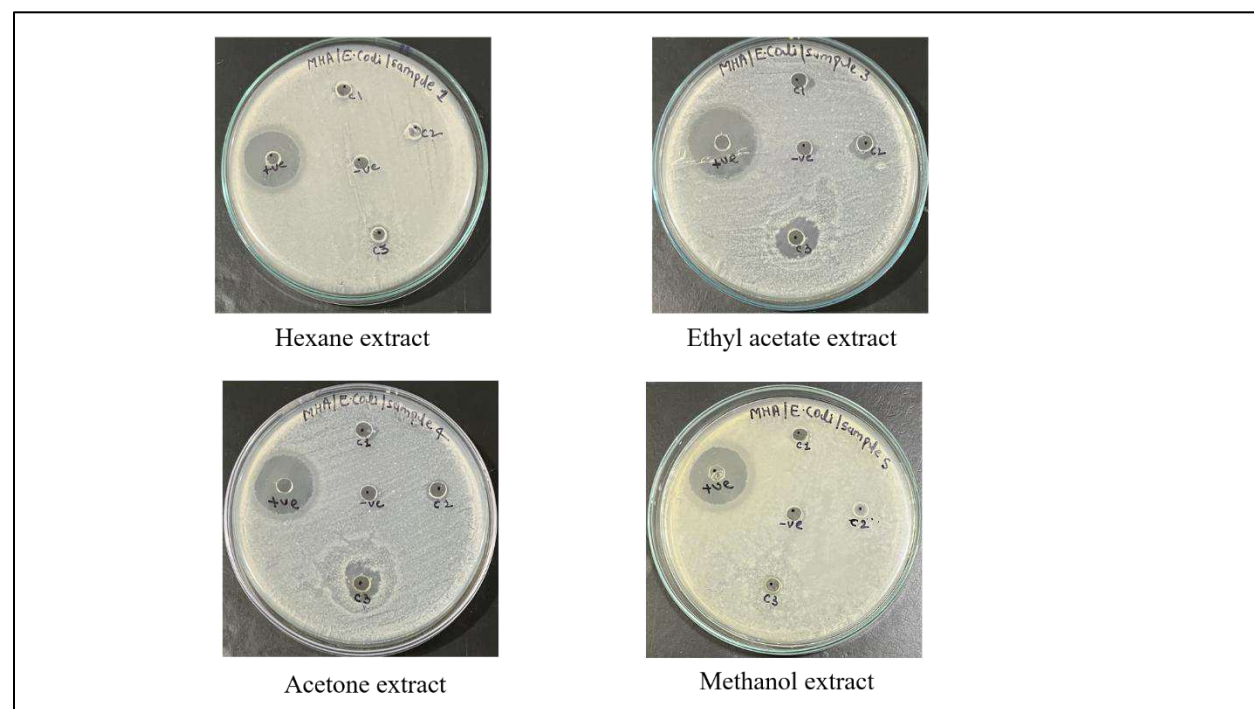


Figure 8. Antibacterial activity of various extracts of *Strobilanthes attenuata* against *Escherichia coli* using the agar well diffusion method (C₁: 250 μg/mL; C₂: 500 μg/mL; C₃: 1000 μg/mL; +ve control: Ciprofloxacin; -ve control: Methanol).

Antifungal screening

The antifungal screening was conducted against *Candida albicans* (MTCC 183). A dose-dependent increase in inhibition was observed for most extracts. SAA showed the strongest response, with moderate to high inhibition (10–19.6 mm) at all the three concentrations tested. SAM exhibited slightly low activity at 500 µg/mL (12.5 mm) and moderate inhibition at 1000 µg/mL (15.8 mm). SAH showed low inhibition (11 mm) at 1000 µg/mL and was ineffective at 250 or 500 µg/mL. Itraconazole, the positive control produced a zone of inhibition ranging from 33–34 mm (Table 7, Fig. 9).

Table 7. Antifungal activity of aerial part extracts of *Strobilanthes attenuata* against *Candida albicans* (MTCC 183) using agar well diffusion method.

Plant extracts	Volume dispensed (µL)	Zone of inhibition (mm)				
		Concentration (µg/mL)			Positive control (15 µL)	Negative control (50 µL)
		C1 (250 µg/mL)	C2 (500 µg/mL)	C3 (1000 µg/mL)		
SAH	20 µL	0	0	11	34	0
SAE		9	10	10	33	0
SAA		10	10	19.6	33	0
SAM		0	12.5	15.8	33	0

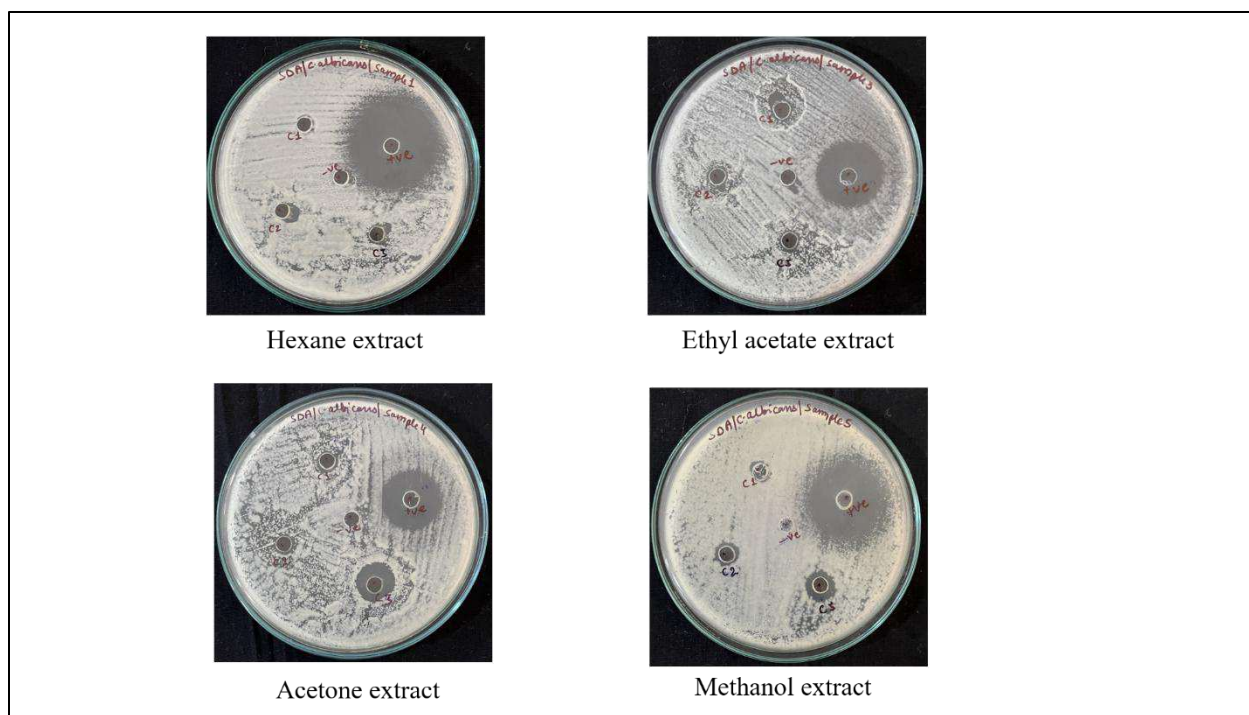


Figure 9. Antifungal activity of various extracts of *Strobilanthes attenuata* against *Candida albicans* using the agar well diffusion method (C₁: 250 µg/mL; C₂: 500 µg/mL; C₃: 1000 µg/mL; +ve control: Itraconazole; -ve control: Methanol).

MTT assay

SAA and SAM extracts, which showed strong antioxidant potential and abundance of phytochemicals in preliminary screening, were selected for cytotoxicity evaluation against MCF-7 cells. Treatment with *S. attenuata* extracts caused a concentration-dependent reduction in MCF-7 cell viability (Fig. 10). DMSO was used as a control. At 62.5 µg/mL, SAM reduced viability to about 66%, while SAA was more effective, lowering viability to nearly 56%. This difference became more evident at 125 µg/mL, where cell viability was ~55% with SAM compared to ~35% with SAA. At higher concentrations (500–1000 µg/mL), both extracts produced strong cytotoxic effects, with viability falling below 20%. The calculated IC₅₀ values further confirmed the greater potency of SAA (61.23 µg/mL) compared to SAM (131.89 µg/mL). The standard drug docetaxel showed the strongest activity, with an IC₅₀ of 20.58 µg/mL.

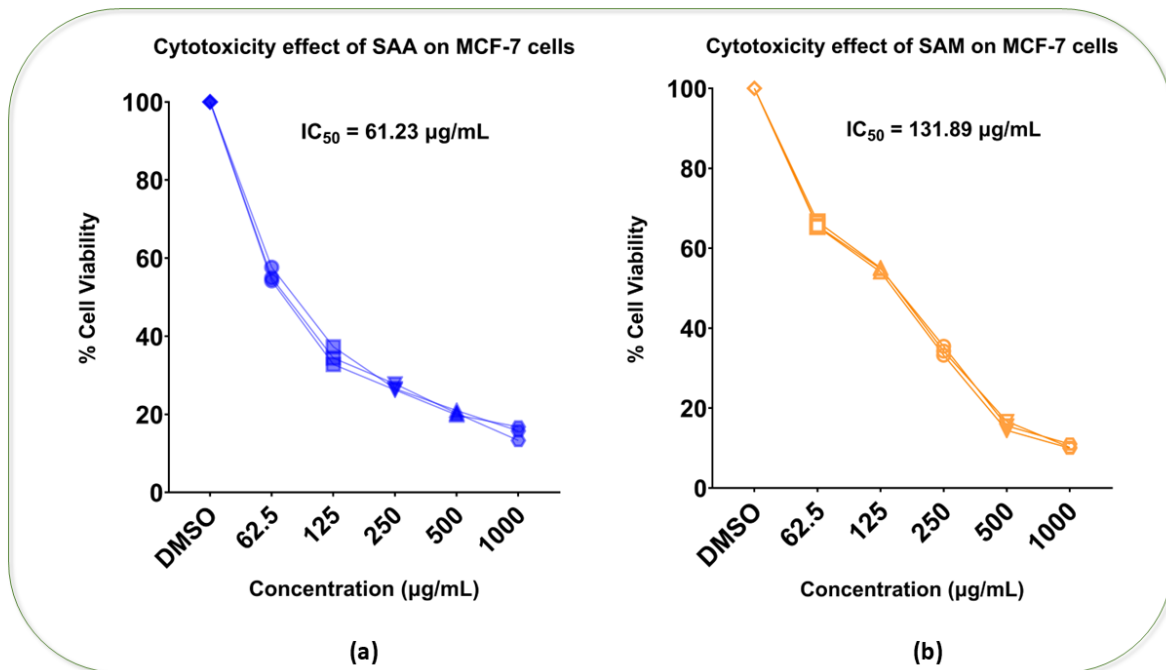


Figure 10. (a) Dose-dependent cytotoxic effect of acetone extract (SAA) and **(b)** methanol extract (SAM) of *Strobilanthes attenuata* on human breast cancer (MCF-7) cells. Cells were treated with extracts (62.5–1000 µg/mL) for 24 h, and cell viability was assessed. Statistical analysis showed a significant effect of concentration for both extracts (SAA: $F(5, 12) = 1426, p < 0.0001$; SAM: $F(5, 12) = 5721, p < 0.0001$). Post-hoc Dunnett’s multiple comparison test confirmed that reductions at concentrations ≥ 250 µg/mL were statistically significant compared with the DMSO control (**** $p < 0.0001$).

Morphological observation

SAA was chosen for morphological observation since the IC_{50} value of SAA was much lower than that of SAM. Phase-contrast microscopy revealed that control MCF-7 cells (DMSO-treated) retained their typical epithelial-like morphology with compact growth and an intact cell layer. In contrast, cells treated with SAA at the IC_{50} concentration appeared shrunken and rounded, with partial detachment from the surface and reduced cell layer coverage, consistent with cytotoxic changes observed in the MTT assay (Fig. 11).

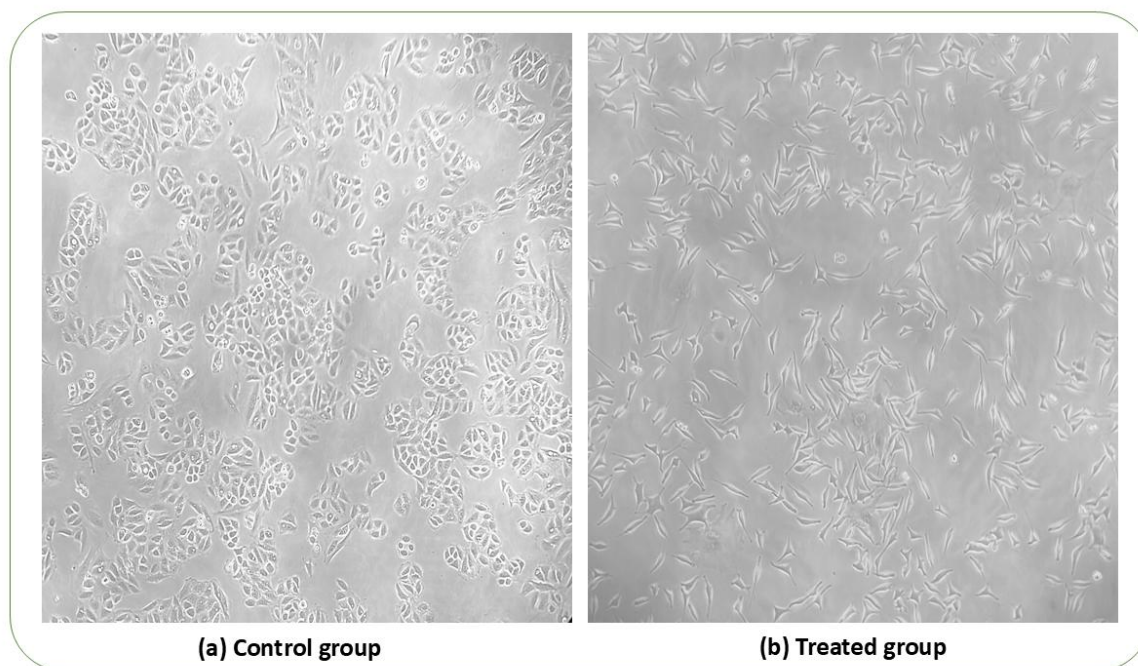


Figure 11. Morphological changes in MCF-7 cells observed under phase-contrast microscope (10x). (a) Untreated control (DMSO) cells showing typical epithelial morphology and intact monolayer. (b) Cells treated with acetone extract of *Strobilanthes attenuata* showing cytotoxic alterations including rounding, shrinkage, detachment and reduced confluency.

GC-MS profiling

GC-MS analysis of the acetone extract of *S. attenuata* was carried out using the NIST 14.L library, and compound identification was based on comparison of retention times and mass spectra with reference standards (Fig. 12). The analysis revealed a complex chemical profile with 130 identified compounds, belonging to diverse classes such as triterpenoids, phytosterols, diterpenoid alcohols and esters, fatty acids and their esters, fatty alcohols, tocopherols, amides, hydrocarbons, lactones and siloxane derivatives (Table 8).

The most abundant compound was lupeol (24.52% RT 44.802 min), followed by γ -sitosterol (10.70% RT 41.944 min), Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- (7.36% RT 37.274 min), squalene (6.50% RT 45.555 min), phytol (4.96% RT 36.554 min), and tocotrienol derivative (3.10% RT 48.961 min). Other significant contributors included 13-Docosenamide, (Z)- (1.84% RT 45.258 min), α -Tocopheryl- β -D-mannoside (1.62% RT 50.417 min), and 5-Octadecenal (1.55% RT 22.512 min).

Several compounds were detected at multiple retention times, indicating the presence of isomers or structurally related derivatives. For example, 3-Octanol was observed at RT 15.255 and 15.979 min, with a combined peak area of 0.39%. Megastigmatrienone occurred at RT 27.940 and 29.154 min (0.11%), while 1-Nonadecene appeared at RT 32.177, 34.792, and 38.090 min, contributing a total of 1.53%. Similarly, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol was identified at two retention times (33.119 and 33.356 min, total 0.93%). Azulenemethanol acetate derivative was found at three peaks (43.228, 44.119, 44.295 min) with a combined area of 2.53%. Tetrapentacontane was repeatedly detected at multiple peaks (39.551–49.789 min), contributing 1.76%. Collectively, siloxane derivatives (cyclooctasiloxane, cyclononasiloxane, tetracosamethylcyclododecasiloxane) accounted for approximately 2.61% of the extract.

In addition to these, many fatty acid derivatives were detected, such as 9,12,15-Octadecatrienoic acid methyl ester (0.89%), 9,12-Octadecadienoic acid (Z,Z)- methyl ester (0.32%), methyl stearate (0.21%), Octadecanoic acid (0.40%), and Tetradecanoic acid (0.26%). Long-chain fatty alcohols like 1-Hexacosanol (0.30%), 1-Pentacosanol (0.12%), and n-Pentadecanol (0.07%) were also present.

The GC-MS profile demonstrated that SAA is dominated by triterpenoids, sterols, fatty acids, tocopherols, and diterpenoid alcohols, along with minor constituents across many chemical classes. This broad chemical diversity indicates a rich phytochemical composition of *S. attenuata*.

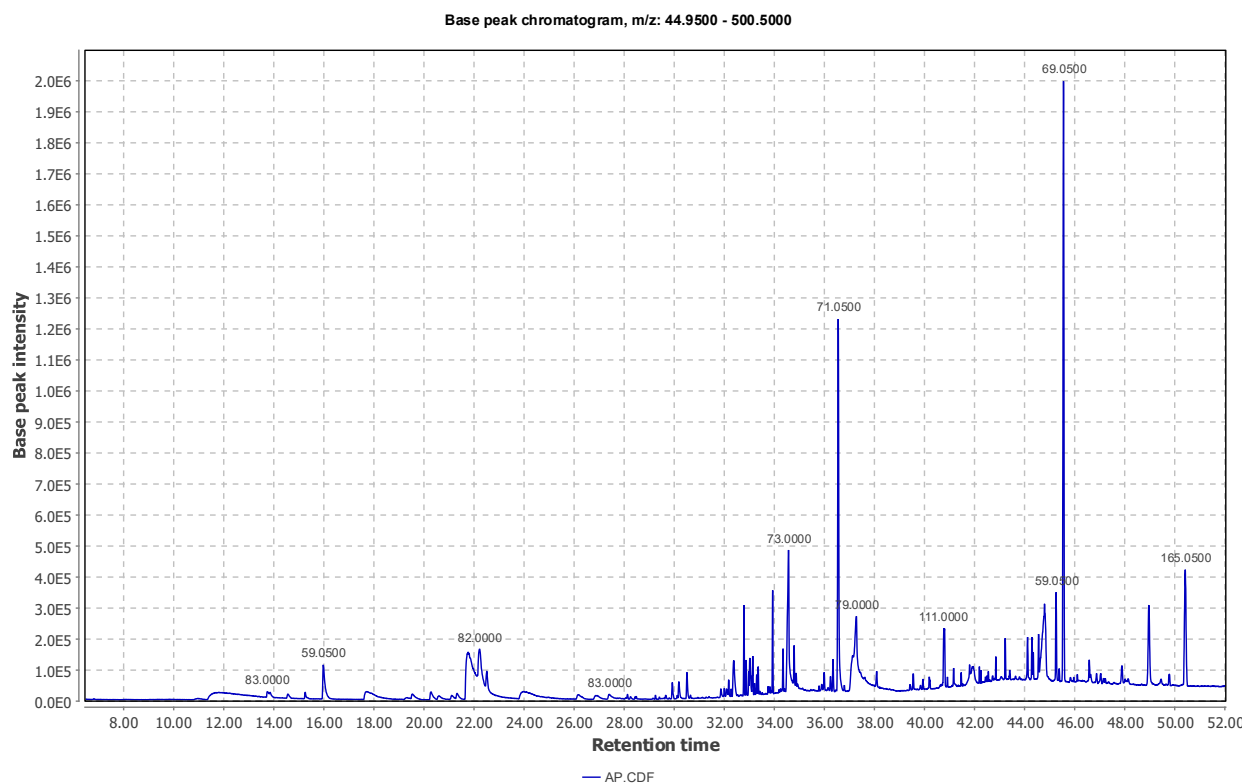


Figure 12. GC-MS chromatogram of acetone aerial part extract of *Strobilanthes attenuata*.

Table 8. GC-MS analysis of aerial part of *Strobilanthes attenuata*.

S. No.	Retention Time (min)	Compound Name	Molecular formula	Mol. weight (g/mol)	RI	Peak Area (%)	Class of compound
1.	15.255	3-Octanol	C ₈ H ₁₈ O	130	979	0.04	Fatty alcohol
2.	15.979	3-Octanol	C ₈ H ₁₈ O	130	979	0.35	Fatty alcohol
3.	20.289	1,5-Diazocine, octahydro-1,5-dinitroso-	C ₆ H ₁₂ N ₄ O ₂	172	1854	0.19	Cyclic dinitroso derivative
4.	21.109	9-Oxabicyclo[3.3.1]nonane-2,6-diol	C ₈ H ₁₄ O ₃	158	1347	0.32	Bicyclic alcohol
5.	21.320	Boranamine, N,N,1,1-tetraethyl-	C ₈ H ₂₀ BN	141	0	0.10	Organoborane

6.	21.770	2-Pyrrolidinone, 5-(ethoxymethyl)-	C ₇ H ₁₃ NO ₂	143	1099	0.25	Pyrrolidinone derivative
7.	22.512	5-Octadecenal	C ₁₈ H ₃₄ O	266	2007	1.55	Unsaturated aldehyde
8.	23.638	Cycloheptasiloxane, tetradecamethyl-	C ₁₄ H ₄₂ O ₇ Si ₇	518	1447	0.04	Organosiloxane
9.	26.145	2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl-	C ₁₀ H ₁₈ O ₃	186	1592	0.18	Lactone
10.	26.311	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	C ₁₁ H ₁₆ O ₂	180	1426	0.06	Benzofuran
11.	26.861	5-Undecanol, 2-methyl-	C ₁₂ H ₂₆ O	186	1312	0.34	Fatty alcohol
12.	27.401	Cyclobutanecarboxylic acid, decyl ester	C ₁₅ H ₂₈ O ₂	240	1702	0.34	Aliphatic ester
13.	27.940	Megastigmatrienone	C ₁₃ H ₁₈ O	190	1454	0.03	Ketone
14.	28.444	Guaiol	C ₁₅ H ₂₆ O	222	1614	0.03	Sesquiterpene alcohol
15.	29.154	Megastigmatrienone	C ₁₃ H ₁₈ O	190	1454	0.08	Ketone
16.	29.252	Cyclooctasiloxane, hexadecamethyl-	C ₁₆ H ₄₈ O ₈ Si ₈	592	1654	0.06	Organosilicon
17.	29.929	4,4,5,8-Tetramethylchroman-2-ol	C ₁₃ H ₁₈ O ₂	206	1705	0.43	Chromanol derivative
18.	30.188	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	C ₁₃ H ₂₂ O ₃	226	1669	0.32	Epoxy alcohol
19.	30.521	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-	C ₁₃ H ₂₀ O ₃	224	1642	0.27	Monoterpenoid

		oxabicyclo[4.1.0]hept-1-yl)-					
20.	30.645	2-Methyltetracosane	C ₂₅ H ₅₂	352	2442	0.05	Alkane
21.	31.276	Chloroacetic acid, undecyl ester	C ₁₃ H ₂₅ ClO ₂	248	1706	0.04	Ester
22.	31.389	2-Methylhexacosane	C ₂₇ H ₅₆	380	2641	0.03	Alkane
23.	31.868	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	1769	0.26	Fatty acid
24.	32.002	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	666	1860	0.11	Siloxane
25.	32.092	Loliolide	C ₁₁ H ₁₆ O ₃	196	1614	0.20	Monoterpenoid lactone
26.	32.177	1-Nonadecene	C ₁₉ H ₃₈	266	1900	0.28	Alkene
27.	32.277	Heptadecane	C ₁₇ H ₃₆	240	1700	0.05	Alkane
28.	32.378	2,3-Bis(1-methylallyl)pyrrolidine	C ₁₂ H ₂₁ N	179	1328	0.55	Pyrrolidine derivative
29.	32.534	Octatriacontyl trifluoroacetate	C ₄₀ H ₇₇ F ₃ O ₂	646	3999	0.04	Ester
30.	32.643	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	1686	0.07	Fatty acid ester
31.	32.700	(E)-Hexadec-2-enal	C ₁₆ H ₃₀ O	238	1808	0.03	Fatty aldehyde
32.	32.979	Methyl triacontyl ether	C ₃₁ H ₆₄ O	452	3179	0.34	Aliphatic ether
33.	33.119	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	2045	0.46	Diterpenoid alcohol
34.	33.212	Dodecylcyclohexane	C ₁₈ H ₃₆	252	1874	0.07	Cycloalkane
35.	33.295	10-Nonadecanone	C ₁₉ H ₃₈ O	282	2046	0.26	Ketone
36.	33.356	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	2045	0.47	Diterpenoid alcohol
37.	33.420	n-Pentadecanol	C ₁₅ H ₃₂ O	228	1755	0.07	Fatty alcohol
38.	33.518	(4aS,7R)-7-(2-Hydroxypropan-2-yl)-	C ₁₅ H ₂₄ O ₂	236	1798	0.08	Sesquiterpenoid

		1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one					
39.	33.751	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C ₁₆ H ₂₂ O ₄	278	1908	0.05	Phthalate ester
40.	33.823	3-Methyl-2-(3,7,11-trimethyldodecyl) furan	C ₂₀ H ₃₆ O	292	1931	0.04	Furanoterpenoid
41.	33.870	6-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	2175	0.05	Fatty acid
42.	34.023	1-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	652	4765	0.05	Fatty acid ester
43.	34.411	7-Hexadecenal, (Z)-	C ₁₆ H ₃₀ O	238	1808	0.06	Fatty aldehyde
44.	34.792	1-Nonadecene	C ₁₉ H ₃₈	266	1900	0.68	Alkene
45.	34.875	Eicosane	C ₂₀ H ₄₂	282	2000	0.10	Alkane
46.	34.928	Borane, trihexyl-	C ₁₈ H ₃₉ B	266	0	0.04	Organoborane
47.	35.456	Docosanoic acid, 1,2,3-propanetriyl ester	C ₆₉ H ₁₃₄ O ₆	1058	7318	0.07	Triacylglycerol
48.	35.782	Cyclooctasiloxane, hexadecamethyl-	C ₁₆ H ₄₈ O ₈ Si ₈	592	1654	0.18	Organosilicon
49.	35.893	Benzocyclodecene, tetradecahydro-	C ₁₄ H ₂₆	194	1581	0.13	Polycyclic hydrocarbon
50.	35.983	Cyclohexane, nonadecyl-	C ₂₅ H ₅₀	350	2570	0.36	Cycloalkane
51.	36.144	17-Pentatriacontene	C ₃₅ H ₇₀	490	3508	0.23	Alkene
52.	36.245	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294	2093	0.32	Fatty acid ester
53.	36.347	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292	2101	0.89	Fatty acid ester
54.	36.461	10-Methylundec-2-en-4-olide	C ₁₂ H ₂₀ O ₂	196	1500	0.05	Lactone

55.	36.554	Phytol	C ₂₀ H ₄₀ O	296	2045	4.96	Diterpenoid alcohol
56.	36.798	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	2077	0.21	Fatty acid ester
57.	37.274	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	C ₂₀ H ₃₈ O ₂	310	2344	7.36	Fatty acid ether
58.	37.416	1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol, TMS	C ₁₈ H ₃₈ OSi	298	1693	0.08	Terpenoid alcohol
59.	37.624	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	2167	0.40	Fatty acid
60.	37.815	8-[(E)-3-Hydroxybut-1-enyl]-1,5-dimethyl-6-oxabicyclo[3.2.1]octane-3,8-diol	C ₁₃ H ₂₂ O ₄	242	1865	0.03	Sesquiterpenoid
61.	37.973	1,13-Tetradecadien-3-one	C ₁₄ H ₂₄ O	208	1529	0.04	Ketone
62.	38.090	1-Nonadecene	C ₁₉ H ₃₈	266	1900	0.57	Alkene
63.	38.229	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	666	1860	0.20	Siloxane
64.	38.600	2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl-	C ₁₃ H ₂₂ O ₂	210	1647	0.07	Cyclohexenone
65.	39.072	Podocarp-7-en-3.beta.-ol, 13.beta.-methyl-13-vinyl-	C ₂₀ H ₃₂ O	288	2114	0.08	Diterpenoid alcohol
66.	39.422	3-Hydroxydecanoic acid	C ₁₀ H ₂₀ O ₃	188	1534	0.10	Hydroxy fatty acid
67.	39.551	Tetrapentacontane	C ₅₄ H ₁₁₀	758	5389	0.32	Alkane
68.	39.847	Benzocyclodecene, tetradecahydro-	C ₁₄ H ₂₆	194	1581	0.14	Polycyclic hydrocarbon
69.	39.942	Cyclohexane, hexadecyl-	C ₂₂ H ₄₄	308	2272	0.11	Cycloalkane
70.	40.200	11-Methyltricosane	C ₂₄ H ₅₀	338	2343	0.37	Alkane

71.	40.336	3,7,11,15-Tetramethylhexadecane-1,2,3-triol	$C_{20}H_{42}O_3$	330	2314	0.04	Fatty alcohol
72.	40.630	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346	2507	0.03	Fatty acid ester
73.	40.685	Podocarpa-6,8,11,13-tetraen-12-ol, 13-isopropyl-, acetate	$C_{22}H_{30}O_2$	326	2366	0.04	Diterpenoid ester
76.	40.776	Cycloheptanone, 3-butyl-	$C_{11}H_{20}O$	168	1370	0.08	Ketone
77.	40.803	3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-enolide	$C_{15}H_{26}O_2$	238	1745	0.10	Lactone
78.	40.918	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	2258	0.20	Lactone
79.	41.167	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	2228	0.46	Amide
80.	41.469	1-Hexacosanol	$C_{26}H_{54}O$	382	2848	0.30	Fatty alcohol
81.	41.528	Heneicosane	$C_{21}H_{44}$	296	2100	0.04	Alkane
82.	41.944	.gamma.-Sitosterol	$C_{29}H_{50}O$	414	2731	10.70	Phytosterol
83.	42.194	Tetrapentacontane	$C_{54}H_{110}$	758	5389	0.20	Alkane
84.	42.253	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	1860	0.29	Siloxane
85.	42.439	1H-Indene, octahydro-	C_9H_{16}	124	981	0.11	Polycyclic hydrocarbon
86.	42.500	Cyclohexane, octadecyl-	$C_{24}H_{48}$	336	2470	0.07	Cycloalkane
87.	42.554	Tetracosane	$C_{24}H_{50}$	338	2400	0.11	Alkane
88.	42.597	3,7,11,15-Tetramethylhexadecane-1,2,3-triol triacetate	$C_{26}H_{48}O_6$	456	2767	0.07	Diterpenoid ester

89.	42.720	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330	2498	0.37	Fatty acid ester
90.	42.804	Octadecanoic acid, 9,10-epoxy-18-(trimethylsiloxy)-, methyl ester, cis-	C ₂₂ H ₄₄ O ₄ Si	400	2413	0.06	Epoxy fatty acid ester
91.	42.853	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390	2704	0.14	Phthalate ester
92.	43.000	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,19,19,19-docosamethyldecasiloxane	C ₂₂ H ₆₆ O ₉ Si ₁₀	754	1991	0.04	Siloxane
93.	43.076	24-Noraleana-3,12-diene	C ₂₉ H ₄₆	394	2635	0.50	Triterpenoid
94.	43.228	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-.alpha.,3,8-tetramethyl-, acetate, [3S-(3.alpha.,5.alpha.,8.alpha.)]-	C ₁₇ H ₂₈ O ₂	264	1804	0.62	Sesquiterpenoid ester
95.	43.403	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	888	2480	0.48	Siloxane
96.	43.634	Humulane-1,6-dien-3-ol	C ₁₅ H ₂₆ O	222	1757	0.29	Sesquiterpenoid alcohol
97.	43.799	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	C ₁₅ H ₂₆ O	222	1530	0.17	Sesquiterpenoid alcohol

98.	43.883	9(1H)-Phenanthrenone, 2,3,4,4a,10,10a- hexahydro-6-hydroxy- 1,1,4a-trimethyl-7-(1- methylethyl)-, (4aS-trans)-	C ₂₀ H ₂₈ O ₂	300	2396	0.06	Sesquiterpenoid
99.	43.990	Tetrapentacontane	C ₅₄ H ₁₁₀	758	5389	0.05	Alkane
100.	44.119	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro- .alpha.,3,8-tetramethyl-, acetate, [3S- (3.alpha.,5.alpha.,8.alpha.)]-	C ₁₇ H ₂₈ O ₂	264	1804	1.49	Sesquiterpenoid ester
101.	44.295	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro- .alpha.,3,8-tetramethyl-, acetate, [3S- (3.alpha.,5.alpha.,8.alpha.)]-	C ₁₇ H ₂₈ O ₂	264	1804	0.42	Sesquiterpenoid ester
102.	44.345	Tetrapentacontane	C ₅₄ H ₁₁₀	758	5389	0.17	Alkane
103.	44.802	Lupeol	C ₃₀ H ₅₀ O	426	2848	24.52	Triterpenoid
104.	45.081	Octacosane, 2-methyl-	C ₂₉ H ₆₀	408	2840	0.04	Alkane
105.	45.258	13-Docosenamide, (Z)-	C ₂₂ H ₄₃ NO	337	2625	1.84	Fatty amide
106.	45.383	Tetracosane	C ₂₄ H ₅₀	338	2400	0.18	Alkane
107.	45.555	Squalene	C ₃₀ H ₅₀	410	2914	6.50	Triterpenoid
108.	45.833	Linoleic acid (2E,6E,10E)- 3,7,11,15-tetramethyl- 2,6,10,14-hexadecatetraen- 1-yl ester	C ₃₈ H ₆₄ O ₂	552	3922	0.08	Diterpenoid ester
109.	45.959	Tetracosamethyl- cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁ 2	888	2480	0.57	Siloxane

110.	46.110	Tetrapentacontane	C ₅₄ H ₁₁₀	758	5389	0.14	Alkane
111.	46.172	.alpha.-Tocospiro A	C ₂₉ H ₅₀ O ₄	462	3195	0.09	Tocopherol derivative
112.	46.364	.gamma.-Sitostenone	C ₂₉ H ₄₈ O	412	2714	0.23	Phytosterol
113.	46.586	Tetracosane	C ₂₄ H ₅₀	338	2400	0.26	Alkane
114.	46.635	1-Hexacosanol	C ₂₆ H ₅₄ O	382	2848	0.10	Fatty alcohol
115.	46.876	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)-	C ₃₀ H ₅₁ BrO	506	3253	0.12	Polyisoprenoid alcohol
116.	47.041	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	C ₂₉ H ₄₈ O	412	2878	0.18	Epoxide
117.	47.644	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	888	2480	0.64	Siloxane
118.	47.889	2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetracosatetraen-10,15-diol	C ₃₀ H ₅₄ O ₂	446	3127	0.31	Polyisoprenoid alcohol
119.	48.024	Tetrapentacontane	C ₅₄ H ₁₁₀	758	5389	0.25	Alkane
120.	48.142	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C ₃₀ H ₅₀ O	426	3058	0.15	Diterpenoid alcohol
121.	48.335	Cholesta-4,6-dien-3-ol, (3.beta.)-	C ₂₇ H ₄₄ O	384	2579	0.04	Sterol
122.	48.674	.beta.-Tocopherol	C ₂₈ H ₄₈ O ₂	416	3036	0.17	Tocopherol
123.	48.961	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-	C ₅₃ H ₈₂ O ₂	750	5594	3.10	Tocotrienol

		(4,8,12,16,20,24,28,32-octamethyl-3,7,11,15,19,23,27,31-tritriacontaoctaenyl)-, [r-(all-E)]					
124.	49.169	3,3'-Diaminobenzidine	C ₁₂ H ₁₄ N ₄	214	2614	0.04	Biphenyl
125.	49.365	Cholesta-4,6-dien-3-ol, (3.beta.)-	C ₂₇ H ₄₄ O	384	2579	0.09	Sterol
126.	49.445	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C ₃₀ H ₅₀ O	426	3058	0.06	Diterpenoid alcohol
127.	49.789	Tetrapentacontane	C ₅₄ H ₁₁₀	758	5389	0.63	Alkane
128.	49.940	1-Pentacosanol	C ₂₅ H ₅₂ O	368	2749	0.12	Fatty alcohol
129.	50.417	.alpha.-Tocopherol-.beta.-D-mannoside	C ₃₅ H ₆₀ O ₇	592	4489	1.62	Tocopheryl glycoside
130.	51.070	Acetic acid, (4-chloro-2-methylphenoxy)-, pentadecyl ester	C ₂₄ H ₃₉ ClO ₃	410	2920	0.21	Phenoxyacetic acid ester derivative

Discussion

Plants produce a wide range of secondary metabolites that help them survive environmental stress and defend against pathogens. Many of these compounds, such as phenols, flavonoids, terpenoids, and alkaloids, are also responsible for the biological activities observed in medicinal plants (Saxena *et al.*, 2013). In the present study, the aerial parts of *S. attenuata* were found to contain diverse phytochemicals as revealed by qualitative phytochemical screening, particularly in SAA and SAM. These extracts were rich in alkaloids, flavonoids, glycosides, phenols, tannins, saponins, terpenoids, and phytosteroids, while SAH and SAE showed limited diversity. Similar solvent-dependent variations in phytochemical content have been reported in related *Strobilanthes* species

such as *S. jomyi* (Suresh and Xavier, 2023) and *S. heyneanus* (Sundaram et al., 2021), supporting the genus's reputation for chemical diversity.

The antioxidant activity of *S. attenuata* extracts was closely related to their phenolic and flavonoid contents. SAM contained a high total phenolic content (112.2 mg GAE/g) and the highest total flavonoid content was exhibited by SAA (191.8 mg QE/g). Phenolic compounds are known to donate hydrogen or electrons to neutralize reactive oxygen species (ROS) (Sepahpour *et al.*, 2018), and this strong correlation was evident in the present study. Among the four extracts, SAA exhibited the highest antioxidant potential, followed by SAM. SAA achieved 63.47% and 90.84% inhibition at 100 µg/mL in the DPPH and ABTS assays, respectively, with IC₅₀ values of 67.94 and 44.71 µg/mL, comparable to the synthetic standards BHA and rutin. SAM also demonstrated substantial scavenging activity, with IC₅₀ = 84.59 and 153.50 µg/mL in the respective assays. These findings are consistent with *S. kunthianus* leaf extracts, which displayed similar radical scavenging potential (Balasubramaniam *et al.*, 2021). When compared with earlier reports, the DPPH IC₅₀ of SAA closely matched that of the acetone stem extract of *S. attenuata* (69.01 µg/mL; Kaur *et al.*, 2025). The essential oil exhibited a much lower IC₅₀ = 24.07 µl/mL (Upadhyay *et al.*, 2023), reflecting the stronger radical scavenging capacity due to the presence of volatile terpenoids such as guaiol and phytol.

The total antioxidant capacity determined by the phosphomolybdenum and FRAP assays further strengthen these results, highlighting the superior total antioxidant capacity and reducing power of SAA. The phosphomolybdenum method quantifies total electron-donating capacity, while the FRAP assay reflects the ability to reduce ferric ions to ferrous form—both correlating with the presence of phenolics and flavonoids (Vyas & Braganza, 2019). Therefore, the enhanced activity of SAA indicates its effectiveness in extracting medium-polarity compounds such as polyphenols and flavonols, which are known to stabilize reactive intermediates through electron transfer or hydrogen atom donation mechanisms (Jafri *et al.*, 2017).

The antimicrobial evaluation demonstrated that the extracts were more effective against Gram-negative bacteria (*E. coli*) than Gram-positive bacteria (*S. aureus*), likely due to structural differences in bacterial cell walls (Ali *et al.*, 2018). SAE displayed high inhibition, while SAA displayed moderate inhibition against *E. coli* at 1000 µg/mL. In contrast, antifungal screening revealed strong activity of SAA against *Candida albicans*, with a 19.6 mm inhibition zone at 1000

µg/mL. The antifungal effect may result from the ability of phenolic compounds and terpenoids to disrupt fungal membranes or interfere with ergosterol biosynthesis (Zonios and Bennett, 2008). Previous *in vivo* study on *Strobilanthes callosus* demonstrated that benzene fractions and the triterpenoid taraxerol exhibit strong antimicrobial activity (Singh *et al.*, 2002), supporting the antimicrobial role of lipophilic compounds in this genus.

Cytotoxicity testing against MCF-7 breast cancer cells revealed dose-dependent effects for both SAA and SAM extracts. SAA was more potent (IC₅₀ = 61.23 µg/mL) than SAM (IC₅₀ = 131.89 µg/mL), consistent with its stronger antioxidant activity. Morphological analysis showed hallmark apoptotic changes such as cell rounding, shrinkage, and detachment. In comparison, our previous study on the acetone stem extract (Kaur *et al.*, 2025) reported a lower IC₅₀ = 42.10 µg/mL, indicating slightly higher cytotoxicity, possibly due to a higher accumulation of triterpenoids and sterols in the stem. Similar anticancer potential has been reported for *S. crispus* and *S. ciliatus*, which induce apoptosis in breast cancer cells via mitochondrial pathways (Chong *et al.*, 2012; Gordani *et al.*, 2017; Sebastian *et al.*, 2023).

GC-MS analysis of the acetone aerial extract of *S. attenuata* revealed 130 compounds distributed across diverse chemical classes, including triterpenoids, sterols, fatty acids, tocotrienols, esters, hydrocarbons, siloxanes, and phenolic derivatives. This complex metabolite spectrum aligns closely with the phytochemical composition reported earlier in the acetone stem extract of the same species (Kaur *et al.*, 2025), indicating both aerial and stem parts share biosynthetically related compounds responsible for strong antioxidant and cytotoxic activities observed.

1. Triterpenoids and sterols

Among the dominant constituents, Lupeol (24.52%) exhibited broad-spectrum activities, including antimicrobial, anticancer, and antioxidant properties (Singh *et al.*, 2025; Liu *et al.*, 2021). Mechanistically, it induces apoptosis in various human cancer cells such as lung and melanoma, while sparing normal cells. Lupeol's ability to suppress drug resistance through inhibition of ATP-binding cassette transporter proteins like ABCG2 further enhances its chemotherapeutic potential (Le *et al.*, 2021).

γ-sitosterol (10.70%) contributes substantially to antioxidant capacity, as shown in *Capparis zeylanica* flower extract (Patel *et al.*, 2026). It has also been isolated from the roots of *Girardinia*

heterophylla—another plant from Uttarakhand—where it modulates components of the extrinsic apoptotic pathway in human lung and breast adenocarcinoma cells, promoting programmed cell death (Tripathi *et al.*, 2013).

Squalene (6.50%), previously detected in the acetone leaves extract of *S. ciliatus* (Sebastian *et al.*, 2023), the stem extract (Kaur *et al.*, 2025) as well as the essential oil of *S. attenuata* (Upadhyay *et al.*, 2023), acts as a potent membrane antioxidant that neutralizes ROS, stabilizes bilayer structures, and repairs oxidative membrane damage (Huang *et al.*, 2009; Du *et al.*, 2024). It also exhibits tumor-suppressive properties by modulating lipid metabolism and redox signaling (Cheng *et al.*, 2024).

Phytol (4.96%), further strengthens this lipid-soluble antioxidant system. It protects cells from oxidative damage by scavenging free radicals, demonstrates antibacterial and antifungal activity, including against drug-resistant bacteria (Saha & Bandyopadhyay, 2020), and possesses immune-modulatory and apoptosis-inducing properties (Islam *et al.*, 2018).

2. Tocotrienols and Vitamin E Derivatives

The detection of 2H-1-Benzopyran-6-ol,3,4-dihydro-2,7,8-trimethyl-2-(4,8,12,16,20,24,28,32-octamethyl-3,7,11,15,19,23,27,31-tritriacontaoctaenyl)-, [r-(all-E)], also known as γ -tocotrienol (3.10%), is particularly notable. Tocotrienols, members of the vitamin E family, possess strong membrane-localized antioxidant and anti-inflammatory properties. γ -tocotrienol modulates lipid metabolism and suppresses oncogenic signaling pathways such as NF- κ B, STAT3, and AKT, thereby attenuating cancer cell proliferation and inducing apoptosis (Fu *et al.*, 2014; Ahsan *et al.*, 2014). Similarly, α -tocopheryl- β -D-mannoside (1.62%), also identified in the acetone stem extract of *S. attenuata* (Kaur *et al.*, 2025), has been shown to induce apoptosis in cancer cells through oxidative stress modulation and mitochondrial dysfunction (Tucker and Townsend, 2005).

3. Fatty acid derivatives and amides

Ethanol, 2-(9,12-Octadecadienyloxy)-,(Z,Z)- (7.36%), a fatty acid ether detected in the essential oil of flaxseed, possesses antibacterial and anti-inflammatory activity (Salem & Lakwani, 2024). 13-Docosenamide, (Z) (1.84%), found in our extract, is a promising anticancer candidate that exhibits activity against human alveolar basal epithelial (A549) cells (Priya *et al.*, 2018) and is being explored for cancer therapy and antibacterial drug development (El-Gazzar *et al.*, 2025). It

also demonstrates radical scavenging ability (Dogara et al., 2025), further supporting its dual antioxidant and anticancer role. 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (0.89%), a methylated linoleic acid derivative, was reported from raw African walnut, this compound exhibits antioxidant, cardio-protective, neural, antiandrogenic, antibacterial properties (Asuzu-Samuel & Nnamdi, 2023).

Octadecanoic acid (0.40%) and Tetradecanoic acid (0.26%), previously observed in the stem extract (Kaur et al., 2025), exhibit antioxidant, anticancer, hypocholesterolemic, and anti-virulence properties (Arora et al., 2017; Juarez-Rodriguez et al., 2021). Tridecanoic acid, 4,8,12-trimethyl-, methyl ester (TAME), though present in minor proportion (0.07%), possesses antibacterial potential via DNA Gyrase B inhibition in *E. coli*, suggesting a mechanism of antimicrobial interference (Misra et al., 2022). These fatty acids and esters likely act through membrane disruption and enzyme inhibition, providing a mechanistic basis for the extract's antibacterial properties.

4. Siloxanes, hydrocarbons, and alcohols

The cyclic siloxane Tetracosamethyl-cyclododecasiloxane (1.69%) possesses antibacterial, antifungal, hepatoprotective, and antioxidant properties (Rizwana et al., 2019; Al Bratty et al., 2020; Pauloi et al., 2024). Long-chain hydrocarbons such as 1-Nonadecene (1.53%) and Eicosane (0.10%), also identified in *S. glutinosus* (Aziz et al., 2022) exhibit antioxidant, antibacterial, and antifungal activities (Heng et al., 2020; Balachandran et al., 2023; Bhat et al., 2024). Bis (2-ethylhexyl) phthalate (0.14%), detected both in the current and previous stem extract (Kaur et al., 2025), possesses cytotoxic, antibacterial, and larvicidal activities (Lotfy et al., 2018; Javed et al., 2022). Tetrapentacontane (1.76%), found in *Santolina chamaecyparissus*, can suppress EGFR expression in MCF-7 cells (Ali et al., 2021), linking to the observed cytotoxicity.

1-Hexacosanol (0.40%), previously isolated from *Launaea taraxacifolia*, shows activity against *Staphylococcus aureus* (Tayman et al., 2013). Tetracosane (0.55%) displayed cytotoxicity against gastric and breast cancer cells with IC₅₀ values between 128.7 and >250 µM (Uddin et al., 2012). 3-Octanol and 5-Octadecenal possess antifungal and antibacterial effects (Zhang et al., 2023; Anjali et al., 2019). 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl- (0.32%) exhibits antioxidant properties and was previously identified in methanolic leaves extract of *Papilionanthe teres* (Ashraf et al., 2025). 2-Methyltetracosane (0.05%) and 2-

Methylhexacosane (0.03%) show radical scavenging, antimicrobial, and hypocholesterolemic effects (Uddin et al., 2024; Ryu et al., 2020).

Heptadecane (0.05%) and 17-Pentatriacontene (0.23%) exhibit anti-inflammatory, antioxidant, and anticancer activities by modulating NF- κ B signaling (Kim et al., 2013; Albratty et al., 2023; Fathi et al., 2022), further reinforcing the mechanistic basis of our cytotoxic findings.

5. Minor compounds

Several minor terpenoid compounds also contribute synergistically. Loliolide (0.20%), isolated from *Codium tomentosum*, is a multifunctional metabolite with antioxidant, antibacterial, antifungal, anticancer and neuroprotective effects (Silva et al., 2021). Guaiol (0.03%), a sesquiterpene alcohol, was identified as a major compound in the essential oil of *S. attenuata* (Upadhyay et al., 2023), exhibits antibacterial and antitumor activity (Yang et al., 2018). 9-Oxabicyclo[3.3.1]nonane-2,6-diol (0.32%) mitigates oxidative stress associated with Type 2 Diabetes Mellitus (Iheagwan et al., 2021), suggesting a ROS-modulatory role. Megastigmatrienone (0.11%), though primarily a flavor compound (Kim et al., 2023), and 4,4,5,8-Tetramethylchroman-2-ol (0.43%), an inhibitor of *Vibrio cholerae* ToxT protein (Rahman et al., 2024), may contribute to antimicrobial effects.

Interestingly, 5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-.alpha.,3,8-tetramethyl-, acetate, [3S-(3.alpha.,5.alpha.,8.alpha.)]- (total 2.53%) was identified at multiple RT. It has been reported from cucumber fruit cultivars but with no documented bioactivity (Zhang et al., 2022). Given its azulene backbone, it may possess antioxidant or antimicrobial potential via free-radical stabilization—a hypothesis warranting future investigation.

Cholesta-4,6-dien-3-ol, (3 β)- (0.13%) exhibits potent anticancer activity via apoptosis induction, DNA fragmentation, and cell cycle arrest in MCF-7 and HeLa cells (Qurishi et al., 2021), along with antifungal activity (Zhu *et al.*, 2018). (E)-Hexadec-2-enal (0.03%) induces programmed cell death and cytoskeletal disruption in multiple mammalian cell lines (Kumar et al., 2011). These compounds likely contribute to the apoptosis-like morphological changes observed in cancer cells treated with the extract in the present study.

Thus, the bioactivity of *S. attenuata* emerges from a synergistic interplay of these compounds rather than from individual molecules alone. The limitation of the present work is that our study

reports the antioxidant, antimicrobial and anticancer activity of *S. attenuata* crude extracts, and the evaluation of the activity of active components in pure form is necessary to understand the reported effect.

Conclusion

S. attenuata extracts showed antibacterial and antifungal property against Gram-negative and fungal strain, and the highest activity was observed against *E. coli* and *C. albicans*. Our findings support the traditional use of *Strobilanthes* genus in the treatment of various diseases. The results obtained in this study indicated that the acetone extract of *S. attenuata* is a valuable source of pharmaceutical natural products, and it can be used for the development of new therapeutic formulations. Based on the detected antioxidant, antimicrobial and anticancer activity, which is reported for the first time in the present study, *S. attenuata* acetone extract can be a promising alternative to antifungal agents or in cancer therapy.

Declaration of competing interest

None.

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