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RESEARCH ARTICLE

Unveiling the therapeutic and insecticidal potential of dangling coneflower: A comprehensive study

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Abstract

Newly described plant species represent valuable yet underexplored sources of bioactive compounds. *Strobilanthes jomyi* (Dangling coneflower) is an endemic species of the Western Ghats, Kerala, India. The current study sought to assess the anthelmintic, insecticidal, antimicrobial and cytotoxic properties of a methanolic extract of *S. jomyi* leaves, stem and root. Extracts were prepared using Soxhlet extraction and screened using standard *in vitro* bioassays. All vegetative parts exhibited significant anthelmintic activity, with the leaf extract showing the shortest death time (91.66 ± 2.08 min), followed by root (115 ± 5 min) and stem (115 ± 5 min) extracts. Insecticidal activity was observed only in the leaf extract (100 % at 25 mg/mL). Anti-microbial activity of all vegetative parts of *S. jomyi* showed mild activity at higher concentrations (10 mg/mL). Cytotoxic evaluation against HeLa, MCF-7 and HT-29 cell lines demonstrated dose-dependent activity, with IC_{50} values within the range of 10–100 µg/mL. GC -MS results proved the presence of bioactive compounds like n-hexadecenoic acid, stigmasterol, phytol, neophytadiene, tris(2,4-di-tert-butylphenyl) phosphate, Squalene, 1-Eicosanol, lupeol and gamma-sitosterol, which in turn could be responsible for the anthelmintic, insecticidal, anti-microbial and cytotoxic activity of *S. jomyi*. Taken together, the findings suggest that *S. jomyi* represents a promising source of bioactive compounds with potential pharmacological and agricultural relevance, warranting further isolation and mechanistic studies.

Keywords: anthelmintic; anti-microbial; cytotoxicity; GC-MS analysis; insecticidal

Introduction

Plant-derived medication is one of the oldest systems of medical practice in the world. Their continued effectiveness has resulted in approximately 80 % of populations in African and Asian countries relying on traditional medicines for primary healthcare needs (1). This efficacy has also positioned medicinal plants as an important source of novel chemical constituents for the discovery of therapeutic drugs and pharmacological innovations (2). The use of medicinal plants in pharmaceutical research has expanded considerably, with studies indicating that approximately 25 % of drugs in developed countries are derived from plant sources (3). Previous studies have demonstrated that plant-derived compounds exhibit anthelmintic, antimicrobial and anticancer activities, sustaining their relevance in pharmaceutical research. In addition, medicinal plants have good agricultural applications due to their insecticidal properties (4-7).

Strobilanthes jomyi is an endemic species of the Western Ghats of Kerala, India. It is commonly known as 'Elathumpadi' in Malayalam. The plant is an anisophyllous shrub that can reach up to 5 m in height (8). The members of the *Strobilanthes* genus (*Acanthaceae*) are widely utilised in the traditional system of medicine across various regions (9). Some economically important species of the same genus, like *S. heyneanus* root extract and *S. crispa* crude extract, were found to possess cytotoxicity activity and anti-microbial activity towards specific cell lines (10, 11). Reports on the Indian system of medicines include *Adhatoda vesica* (*Acanthaceae*) root, which is used for treating gastrointestinal nematodes because of its anthelmintic activity. Similar properties are exhibited by *Barleria* spp. and *Hygrophila spinosa* (12-14). Research has demonstrated that *Andrographis paniculata* Burm. f. (*Acanthaceae*) showed that their extract exhibited insecticidal activity against *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes (15). Based on this background, the present study aims to evaluate the cytotoxic, anthelmintic, insecticidal and antimicrobial properties of *S. jomyi*. Additionally, it also includes GC-MS analysis of its leaves, stem and root of the plant. These analyses provide baseline scientific data on *S. jomyi*, supporting its potential relevance in future pharmacological and agricultural research.

Materials and Methods

Collection and identification

Fresh leaves, stem and root of *S. jomyi* were collected from Kasaragod district, Kerala, India in December 2020. The voucher herbarium specimen (Voucher No. FRLH-123445) was identified and authenticated at FRLHT (Foundation for Revitalisation of Local Health Traditions), Bengaluru, Karnataka, India.

Processing of plant materials

The leaves, stem and root of *S. jomyi* were washed thoroughly with tap water, followed by distilled water. The cleaned plant parts were shade-dried at room temperature for approximately 10 days and then separately pulverised using a mechanical grinder. The resulting powdered samples were stored in airtight containers for further analysis (16).

Preparation of plant extract

The shade-dried leaves, stem and root of *S. jomyi* were separately pulverised into a coarse powder. For extraction, 50 g of each powdered plant material was extracted with 500 mL of methanol (plant material to solvent ratio 1:10, w/v) using a Soxhlet apparatus. The extraction was carried out for 16 hr, corresponding to approximately 8–10 continuous siphoning cycles, until the solvent in the siphon tube became colourless, indicating exhaustive extraction. The resulting methanolic extracts were filtered and concentrated using a rotary evaporator under reduced pressure at a controlled temperature of 37 °C, which helps preserve thermolabile secondary metabolites. The concentrated crude extracts were transferred into sterilised Falcon tubes, tightly sealed and stored at 4 °C in dark conditions to prevent degradation (17).

Estimation of bioactive compounds by GC-MS analysis

Secondary metabolites of the leaves, stem and root of *S. jomyi* were analysed using a Shimadzu GC-MS QP2010SE system. Different vegetative parts of *S. jomyi* were separately mixed with 10 mL of ethyl acetate and kept in a rotary shaker for 24 hr at room temperature. The extracts were then filtered through Whatman No. 1 filter paper and dried using a hot air oven. The dried crude extract was transferred into an Eppendorf tube and re-dissolved in 0.5 mL of ethyl acetate, from which 1 µL of the sample was injected into the GC-MS system.

Chromatographic separation was achieved using a capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250 °C and the injection was performed in split mode. The oven temperature was programmed as follows: initial temperature of 60 °C (held for 2 min), increased at a rate of 10 °C/min to 280 °C and held for 10 min. The mass spectrometer was operated in electron ionisation (EI) mode at 70 eV, with an ion source temperature of 230 °C and an interface temperature of 280 °C. The mass spectra were recorded over a scan range of m/z 40–600.

Identification of compounds was performed by comparing the obtained mass spectra with those in the NIST 2017 MS library, based on similarity indices. The identified compounds were tentatively assigned and their relative abundance was calculated based on peak area normalisation. Due to the exploratory nature of the study, identification was not further confirmed using authentic standards or retention indices (18).

Anthelmintic assay

An average-sized (6–8 cm) *Perionyx excavatus* (Indian blue earthworm) was collected from the Christ (Deemed to be University) vermicompost unit. *Perionyx excavatus* was used for the anthelmintic assay due to its morphological and physiological similarity to human gastrointestinal helminths, particularly in neuromuscular organisation. The anthelmintic activity of the leaves, stem and root of *S. jomyi* was carried out with minor modifications. Freshly prepared methanolic extracts (5, 15 and 25 mg/mL) were transferred to Petri dishes. In each Petri plate containing 30 mL of extract solution (1 % methanol), three worms were introduced and three worms were used per treatment group. The time taken for paralysis and death was recorded for a maximum of 3 hr. analysis was defined as loss of spontaneous movement and no response to mechanical stimulation, while death was confirmed by complete absence of movement and loss of body tone. Albendazole (16 mg/mL) served as the reference standard and 1 % methanol was used as the negative control. All experiments were conducted in triplicate under uniform conditions to minimise observer bias (19).

Insecticidal assay

Two-day-old *Drosophila melanogaster* flies were collected from mashed banana culture media and isolated based on morphological characteristics. Insecticidal activity was estimated using a soji-jaggery-agar medium with slight modification. A mixture of jaggery (10 g), soji (10 g) and agar-agar (2 g) in 100 mL of distilled water was boiled for 2–3 min. After the addition of 0.7 mL propanoic acid, the medium was poured into sterile boiling tubes (20). Thereafter, 200 µL of different concentrations (5, 15 and 25 mg/mL) of leaves, stem and root (1 % methanolic) extracts were added to the medium and dried under a fume hood at room temperature for 5 min. 1 % methanol was used as the negative control and carbofuran (10 mg/mL) was used as the reference insecticide. 10 adult *D. melanogaster* were introduced into each tube and closed. Three independent replicates were maintained for each concentration, with a total of 30 flies per treatment group. Mortality was recorded at 24, 48 and 72 hr. The observed mortality in treated groups was corrected using Abbotts' formula to account for control mortality. The LD_{50} value was calculated from corrected mortality data using probit analysis, based on dose–response relationships (21).

Anti-microbial assay by the well diffusion method

The bacterial strains, such as Gram-positive and Gram-negative organisms, such as *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (ATCC 2146-01060P) and *Pseudomonas aeruginosa* (ATCC 27853), were used for the anti -bacterial assay, whereas for anti-fungal activity, *Aspergillus flavus* (ATCC 9643), *Candida glabrata* (MTCC3019), *Candida parapsilosis* (ATCC 22019) and *Candida albicans* (ATCC90028) were used.

Anti-bacterial activity

The nutrient agar (2.8 g in 100 mL) was autoclaved and transferred into sterile petri dishes. The different bacterial inocula were then transferred into each petri plate and spread by using a glass spreader. A sterile well cutter was used for the holes of size 6–8 mm to be made separately under aseptic conditions. Different concentrations of leaves, stem and root methanolic extracts (2–10 mg/mL of *S. jomyi* were transferred into each well and incubated overnight at 37 °C. After 24 hr of incubation, the zone of inhibition was measured and expressed in millimetres (mm). Chloramphenicol (1 mg/mL) was used as a control (22).

Antifungal activity

Yeast peptone dextrose agar (2.4 g in 100 mL) was added and petri plates were autoclaved under sterile conditions. Once the medium was cooled, each fungal broth was transferred and spread by a glass spreader and a sterile well cutter was used for making wells

of 6–8 mm. The various concentrations ranging from 2 mg/mL to 10 mg/mL of leaves, stem and root of *S. jomyi* extract were then transferred into the wells, including standard (1 mg/mL fluconazole) and incubated at room temperature (28 ± 2 °C) for about 3–4 days. The zone of inhibition was then measured in millimetres (mm) (22).

Cytotoxicity assay

The anti-cancerous activity of the methanolic extract of *S. jomyi* was tested on HeLa (Passage number 33), MCF-7 (Passage number 41) and HT-29 (Passage number 30) cell lines obtained from NCCS Pune. The activity was analysed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay with a slight modification. The cell lines were cultured in MEM medium supplemented with fetal bovine serum for 37 °C at 24 hr with 5 % CO_2. After 24 hr of incubation, the culture medium from the cancer cells was replaced with fresh media. Test samples at different concentrations (10 to 100 µg/mL) in triplicate were added to the cells. The cell seeding density was 1×10^4 cells/well. After the incubation of the sample with cells at 37 ± 1 °C for 18 to 24 hr, MTT (1 mg/mL) was added to all the wells and incubated for 4 hr. Thereafter, DMSO (100 %) was added to the wells and optical density was read at 570 nm using a microplate reader. The electronic image of the cancer cells against plant extracts was taken using an inverted phase contrast microscope. From the obtained data, a graph was plotted with concentration (µg) on the x-axis and the percentage of cytotoxicity on the y-axis along the y-intercept (y = mx + c) and the IC_{50} value was calculated. Cytotoxicity and cell viability were calculated by the formula mentioned below (23).

$$\text{Cytotoxicity} = \frac{\text{Control - Treated}}{\text{Control}} \times 100 \quad \text{(Eqn. 1)}$$

Statistical analysis

The statistical analysis of the insecticidal and cytotoxicity assay of *S. jomyi* was estimated using SPSS software version 22.0.0.0. The findings were statistically interpreted by a two-way analysis of variance (ANOVA) for insecticidal activity and one-way ANOVA for cytotoxic studies. Post-hoc analysis was performed using Tukey's test to analyse significant variance probability less than or equal to $P \leq 0.05$.

Results and Discussion

GC-MS analysis

GC-MS analysis of the leaves, stem and root of *S. jomyi* indicated the presence of several bioactive compounds, including n-Hexadecenoic acid, stigmasterol, phytol, *neophytadiene*, Tris(2,4-di-tert-butylphenyl) phosphate, Squalene, 1-Eicosanol, lupeol, gamma-sitosterol and 2,4-Di-tert-butyl phenol (Table 1; Fig. 1–3). Research has demonstrated that several of these compounds exhibit anti-inflammatory activity, anti-osteoarthritic activity, anti-tumour, cytotoxicity, antioxidant, anti-bacterial, hepatoprotective and even anti-fungal activities (24-33). Similar phytochemical profiles have been reported in *Strobilanthes crispus*, suggesting phytochemical conservation within the genus. The secondary metabolite composition of *S. jomyi* showed notable similarity to different vegetative parts of *S. crispus*, particularly with respect to lupeol, stigmasterol and squalene (34).

Anthelmintic activity on *Perionyx excavatus*

The anthelmintic activity of the leaves, stem and root of *S. jomyi* methanolic extract is represented in Table 2 and Fig. 4. Three different concentrations (5, 15 and 25 mg/mL) of all vegetative parts of *S. jomyi* expressed paralysis, with the highest expression in

Table 1. GC-MS analysis of *Strobilanthes jomyi*

Sl. No	Name of compound	Area (%)	RT	Part used
Leaves				
1	2-Octylcyclopropene-1-heptanol	19.564	14.71	leaf
2	9(E),11(E)-Conjugated linoleic acid	5.98	19.785	leaf
3	n-Hexadecanoic acid	5.51	18.313	leaf
4	Stigmasterol	3.69	31.372	leaf
5	Phytol	3.62	19.197	leaf
6	Neophytadiene	3.50	16.697	leaf
7	Tris(2,4-di-tert-butylphenyl) phosphate	3.47	26.330	leaf
8	Octadecanoic acid	2.95	19.651	leaf
9	Squalene	2.67	23.689	leaf
10	1-Eicosanol	0.43	21.677	leaf
Stem				
11	Lupeol	25.19	35.614	Stem
12	Stigmasterol	12.83	31.299	Stem
13	Gamma. -Sitosterol	9.08	32.895	Stem
14	Campesterol	4.74	30.650	Stem
15	Neophytadiene	3.64	16.676	Stem
16	n-Hexadecanoic acid	3.45	18.267	Stem
17	1-Tetradecene	1.99	11.837	Stem
18	Cetene	1.95	13.975	Stem
19	Phytol	1.07	19.183	Stem
Root				
20	Lup-20(29)-en-3-one	20.45	34.521	Root
21	Stigmasterol	9.21	31.285	Root
22	Lupeol	6.18	35.730	Root
23	Gamma-Sitosterol	6.85	32.894	Root
24	Campesterol	6.64	30.634	Root
25	n-Hexadecanoic acid	3.85	18.262	Root
26	2,4-Di-tert-butylphenol	3.39	13.435	Root
27	Squalene	2.18	23.661	Root
28	Cetene	1.54	13.973	Root
29	Octacosane	1.42	21.231	Root

*RT: Retention time.

Table 2. Anthelmintic activity of *Strobilanthes jomyi* using *Perionyx excavatus*

Groups	Concentration (mg/mL)	*Perionyx excavatus*	
		Paralysing time (min)	Death time (min)
Leaves	5	146 ± 1	NA
	15	105 ± 3	172.3 ± 3.05
	25	25.66 ± 2.08	91.66 ± 2.08
Stem	5	168 ± 1	NA
	15	127.33 ± 2.08	NA
	25	58.47 ± 0.72	130.33 ± 1.52
Root	5	160.66 ± 0.57	NA
	15	171.66 ± 0.57	NA
	25	29.53 ± 0.41	115 ± 5
Albendazole	16	19.58 ± 0.79	77.33 ± 2.51

Results are means of triplicate (Mean ± SD). NA: No activity found.

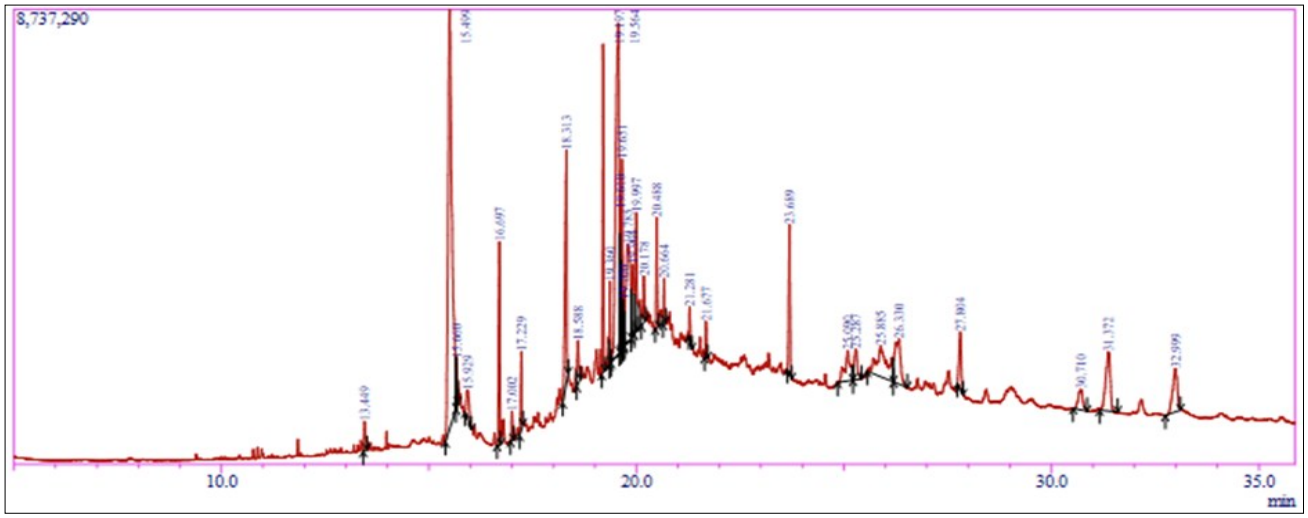

Fig. 1. GC-MS chromatogram of *S. jomyi* ethyl acetate leaf extract.

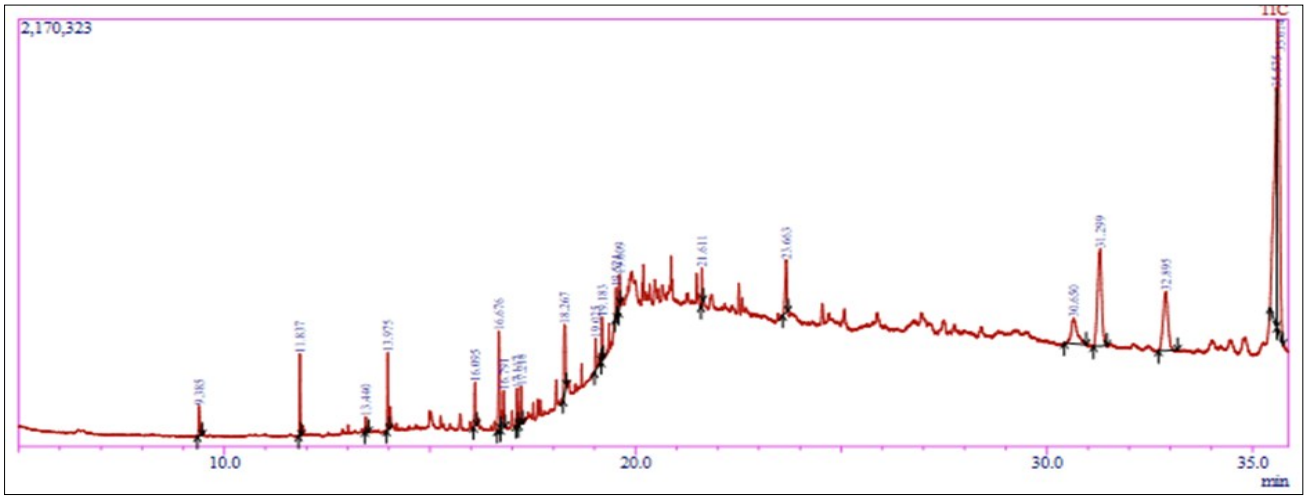

Fig. 2. GC-MS chromatogram of *S. jomyi* ethyl acetate stem extract.

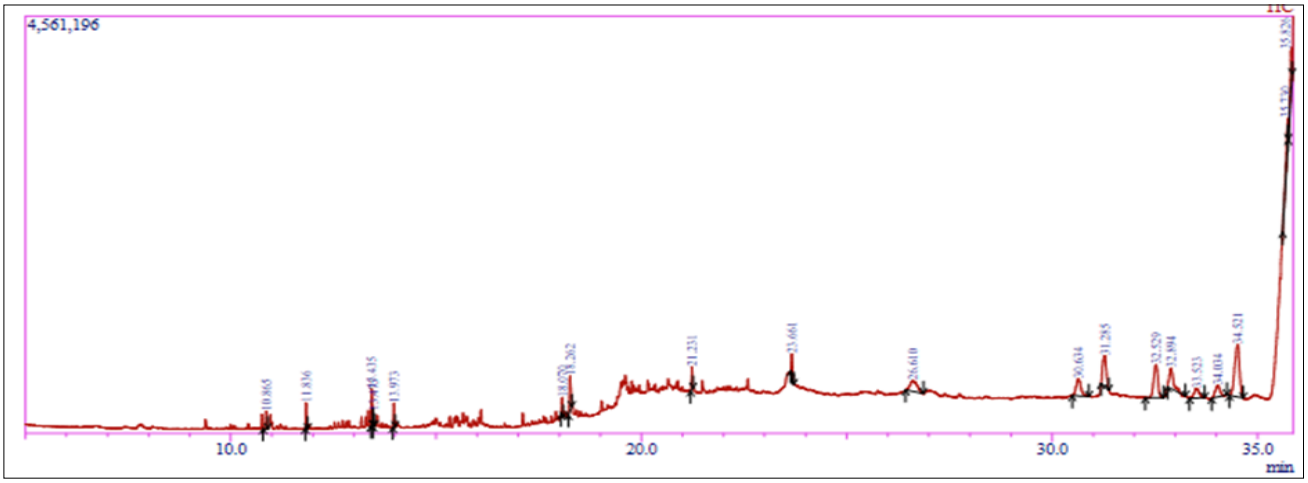

Fig. 3. GC-MS chromatogram of *S. jomyi* ethyl acetate root extract.

Fig. 4. Anthelmintic activity of *S. jomyi* methanolic extract: (A) Leaves (25 mg/mL), (B). stem (25mg/mL), (C). Root (25mg/mL), (D). standard (16 mg/mL).

leaves, followed by root and stem. Similarly, in higher concentrations (25 mg/mL), the extracts of leaves (25.66 ± 2.08), stem (58.47 ± 0.72) and root (29.53 ± 0.41) exhibited lower paralysing time in higher concentrations. The time of death for *P. excavates* in each vegetative part was shortest in the methanolic extract of leaves at a 25 mg/mL concentration. Standard albendazole 16 mg/mL concentration caused paralysis at 19.58 ± 0.79 min and death was observed at 77.33 ± 2.51 min, which was a much shorter time duration than the plant samples. Standard albendazole acts by increasing the chloride ion conductance of the worm muscle membrane, thus inducing hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. The previously documented studies suggest that the anthelmintic activity may be due to the presence of tannin content in the plant body, which can bind with the free protein in the gastrointestinal tract or any glycoprotein that binds with the cuticle that appears in the host animal, leading to the death of the organisms (19). Research has demonstrated that *Barleria gibsoni* Dalz. and *Adhatoda vasica* Nees (family *Acanthaceae*) have demonstrated significant anthelmintic activity against *Eudrilus eugeniae* (10.4350 ± 0.1434 min) and *Pheretima posthuma* (78 ± 0.025 min) at higher concentrations of leaf extracts (15 and 50 mg/mL) (12, 13). The leaves of *S. jomyi*, another member of the *Acanthaceae* family, also exhibited notable anthelmintic activity, indicating their potential use in Ayurvedic medicine due to high efficacy and minimal side effects.

Effect of *S. jomyi* extract on *Drosophila melanogaster*

Three different concentrations (5, 15 and 25 mg/mL) of *S. jomyi* leaves, stem and root extract were mixed with soji-jaggery-agar medium and fed to *D. melanogaster*. The stem and root extract did not exhibit any insecticidal properties against fruit flies. The percentage of mortality of *S. jomyi* leaves at a 5 mg/mL concentration was revealed to be 46.66 % at 24 hr, whereas in higher concentrations (25 mg/mL), the mortality percentage was around 86.66 %. This maximum percentage of mortality was observed at 15 mg/mL concentration and 25 mg/mL concentrations of leaf extract at 72 and 42 hr, respectively. Furadon 10 mg/mL concentration revealed 100 % of insecticidal activity within 24 hr (Table 3). Based on previous studies, in higher concentrations, the percentage of mortality is directly proportional to longer time intervals. LC_{50} value of leaf extract of *S. jomyi* against *D. melanogaster* was found to be 5.55 mg/mL. Statistically, the different concentrations of leaf extract with different time intervals showed significant variance probability, $P \leq 0.05$. Previous studies on *Andrographis paniculata* (*Acanthaceae*) revealed insecticidal activity against adult *Aedes aegypti and Culex pipiens* mosquitoes,

Table 3. Insecticidal activity of *Strobilanthes jomyi* using *Drosophila melanogaster*

Samples	5 mg/mL	15 mg/mL	25 mg/mL	Positive control (within 24 hr)
	Mortality (%)	Mortality (%)	Mortality (%)	Mortality (%)
Leaves				
24 hr				
	46.66 ± 11.54	70 ± 10	86.66 ± 5.7	100 ± 0.00
48 hr				
	63.33 ± 11.54	83.33 ± 5.77	100 ± 0.00	100 ± 0.00
72 hr				
	83.33 ± 11.54	100 ± 0.00	100 ± 0.00	100 ± 0.00

Results are means of triplicate (Mean ± SD). Data are corrected for mortality in the treated groups with Abbotts' formula. Leaf extract with different time intervals showed significant variance probability $P \leq 0.05$.

even at lower concentrations of plant extracts, exhibited excellent insecticidal properties on different solvent extracts when compared to *S. jomyi* (15). Studies suggested that bioactive compounds like phytol, n-Hexadecenoic acid, 1-Eicosnol are responsible for the insecticidal activity (34–36). Based on the GC-MS study, the number of all these secondary metabolites is observed to be highest in leaves, followed by the stem and the root. In the current study, *S. jomyi* leaf extract functions even at low concentrations as a possible pesticide for *D. melanogaster* when compared to plants of other genera (21). *S. jomyi* extracts posing less of a threat to the environment can therefore be used in conjunction with integrated pest management for boosting agricultural productivity.

Anti-microbial assay

An anti-microbial study was done using the leaves, stems and roots of *S. jomyi*. Anti-bacterial and anti-fungal studies were carried out using the methanolic extract of the plant represented in Table 4-5 and Fig. 5-6. Different concentrations were taken, ranging from 2 mg/mL to 10 mg/mL, to analyse the anti-microbial properties of *S. jomyi*. Based on the current investigation, anti-bacterial activity was not observed in the methanolic extracts of the leaves for all the bacterial strains. However, mild anti-bacterial activity was observed in *S. aureus* and *P. aeruginosa* cultures treated with methanolic extract of the stem. Among anti-bacterial studies, the methanolic extract of the root revealed a minute zone of clearance in all the bacterial strains at higher concentrations. The anti-fungal activity of *S. jomyi* methanolic leaf extract was observed in leaves and stem, whereas the activity was absent in the methanolic root extract. The stem extract of *S. jomyi* is an effective drug against fungal pathogens like *A. flavus*, *C. glabrata*, *C. parapsilosis* and *C. albicans*.

Based on previous studies on *Strobilanthes hyneus* (root) and *Strobilanthes glutinosus* (bark and leaf), their methanolic extractsrevealed anti-bacterial activity on *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* (10). Similarly, *S. glutinosus* also exhibited comparable anti-fungal activity on *Aspergillus niger* and *Aspergillus oryzae* (37). In comparison with the available data on other *Strobilanthes* genera, few vegetative parts of *S. jomyi* possess a reasonable anti-microbial activity because it contains secondary metabolites like n-hexadecanoic acid, phytol, neophytadiene, octadecanoic acid, squalene, cetene, stigmasterol and lupeol (38-44). *S. jomyi* can be used in a variety of pharmaceutical formulations to treat microbial infections like pneumonia and skin disorders in humans due to its low risk of side effects.

Cytotoxicity

Cytotoxic activity of methanolic extract of *S.jomyi* leaves, stem and root on HeLa, MCF-9 and HT-29 cancer cell lines was investigated using MTT assay, shown in Fig. 7. MTT is a colourimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by active mitochondrial succinate dehydrogenase present in viable cells. The MTT solution

Table 4. Zone of inhibition for varying concentrations of *Strobilanthes jomyi* in different bacterial strains

Strains	Zone of inhibition (mm)					
	Control (1 mg/mL)	2 mg/mL	4 mg/mL	6 mg/mL	8 mg/mL	10 mg/mL
Leaves methanolic extract						
S. aureus	23	-	-	-	-	-
B. subtilis	23	-	-	-	-	-
K. pneumoniae	24	-	-	-	-	-
P. aeruginosa	23	-	-	-	-	-
Stem methanolic extract						
S. aureus	23	-	-	-	-	11
B. subtilis	23	-	-	-	-	-
K. pneumoniae	24	-	-	-	-	-
P. aeruginosa	23	-	-	-	-	10
Root methanolic extract						
S. aureus	23	-	-	-	-	11
B. subtilis	23	-	-	-	-	11
K. pneumoniae	24	-	-	-	-	11
P. aeruginosa	23	-	-	-	-	11

'-': Absent.

Table 5. Zone of inhibition for varying concentrations of *Strobilanthes jomyi* in different fungal strains

Strains	Zone of inhibition (mm)					
		Control (1 mg/mL)	2 mg/mL	4 mg/mL	6 mg/mL	8 mg/mL
Leaves methanolic extract						
A. flavus	16	-	-	-	-	-
C. glabrata	15	-	-	-	11	12
C. parapsilosis	14	-	-	-	11	12
C. albicans	16	-	-	12	14	15
Stem methanolic extract						
A. flavus	14	-	-	-	11	12
C. glabrata	15	-	-	-	11	12
C. parapsilosis	15	-	-	-	12	13
C. albicans	15	-	-	-	-	13
Root methanolic extract						
A. flavus	14	-	-	-	-	-
C. glabrata	15	-	-	-	-	-
C. parapsilosis	15	-	-	-	-	-
C. albicans	15	-	-	-	-	-

'-': Absent

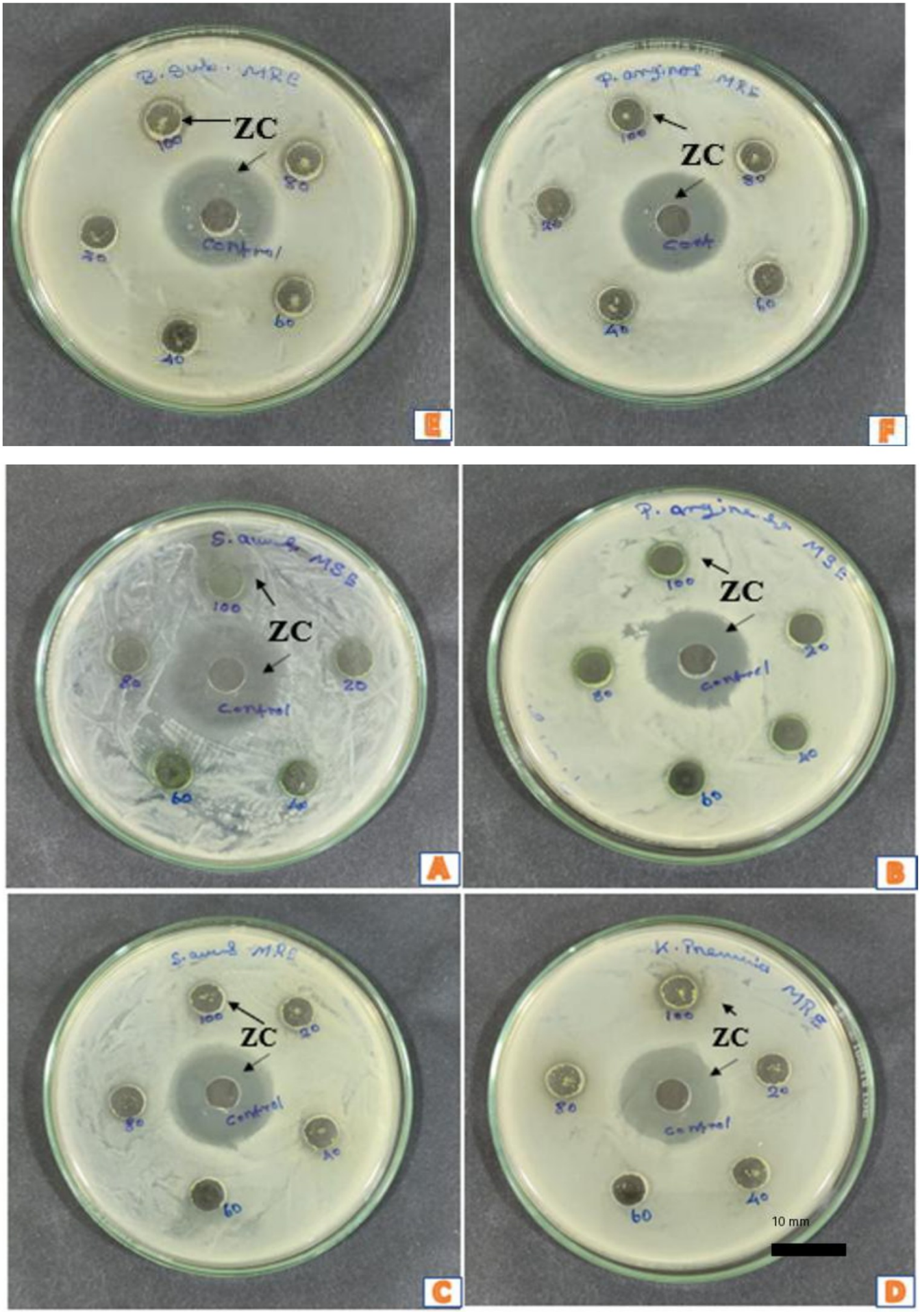

Fig. 5. Anti-bacterial activity of stem and root of *S. jomyi*: (A) Stem extract on *S. aureus*. (B) Stem extract on *P. aeruginosa*, (C) Root extract on *S. aureus*, (D) Root extract on *K. pneumoniae*, (E) Root extract on *B. subtilis*, (F) Root extract on *P. aeruginosa*. ZC: Zone of clearance.

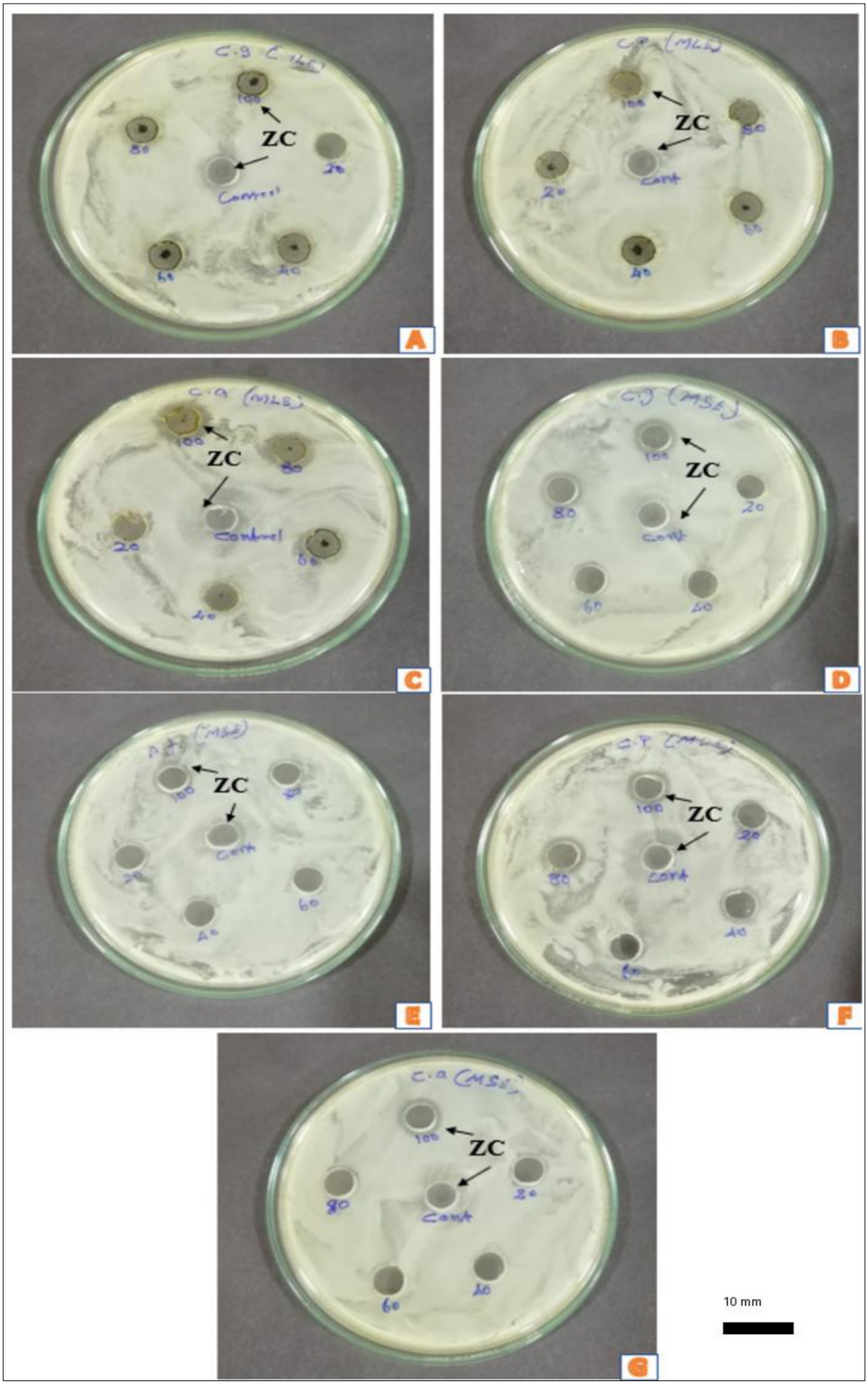

Fig. 6. Anti-fungal activity of leaves and stem of *S. jomyi*: (A) Leaves extract on *C. glabrata*, (B) Leaves extract on *C. parapsilosis*, (C) Leaves extract on *C. albicans*, (D) Stem extract on *C. glabrata*, (E) Stem extract on *A. flavus*, (F) Stem extract on *C. parapsilosis*, (G) Stem extract on *C. albicans*. ZC: Zone of clearance.

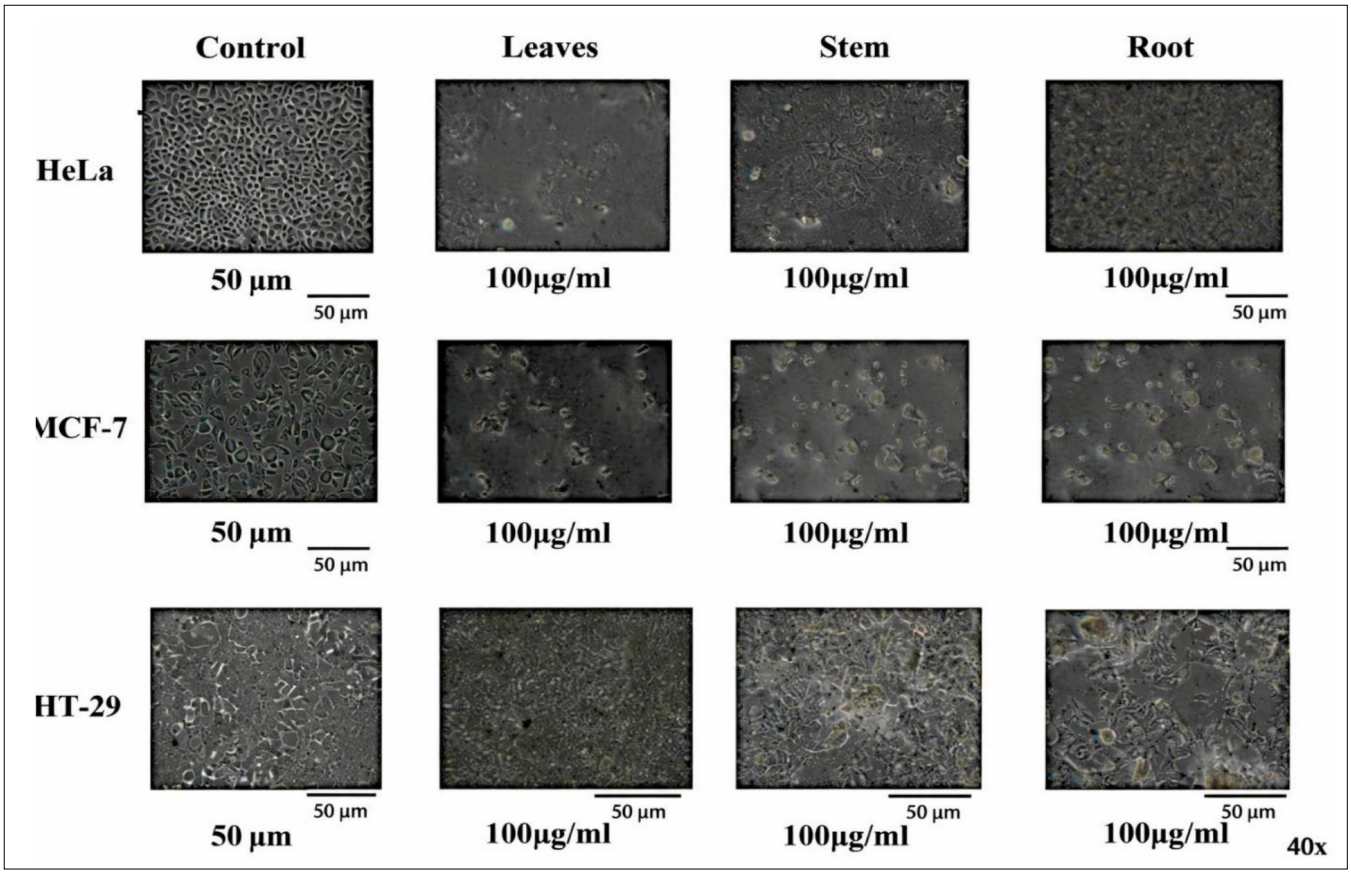

Fig. 7. The cytotoxic activity of *S. jomyi* leaves, stem and root methanolic extract in different cell lines, like HeLa, MCF-7 and HT-29, at a maximum concentration (100 µg/mL).

enters the cells and reacts with the mitochondrial enzymes, where it is reduced into formazan, an insoluble and coloured (dark purple) product. The formazan crystal within the cells is then solubilised with an organic solvent, DMSO and the released, solubilised purple-coloured formazan reagent is measured spectrophotometrically (45, 46).

All extracts exhibited dose-dependent cytotoxic activity across the tested cell lines (Table 6). The cytotoxic activity of all vegetative parts showed severe activity against HeLa cell lines in the order of leaves (IC_{50} 14.96 µg/mL)> stem (IC_{50} 17.53 µg/mL)> root (IC_{50} 21.13 µg/mL). Similarly, cytotoxic activity was exhibited in MCF-7 cell lines in order of stem (IC_{50} 33.23 µg/mL), then leaves (IC_{50} 35.7 µg/mL), followed by root (IC_{50} 39.7 µg/mL). Studies on HT-29 revealed that the stem (IC50 20.48 µg/mL) of *S. jomyi* possessed more cytotoxic activity than leaves (IC_{50} 24.28 µg/mL) and root (IC_{50} 26.76 µg/mL). All the cell lines study are statistically significant within the sample by $p \leq 0.05$, calculated using a single variance of ANOVA.

Table 6. Cytotoxicity evaluation of *Strobilanthes jomyi*

Part used	Cell lines used	IC_{50}
Leaves	HeLa	14.96 ± 0.16 µg/mL
Stem	HeLa	17.53 ± 0.22 µg/mL
Root	HeLa	21.13 ± 0.11 µg/mL
Leaves	MCF-7	35.77 ± 1.92 µg/mL
Stem	MCF-7	33.23 ± 1.45 µg/mL
Root	MCF-7	39.77 ± 2.97 µg/mL
Leaves	HT-29	24.28 ± 0.06 µg/mL
Stem	HT-29	20.48 ± 0.11 µg/mL
Root	HT-29	26.76 ± 0.18 µg/mL

Results are means of triplicate (Mean ± SD). Significantly different among the samples at $p \leq 0.05$.

Prior studies on the flower and vegetative parts of *S. kunthiana* (*Acanthaceae*) also indicated mild cytotoxic activity against HeLa cell lines (47). Ethanolic extract of *S. crispus* leaves expressed severe cytotoxic activity against the HeLa cell line (IC_{50} 78 ± 1.5 µg/mL), HT-29 cell lines (IC_{50} 52 ± 6.3 µg/mL) and MCF-7 cell lines (IC_{50} 30 µg/mL) after 72 hr of incubation. The extract has the potential to produce bioactive metabolites that can be employed as a cancer chemoprevention agent, as evidenced by IC_{50}values that are lower than 100 g/mL (47, 48). So, based on the current study, all the vegetative parts of *S. jomyi* showed exceptional anti-cancerous activity against HeLa, MCF-7 and HT-29 cancer cell lines when compared to plants of other *Strobilanthes* genera.

The current study also proved that the anticancer activity of *S. jomyi* is mainly based on the secondary metabolites present in the plant samples. The higher anti-cancerous activity towards HeLa, MCF-7 and HT-29 observed in all parts of *S. jomyi* may be due to cancer-targeted bioactive compounds like lupeol, phytol, gamma-sitosterol and squalene by analysis (49). The present study proved that the vegetative parts of *S. jomyi* are a source of many bioactive compounds, which can also be used in future plant-based medication for the treatment of human breast cancer, cervical cancer and even colon cancer with the least side effects as compared to targeted drug therapy and chemotherapy. However, to identify the compounds responsible for specific cancer cell lines, further isolation and purification of bioactive compounds from *S. jomyi* are required.

Conclusion

The present study supports the conventional use of plants for therapeutic purposes and provides preliminary evidence that medicinal plants may possess antimicrobial, anthelmintic, insecticidal and cytotoxic activities. The findings indicate that the leaves, stem and root of *S. jomyi* exhibit variable biological activities, with measurable antimicrobial, anthelmintic and cytotoxic effects, while insecticidal activity was predominantly observed in leaf extracts. The presence of secondary metabolites of therapeutic relevance was further substantiated by GC–MS analysis of *S. jomyi*, suggesting a possible contribution of these compounds to the observed bioactivities. As a first comprehensive report documenting the antimicrobial, anthelmintic, insecticidal and cytotoxic potential of *S. jomyi*, this study provides a baseline for future research. However, the results are based on preliminary *in vitro* assays using crude extracts and therefore, conclusions regarding direct pharmaceutical or agricultural applications should be interpreted with caution. Further investigations involving extract standardisation, isolation of active compounds, detailed dose–response studies and in vivo validation are necessary to establish efficacy, safety and practical applicability.

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Authors' contributions

AS and JX carried out the experiments, processed the results and wrote the paper. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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