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Research Article
Comprehensive Phytochemical, Anti-Oxidant and GC-MS Analysis of *Strobilanthes jomyi* P. Biju, Josekutty, Rekha & J.R.I.Wood

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Abstract

Background and Objective: Plant-based medication is one of the most established practices in the Indian medical field. Earlier, raw parts of plants were directly used to treat many health conditions. Later, the most valuable part was identified, separated the chemical compounds and treated various diseases. The plant *Strobilanthes jomyi* belongs to the family Acanthaceae, commonly called Elathumpadi. The study aimed to evaluate the physicochemical, mineral composition, phytochemical, anti-oxidant and GC-MS analysis of leaves stem and root of *S. jomyi*. **Materials and Methods:** Different vegetative parts of *S. jomyi* were extracted with the Soxhlet extraction method by using methanol as solvent. Physicochemical, phytochemical, mineral composition, anti-oxidant and GC-MS analyses were evaluated by different standard protocols. **Results:** The phytochemical analysis revealed that leaves contained more phenolic (87.4±0.44 mg g^{-1} of GAE), flavonoid (66.23±0.53 mg g^{-1} equivalent of QE), carbohydrate (44.7±1.28 mg g^{-1} of fresh weight), protein (17.7±0.76 mg g^{-1} of fresh weight), proline (46.8±0.15 mg g^{-1} of fresh weight) and chlorophyll (46.8±0.15 mg g^{-1} of fresh weight) content than the root and stem of methanolic extract. The non-enzymatic anti-oxidant assays of the methanolic extract showed the presence of higher anti-oxidant activities in leaves, followed by root and stem. The GC-MS study of the root, stem and leaves revealed medicinally important bioactive compounds like 2,4-di-tert-butyl phenol, phytol, squalene, phenol, neophytadiene and lupeol. **Conclusion:** *Strobilanthes jomyi* can be used as an alternative source of the ayurvedic system of medicine based on its phytochemical and antioxidant activity.

Key words: Phytochemistry, anti-oxidant assay, GC-MS analysis, physicochemical activity, mineral analysis, flavonoids, analgesic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

India is famous for its traditional therapeutic practices and about 8,000 medicinal plants commonly used for Ayurveda, Unani and Siddha systems of medicines[1]. Seventy percent of the rural Indian population of India still depends upon the traditional ayurvedic system of medicine. Plant-based medical practice is crucial and standard in the Indian medical field. There are 2,50,000 registered ayurvedic medicinal practitioners compared to 7,00,000 modern medicines, yet, very few of them are studied chemically and pharmacologically for the therapeutic values of plants[2].

Phytochemicals like phenolics, saponins, alkaloids, tannins, terpenoids, steroids and flavonoids are considered to be of therapeutic value and are also in high demand in industries. Hence it is vital to conduct phytochemical analysis to identify these compounds[1].

The members of the *Strobilanthes* genus are one of the economically important genera in the environment and are used in the Indian system of medicine[3]. Strobilanthe's name is derived from the Latin words "Strobilos", which means cone and "anthos", which means flower or shoot[4]. Strobilanthes blume is the second most diverse genera in the Acanthaceae family. Around 150 *Strobilanthes* species have been reported in India. Out of that, 62 species are from the Southern Western Ghats of South India[5].

Strobilanthes jomyi was recently identified and provincially known as Elathumpadi and comes under the family Acanthaceae. *S. jomyi* is an endemic species of the Western Ghats and is only observed in the Pandy and Periya localities of the Kasaragod district of Kerala, India. However, this species is also reported in another Western Ghats area of Karnataka. The flowering of *S. jomyi* is also like other "kurinji" species having certain flowering intervals. The flowering intervals of this particular species are still unknown. The new species, *S. jomyi,* is named after Dr. Jomy Augustine, Professor in Botany, St. Thomas College Pala, Kerala, India, who studied *Strobilanthes* species for thirty years[6].

S. jomyi is an anisophyllous shrub that can grow up to 5 m tall. Leaves are simple, ovate to elliptic, acuminate, margins are crenate in shape and the leaves base is oblique. The inflorescence is branched axillary spikes. The calyx is 5 lobed and white. Corolla is 14-16 mm in length, bluish-white in colour and contains a basal tube. The fruit is hairy with glandular and non-glandular hairs, showing deep purple coloration[6].

S. jomyi shows the morphological similarity between *Strobilanthes ciliatus* and *Strobilanthes ixiocephala*[6]. Vegetative parts of *S. ciliatus* are exclusively utilized in the Indian medical field because they contain a good number of medicinal properties like anti-inflammatory, analgesic, anti-microbial, antifungal, anti-diabetic, etc. Kurinji kuzhambu is a medicinal preparation from *S. ciliatus* used to treat pregnancy-related issues[3]. Phytochemical screening of the leaves and stem of *S. ciliatus* shows the presence of alkaloids, saponins, glycosides, flavonoids, steroids, sterols, carbohydrates, tannins and terpenoids[7]. The GC-MS study confirmed the existence of medicinally essential secondary metabolite lupeol, which acts as an anti-inflammatory, antitumor, antiprotozoal and antimalarial agent[3].

The objectives of the present study were to analyze the mineral composition, physicochemical characters, phytochemical analysis and anti-oxidant assay and to find out the bioactive compounds by GC-MS analysis of leaves, stems and roots.

MATERIALS AND METHODS

Study area: The plants were collected from Pandi, Kasaragod District of Kerala, India (latitude-12.528705, longitude-75.213831) in December, 2020. The plants were then raised in the greenhouse inside the university throughout this study from January to August, 2021. The plants are still maintained for future studies and analysis.

Identification of plant material: The plant was authenticated and identified by Biju. P. Assistant Professor, Department of Botany, Government College Kasaragod, Kerala. The voucher specimens (Voucher No. FRLH-123445) were deposited at FRLHT Bengaluru.

Preparation of plant extract: Fresh leaves, stems and roots of *Strobilanthes jomyi* were cleaned with tap water followed by distilled water. The cleaned parts were shade dried at room temperature. Then the dried samples were powered by a mechanical grinder and stored in an airtight container[8].

Methanolic extraction: Shade-dried leaves, stems and roots of *S. jomyi* were extracted with methanolic solvents by Soxhlet apparatus for 9 hrs. The solvents were vaporized using a rotary evaporator under a vacuum at 37°C. Later the extracted plant materials were stored in sterile falcon tubes in cold conditions for future studies[8]. In qualitative analysis, 2 g of dried samples were dissolved in 20 mL of methanol and sieved through Whatman No. 1 filter paper. The final extract was used for further qualitative tests[9].

Physicochemical characters

Total ash value: A cleaned silica crucible was heated for about half an hour in an electric muffins furnace until it became red hot and cooled in a desiccator. The empty weight of the silica crucible was then taken. A total of 3 g of leaves, stem and root powders were taken separately in the silica crucible and kept in an electric muffins furnace (R.V Lab Care Technologies, Karnataka, Bengaluru, India) at 600-625°C for 2 hrs. Once the powdered plant material was turned to carbon-free ash, the crucible was then kept in a desiccator to cool and the weight of the carbon-free ash was noted down to find out the total ash content[10].

Acid insoluble ash: The carbon-free ash acquired from the total ash was boiled with 25 mL of 2 M hydrochloric acid for about 5 min. Then insoluble ash was filtered using ashless filter paper, which was then cleaned with regular hot water to remove the traces of acid content and ignited in electric muffins furnace at 450°C for 1 hr. Finally, the crucible was cooled in a desiccator (Borosil, Mumbai, India). The weight of the sample was taken to obtain the percentage of acid-insoluble ash[10].

Water-soluble ash: The ash acquired from the total ash was heated with 25 mL of distilled water with continuous stirring for 5 min. The filtrate was then transferred through ashless filter paper and ignited in electric muffins furnace at 450°C for 1 hr. Crucible was then cooled and weighed the sample to obtain the percentage of water-soluble ash[11].

Determination of moisture content: A total of 0.5 g of powdered sample was heated at 105°C till all the moisture content was removed. The powdered materials were then placed in a Shimadzu-unibloc moisture analyzer (Shimadzu Corporation, Kyoto, Japan) to analyze the sample's moisture content[12].

Mineral assay using atomic absorption spectroscopy: About 0.5 g of plant sample was mixed with 2 mL of 65% HNO_3 and 30% of 1M H_2O_2 and incubated overnight at room temperature. The samples underwent an acid digestion process at 220°C for 5 min using Whatman No. 1 filter paper. Already made up (distilled water) samples were then filtered and determined the mineral content using Shimadzu atomic spectrometer-AA-6880 (Shimadzu Corporation, Kyoto, Japan)[13].

Qualitative estimation of phytochemical analysis

Detection of alkaloid

Dragendorff's Test: Almost 3 mL of crude extract was mixed with 1 mL of dilute hydrochloric acid followed by 1-2 mL of Dragendorff's reagent. Orange-brown precipitation indicated the presence of alkaloids[14].

Detection of flavonoid

Ammonia Test: A total of 5 mL of diluted ammonia solution was added with 3 mL of extract and 1 mL of concentrated H_2SO_4. Yellow colure indicated the presence of flavonoids[15].

Detection of saponin: The amount of 1 mL of plant extract was mixed with 2 mL of distilled water and a few mL of sodium bicarbonate solution. The appearance of stable honeycomb froth after vigorous shaking indicated the presence of saponins[16].

Detection of tannin: A total amount of 0.5 mL of 5% lead acetate solution was treated with 2 mL of plant extract. The white colour revealed the presence of tannins[17].

Detection of terpenoids: Detection of terpenoids with slight modification. A total of 5 mg of plant extract was mixed with 5 mL of methanol followed by 2 mL of chloroform. The whole solution was then warmed and cooled. About 1 mL of the concentrated H_2SO_4 was added to the test tube. The reddish-brown colour in the interface revealed the existence of terpenoids[17].

Detection of steroids: About 1 mL of chloroform was treated with 1 mL of extract, followed by concentrated H_2SO_4. The appearance of brownish colour shows the presence of steroids[18].

Detection of resins

Turbidity Test: In 1 g of dried plant power, 10 mL of distilled water was added and ultrasonicated for 15 min at 30. The mixture was then filtered, the formation of turbidity indicated the existence of resin content[19].

Detection of phlobatannin: A few drops of 1% HCl were added with 2 mL of plant extract. The formation of red precipitation reveals the existence of phlobatanni[8].

Detection of carbohydrate

Benedict's Test: About 1 mL of test solution was suspended with 1 mL of Benedict reagent and boiled for 2-3 min. Red, green or yellow colour denotes the existence of reducing sugar[20].

Detection of protein and amino acids: A total of 2 mL of extract was mixed with 1-2 drops of freshly prepared Ninhydrin reagent and heated for 2-3 min. The development of blue colour denotes the presence of amino acids, proteins, or peptides[20].

Quantitative estimation of phytochemical analysis

Total phenol estimation: Total Phenolic estimation was determined by 96 well-microtiter plates using Folin-Ciocalteu Method with slight changes. Maximum 25 µL of leaves, stem and root extracts of (1 mg mL^{-1}) *S. jomyi* were diluted with 75 µL of water and mixed with 25 µL of 1:1 diluted Folin-Ciocalteu Reagent. Later 96 -well microplate was shaken for about 1 min and allowed to stand for 4 min. The 100 µL of 5% (w/v) Na_2CO_3 solution was also added after 4 min of incubation and then shaken for about 1 min at a constant speed. After 2 hrs of incubation, absorbance was taken at 700 nm. The results were expressed as mg GAE/g[21].

Total flavonoid estimation: The total flavonoid content of *S. jomyi* leaves, stem and roots was estimated by an aluminium chloride assay with minor changes. About 50 µL of extract were treated with 150 µL of 100% methanol, followed by 10 µL of 10% aluminium chloride and 10 µL of 1M sodium acetate. At room temperature, the reaction mixture was kept in dark conditions for 40 min and absorbance was taken at 415 nm (using a microtiter plate). The results were expressed as mg QE/g[21].

Total carbohydrate estimation: Estimation of the total carbohydrate content of roots, stem and leaves of *S. jomyi* was done by the phenol-sulphuric acid method with minor modification. About 0.1 g of crushed fresh plant material was taken in a boiling tube and treated with 2.5 N of 5 mL HCl. The boiling tube was incubated in the water bath at 100°C and cooled to room temperature. Sodium carbonate was then added until saturation point is obtained. Distilled water was used to make up the volume to 25 mL. Then the reaction mixture was centrifuged for 10 min at 10000 rpm. A total of 100 µL of supernatant was taken and treated with 100 µL of 5% phenol, followed by 500 µL of 96% H_2SO_4. The test tubes were then kept in a water bath for 20-30 min and vortexed for 10 min. Absorbance was taken at 490 nm. D-Glucose was taken as a standard and expressed as mg g^{-1} of fresh weight of the samples[22].

Total protein estimation: The total Protein Content of *S. jomyi* was estimated by Lowry's Method with slight modification. A total of 0.5 g of fresh leaf, stem and root of *S. jomyi* were ground with 10 mL of buffer solution (0.2 M of NaH_2PO_4 and 0.2 M of Na_2HPO_4 at a pH of 7). After centrifugation, the supernatant was collected. The 100 µL of the sample was treated with 1000 µL Reagent C (50 mL of Reagent A was prepared using 2% Na_2CO_3 was added to 0.1 N of NaOH and 1 mL of Reagent B was prepared using 1% Potassium sodium tartrate was added with 0.5% $CuSO_4$). The entire mixture was vortexed and allowed to stand for 10 min. The reaction mixture was incubated in dark after adding 100 µL of 1:1 Folin-Ciocalteu Reagent (Reagent D). Using a microtiter plate, the absorbance was read at 660 nm. Bovine Serum Albumin was taken as standard for the assay and results were expressed as mg g^{-1} of fresh weight of the samples[22].

Total proline estimation: In the experiment, 1 g of fresh sample (fresh leaves, stem and roots, respectively) was ground with 10 mL of sulphosalicylic acid (3%) and the filtrate was taken for further use. About 2 mL of the filtrate was allowed to react with 2 mL of glacial acetic acid and freshly prepared acid Ninhydrin. It was then kept in a water bath at 100°C for 1 hr. The tubes were placed in an ice bath to arrest the reaction process. To the reaction mixture, 4 mL of toluene was poured and stirred for 30 sec. In the microtiter plate, the coloured toluene layer was taken and absorbance was read at 520 nm. Standard was taken as proline and results were then expressed as mg g^{-1} of fresh weight of the samples[22].

Total chlorophyll estimation: The total chlorophyll of *S. jomyi* (fresh leaves, stem and root) was determined by the acetone method[22]. The test solution was prepared using 1 g of the fresh sample which was pulverized with 20 mL of 80% acetone. The mixture was centrifuged for 5 min at 5000 rpm. The supernatant was separated from the pellet. The same procedure was continued 2-3 times until the pellet became colourless with 80% acetone. The volume of supernatant was made up to 30 mL. Absorbance was taken at 645,652 and 663 nm, respectively, by using a microtiter plate. About 80% acetone was used as a blank[22]. Results were then expressed as mg g^{-1} of fresh weight of the samples[22]:

$$\text{Chlorophyll a} = 12.7\,(AB_{663}) - 2.69\,(AB_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9\,(AB_{645}) - 4.68\,(AB_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = 20.2\,(AB_{645}) + 8.02\,(AB_{663}) \times \frac{V}{1000 \times W}$$

Where:

AB = Absorbance
V = Final volume of test solution
W = Initial weight of the fresh sample

Determination of anti-oxidant activities: The antioxidant activity of the plant extracts was estimated by non-enzymatic and enzymatic methods like DPPH (2,2 -diphenyl-1-picrylhydrazyl), phosphomolybdate assay, Ferric reducing power assay and metal chelating assay.

Determination of antioxidant activity by DPPH radical scavenging assay: Estimation of antioxidant activity of the leaves, stem and root methanolic extract of *S. jomyi* was estimated by the revised DPPH (2,2 -diphenyl-1-picrylhydrazyl) procedure. About 500 µL of methanolic plant extract was made up of 2.5 mL of methanol followed by 1 mL of 0.004% (0.002 g in 100 mL of methanol) DPPH solution and kept in dark condition for 30 min. The wavelength was read at 517 nm by using a UV-VIS spectrophotometer-1800 (Shimadzu Corporation, Kyoto, Japan). Methanol was taken as a blank and ascorbic acid was used as standard[23]. The antioxidant activity was calculated as[23]:

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

where, $X_{control}$ and X_{sample} are the absorbance of control without plant extract and sample with plant extract, respectively.

Determination of antioxidant activity by phosphomolybdate assay: The antioxidant activity was estimated by the Phosphomolybdate assay. To 0.1 mL of plant extracts, 1 mL of phosphomolybdate reagent was added and incubated for about 90 min in a water bath at 90°C. The whole test solution was then cooled at room temperature and absorbance was measured at 695 nm using a UV-VIS spectrophotometer. About 0.1 mL of methanol and 1 mL of phosphomolybdate reagent mixture were taken as a blank followed by ascorbic acid taken as standard. Equal volumes of 28 mM Na_3PO_4, 4 mM $(NH_4)_2MoO_4$ and 0.6 M H_2SO_4 were used for the preparation of Phosphomolybdate reagent[23].

Determination of antioxidant activity by (FRAP) ferric reducing power assay: The antioxidant activity was estimated by FRAP assay. A total of 1 mL of methanolic extracts of leaves, stem and root were suspended with 2.5 mL of phosphate buffer (0.2 M at pH of 6.5), followed by 2.5 mL of 1% ferricyanide. The final reaction mixture was then enveloped with foil paper and kept in a water bath for 20 min at 50°C. After 20 min, 2.5 mL of 10% TCA (trichloroacetic acid) was added and centrifuged at 3000 rpm for 10 min. Later 2.5 mL of supernatant was collected and suspended with 2.5 mL of distilled water, followed by 0.5 mL of $FeCl_3$. The wavelength was measured at 700 nm by using a UV-VIS spectrophotometer. Methanol was taken as blank and ascorbic acid as standard[23].

Determination of antioxidant activity by metal chelating assay: The non-enzymatic anti-oxidant assay was performed by metal chelating assay with slight modification. A total of 1 mL of methanolic plant extract was mixed with 1 mL of 0.1 mM $FeSO_4$ followed by 2 mL of 0.25 mM of ferrozine. After 10 min of incubation, absorbance was measured at 562 nm using a UV-VIS spectrophotometer. Methanol was taken as standard and in addition to methanol, a remaining reagent was used as control[24]:

$$\text{Metal chelating activity (\%)} = \frac{Z_{control} - Z_{sample}}{Z_{control}} \times 100$$

where, $Z_{control}$ and Z_{sample} are the absorbance of control without plant extract and sample with plant extract, respectively.

Gas chromatography-mass spectrometry analysis

Extract preparation: Extract prepared for GC-MS analysis with slight modification. As 1 g of powdered samples of leaves, stem and roots of *S. jomyi* was immersed with 10 mL of chloroform and kept for three days for incubation. The extract was filtered and evaporated at dryness. After evaporation, the solid extracts were transferred to vials and mixed with 0.5 mL of chloroform, respectively[25].

Gas chromatography and mass spectrometry analyses of the chloroform of *S. jomyi* were carried out in the Shimadzu GC-MSQP2010SE system. The mass spectrometer consisted low polarity phase: Crossbond™ Silarylene Phase 1,4 bis (dimethylsiloxysil) phenylene dimethyl polysiloxane with ID:025 mm and 0.25 µDF, length 30 m Column. The column temperature was maintained at a maximum of 280°C throughout the analysis and the helium gas flow rate was at 1.25 mL/min. The ionization voltage was at 70 eV. The mass spectrum was obtained in the range of 1.5-1000 m/z. The peaks of GC-MS compounds in plant extracts were compared with the standard MS (NIST 2017) data library.

Statistical analysis: The statistical analysis of the present study was analyzed by SPSS statistics software version 22. The results were interpreted by One-way Analysis of Variance (ANOVA) to verify the significance by using Tukey's Test by the probability less than $p \leq 0.05$.

RESULTS

Physicochemical characters: The results of physicochemical characteristics like acid-insoluble ash, water-soluble ash, total ash value and moisture content were represented in Table 1. The total ash values and water-soluble ash values decreased in the order stem>leaves>root. Similarly, acid-insoluble ash values were higher in leaves, then stem, followed by root. The moisture content of the samples was observed to be higher in leaves than in roots followed by the stem.

Mineral assay using atomic absorption spectroscopy: Micro and Microelements of vegetative parts like stem, root and leaves of *S. jomyi* were shown in Table 2. The Ca content was observed to be higher in concentration in different vegetative parts of *S. jomyi.* The number of minerals was in the order Mg>Fe>Zn>Mn>Cu>Pb>Cd, respectively.

Qualitative phytochemical analysis: The preliminary qualitative phytochemical study of *S. jomyi* in different vegetative parts of methanolic extracts was presented in Table 3. The methanolic extract of leaves exhibited the existence of saponins, tannins, alkaloids, steroids, phlobatannin, carbohydrates, flavonoids, proteins, amino acids, terpenoids and resins tested negative in the methanolic extract of leaves. Similarly, qualitative estimation of methanolic stem extract also revealed the presence of tannins, flavonoids, carbohydrates, alkaloids, proteins, saponins and amino acids. The root extract of methanolic showed the existence of alkaloids, flavonoids, saponins, carbohydrates, proteins and amino acids. In contrast, resins showed a negative result in methanolic extracts of leaves, stems and roots.

Quantitative phytochemical analysis: The total phenolic, flavonoid, carbohydrate, protein, chlorophyll and proline Content of the methanolic extract of leaves, stem and root of *S. jomyi* were shown in Table 4-6. The studies revealed that leaves (87.43±0.44 mg GAE/g) contain more phenols than stem (5.89±1.77 mg GAE/g) and root (73.33±1.17 mg GAE/g). Similarly, flavonoid content was also observed to be higher in leaves (66.23±0.530 mg QE/ g), followed by root (12.02±0.30 mg QE/g) and stem (12.02±0.30 mg QE/g). Total carbohydrate content in fresh samples of leaves (44.75±1.28) expressed a higher value than root (36.48±0.98) and stem (24.82±2.27). Moreover, protein analysis revealed a higher quantity in leaves (17.78±0.76 mg g^{-1} of fresh weight) compared to the root (10.38±0.26 mg g^{-1} of fresh weight) and stem (5.15±0.34 mg g^{-1} of fresh weight). The statistically significant difference in total phenol, flavonoid and carbohydrate-protein content was observed between leaves, root and stem. Similarly, the statistically significant difference in total proline content was observed to be higher in leaves (46.83±0.15 mg g^{-1} of fresh weight) followed by the stem (21.15±2.47 mg g^{-1} of fresh weight) and root (13.68852±0.00 mg g^{-1} of fresh weight). And even the same trend was observed in total chlorophyll content in a different part of *S. jomyi* in leaves (2.76±0.02 mg g^{-1} of fresh weight), then Stem (0.20±0.001 mg g^{-1} of fresh weight) followed by root (0.04±0.006 mg g^{-1} of fresh weight).

Anti-oxidant activity: The anti-oxidant assay in different vegetative parts of *S. jomyi* was estimated by DPPH, ferric reducing power assay, phosphomolybdate method and metal chelating assay (Table 7). The methanolic extract of *S. jomyi* leaves (83.64±0.46%) showed a higher percentage of anti-oxidant activities by DPPH assay than root (77.70±0.46%) and stem (21.26±0.17). Although industrially available the ascorbic acid standard showed higher anti-oxidant activity (96.31±0.001%) than plant samples. Anti-oxidant activity by ferric reducing power assay showed higher anti-oxidant activity in standard ascorbic acid (0.82±0.007 nm) by comparing with the samples like leaves, stem and root. Leaves (0.39±0.004 nm) showed higher values then followed by root (0.22±0.002 nm) and stem (0.09±0.001 nm). Phosphomolybdate assay also showed higher anti-oxidant activity in leaves (180.28±1.95 µg mg^{-1} of extract) than root (150.90±6.84 µg mg^{-1} of extract) and stem (27.17±5.95 µg mg^{-1} of extract). Non-enzymatic assay like metal chelating assay revealed higher anti-oxidant potential in leaves (64.78±0.40%) then root (42.20±0.32%) and stem (33.70±0.32%). Statistical analysis of different anti-oxidant assays revealed significant differences between leaves, roots and then the stem.

Gas chromatography-mass spectrometry analysis: The volatile secondary metabolite present in the root, stem and leaves of *S. jomyi* was identified by GC-MS analysis (Table 8). The chromatograms of each vegetative part of the chloroform extract were expressed in Fig 1-3. Bioactive compounds like 2,4-di-tert-butylphenol, phytol, squalene, neophytadiene, chloromethyl 5-chloroundecanoate and Lupeol were the most common secondary metabolites present in the chloroform extract. The retention time, molecular weight, molecular formula, percentage of area and compound name of secondary metabolites present in *S. jomyi* were presented in Table 6.

Table 1: Physicochemical characters of leaves, stem and root of *Strobilanthes jomyi*

Part used	Total ash value (%)	Water soluble ash (%)	Acid insoluble ash (%)	Moisture content (%)
Leaves	22.21±0.2516	3.33±0.024	2.18±0.470	11.43±0.25
Stem	26.12±0.050	11.72±0.037	1.85±0.069	9.41±0.42
Stem	19.50±0.056	2.94±0.260	2.17±0.050	10.13±0.17

Results are means of triplicates (Mean±SD)

Table 2: Mineral assay in leaves, stem and root of *Strobilanthes jomyi*

Types of elements	Samples	Concentration in PPM
Macro-elements		
Ca	Leaves	46.52±0.29
	Stem	43.67±0.46
	Root	46.05±0.48
Mg	Leaves	1.38±0.001
	Stem	1.35±0.001
	Root	1.29±0.000
Fe	Leaves	1.32±0.006
	Stem	0.542±0.006
	Root	2.825±0.008
Zn	Leaves	0.757±0.006
	Stem	0.809±0.004
	Root	0.796±0.001
Micro-elements		
Cu	Leaves	0.118±0.006
	Stem	0.062±0.002
	Root	0.175±0.003
Mn	Leaves	0.147±0.002
	Stem	0.536±0.001
	Root	0.666±0.003
Pb	Leaves	0.086±0.019
	Stem	0.098±0.021
	Root	0.068±0.020
Cd	Leaves	0.0008±0.00
	Stem	0.001±0.000
	Root	0.002±0.000

Results are means of triplicates (Mean±SD)

Table 3: Qualitative estimation of methanolic extract of *Strobilanthes jomyi*

Phytochemical	Leaves	Stem	Root
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponin	+	+	+
Tannin	+	+	+
Terpenoids	-	-	-
Steroids	+	-	-
Resin	-	-	-
Phlobatannins	+	-	-
Carbohydrate	+	+	+
Protein and amino acids	+	+	+

+: Present and -: Absent

Table 4: Phenolic and flavonoid content in methanolic extract of leaves, stem and root of *Strobilanthes jomyi*

Part used	Solvent	Total phenolic content (mg g^{-1} of GAE)	Total flavonoid content (mg g^{-1} the equivalent of QE)
Leaves	Methanol	87.4±0.44^{a}	66.23±0.53^{a}
Stem	Methanol	5.89±1.77^{c}	3.04±0.08^{c}
Root	Methanol	73.3±1.17^{b}	12.02±0.30^{b}

Results are means of triplicates (Mean±SD), significantly different among the samples at $p \leq 0.05$ and upper case letters denote the significant differences

Table 5: Total carbohydrate, protein, and proline content in leaves, stem and root of *Strobilanthes jomyi*

Part used	Total carbohydrates (mg g^{-1} of fresh weight)	Protein (mg g^{-1} of fresh weight)	Proline (µg g^{-1} of fresh weight)
Leaves	44.7±1.28^{a}	17.7±0.76^{a}	46.8±0.15^{a}
Stem	24.8±2.27^{c}	5.15±0.34^{c}	21.1±2.47^{b}
Root	36.4±0.98^{b}	10.38±0.26^{b}	13.6±0.00^{c}

Results are means of triplicates (Mean±SD), significantly different among the samples at $p \leq 0.05$ and upper case letters denote the significant differences

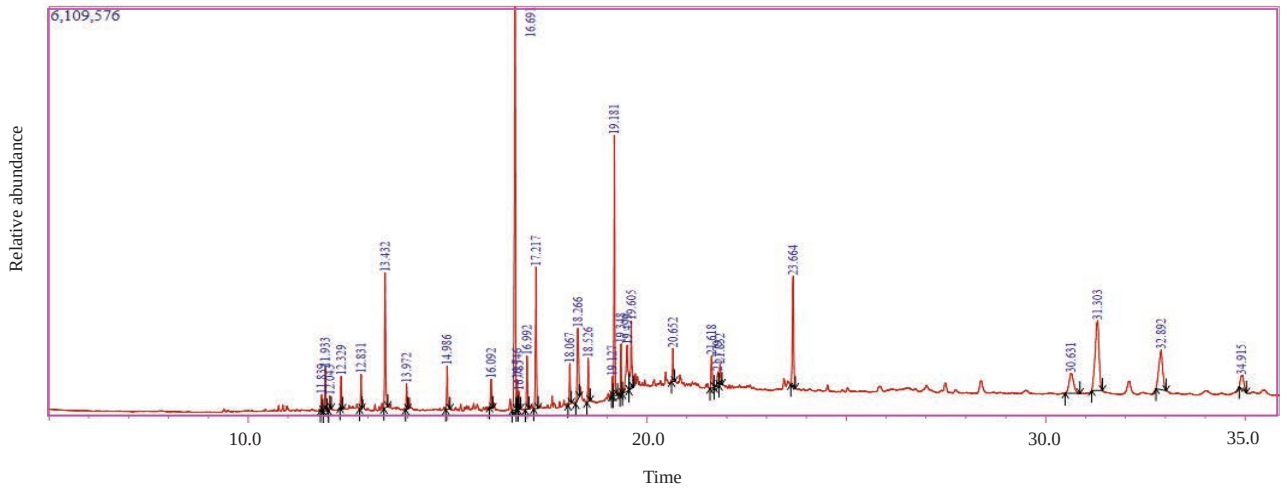

Fig. 1: GC-MS chromatogram of *Strobilanthes jomyi* chloroform leaves extract

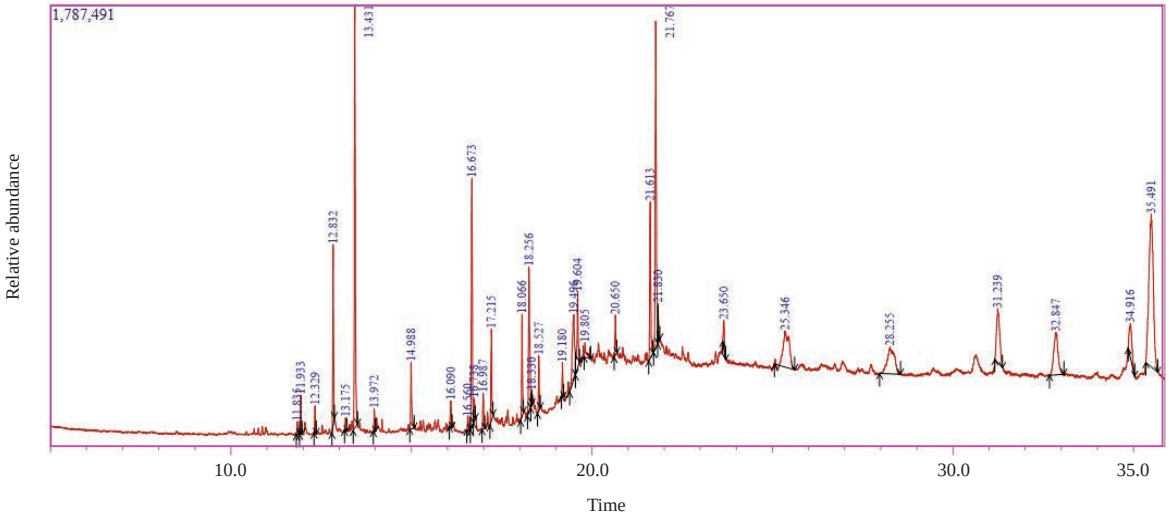

Fig. 2: GC-MS chromatogram of *Strobilanthes jomyi* chloroform stem extract

Table 6: Total chlorophyll content in leaves, stem and root of *Strobilanthes jomyi*

Part used	Total chlorophyll content (mg g^{-1} of fresh weight)
Leaves	2.76±0.024[a]
Stem	0.20±0.001[b]
Root	0.04±0.006[c]

Results are means of triplicates (Mean±SD), significantly different among the samples at $p \leq 0.05$ and upper case letters denote the significant differences

Table 7: Anti-oxidant activity in *Strobilanthes jomyi* leaves, stem and root

		Samples		
Name of the assay	Unit expressed as	Leaves	Stem	Root
DPPH	Percentage	83.64±0.46[a]	21.26±0.17[c]	77.70±0.46[b]
FRAP	Absorbance	0.39±0.004[a]	0.09±0.001[c]	0.22±0.002[b]
Phosphomolybdate	µg mg^{-1} of extract	180.28±1.95[a]	27.17±5.95[c]	150.90±6.84[b]
Metal chelating	Percentage	63.76±0.40[a]	33.70±0.32[c]	42.20±0.32[b]

Results are means of triplicates (Mean±SD), significantly different among the samples at $p \leq 0.05$ and upper case letters denote the significant differences

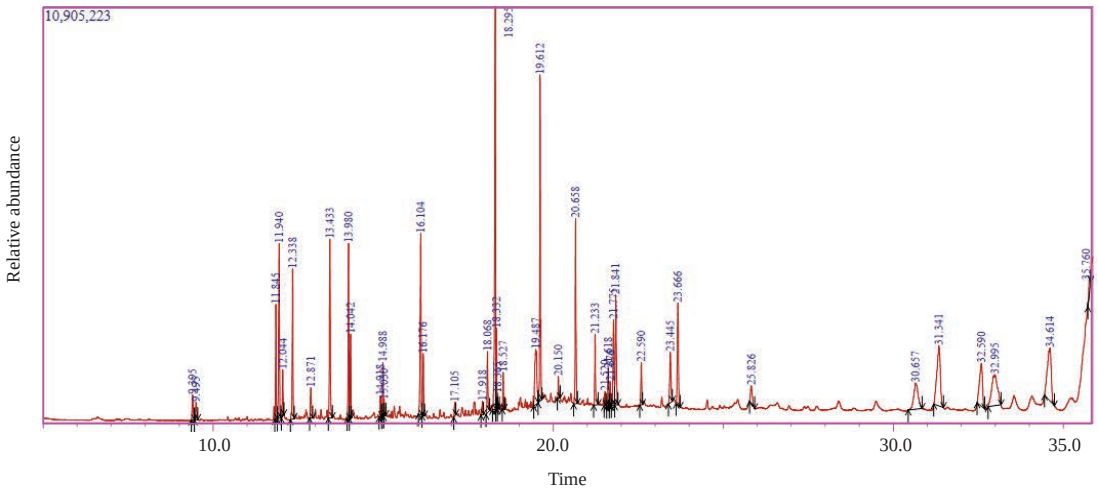

Fig. 3: GC-MS chromatogram of *Strobilanthes jomyi* chloroform root extract

Table 8: GC-MS analysis of *Strobilanthes jomyi* leaves, stem and root of chloroform extract

Sl. No.	Name of compounds	Area (%)	RT	Molecular formula	Molecular weight	Part used	Solvent
1	Phytol	8.08	19.180	$C_{20}H_{40}O$	296	Leaf	Chloroform
2	3,7,11,15-Tetramethyl 2-hexadecen-1-ol	5.84	17.215	$C_{20}H_{40}O$	296	Leaf	Chloroform
3	2,4-di-tert-butylphenol	5.52	13.430	$C_{14}H_{22}O$	206	Leaf	Chloroform
4	Squalene	5.78	23.665	$C_{30}H_{50}$	410	Leaf	Chloroform
1	Lupeol	17.06	35.490	$C_{30}H_{50}O$	426	Stem	Chloroform
2	2,4-di-tert-butylphenol	10.30	13.430	$C_{14}H_{22}O$	206	Stem	Chloroform
3	Neophytadiene	6.64	16.675	$C_{20}H_{38}$	278	Stem	Chloroform
4	Chloromethyl5-chloroundecanoate	9.89	21.765	$C_{12}H_{22}C_{l2}$	268	Stem	Chloroform
5	n-Hexadecanoic acid	4.45	18.255	$C_{16}H_{32}O_2$	256	Stem	Chloroform
1	1-Heptadecene	10.04	18.295	$C_{17}H_{34}$	238	Root	Chloroform
2	1-Docosene	6.64	19.610	$C_{22}H_{44}$	308	Root	Chloroform
3	1-Octadecene	4.94	16.105	$C_{18}H_{36}$	252	Root	Chloroform
4	Cetene	3.19	13.980	$C_{16}H_{32}$	224	Root	Chloroform
5	2,4-di-tert-butylphenol	4.38	13.430	$C_{14}H_{22}O$	206	Root	Chloroform

DISCUSSION

The present study focused on the qualitative and quantitative profiles of the physicochemical characters, micro and macro elements, phytochemicals, anti-oxidants and GC-MS analyses of the leaves, stem and root of *Strobilanthes jomyi*.

Ash values were used to detect purity, quality of impurities, adulteration and authenticity of the samples. The water-soluble ash values express the major inorganic component present in the sample. Estimation of the acid-insoluble ash value was used to determine the amount of silica. The minimal amount of these three parameters represents a smaller number of inorganic substances and silica[26]. The current study proved that the total ash value of *S. jomyi* stem showed the highest percentage followed by leaves and roots. Compared with the available data on *Strobilanthes ciliatus* total ash value of stem showed a higher value (15.4%) and leaves (12.3 %) which were a much lesser ash value than the *S. jomyi*. The water-soluble ash value of *S. ciliatus* were 6.1% for leaves and 8.2% for the stem. Similarly, the acid insoluble ash value was 2.2% for leaves and 2.4% for the stem in *S. ciliatus*[7]. The previous studies on *Acanthus montanus* (Nees) T. Anderson leaves which also belongs to the Acanthaceae family disclosed an abundant amount of moisture content compared to *S. jomyi* leaves[27]. Higher moisture content leads to higher enzymatic activity, which initiates the disintegration of specific phytochemical substances[13].

Mineral assay of *S. jomyi* revealed the presence of micro and macro elements. Calcium was higher in concentration and the least was cadmium. Another Acanthaceae member *Andrographis echioides* (L.) Nees. also revealed a higher concentration of calcium (2203 mg/100 g) and the least of Ag (0.01 mg/100 g)[28]. However, *S. jomyi* showed a good amount of these elements than *A. echioides*. Elements like calcium improve the development of bones and contraction of muscles and help with the transmission of nerve impulses[29].

The Mg can turn on enzymes and control various concomitants such as blood pressure, glycemic control, myocardial contraction, etc.[30]. It can also help in the synthesis of hemoglobin and myoglobin[31]. The Zn is another essential element for DNA synthesis and carbohydrate metabolism[13]. The Cu has a significant role in the development and growth of bones and medullary sheath in the nervous system[29]. The Mn plays a vital role in blood clotting and bone and tissue growth[32]. Heavy metals like Pb and Cd are highly toxic to the human body and categorized as non-essential elements[33]. Because of a good amount of micro and macro elements observed in the *S. jomyi*, vegetative parts can be used in various forms for the deficiency of any elements in the human body.

The qualitative phytochemical analysis is mainly dealing with the presence and absence of phytochemicals. In contrast, quantitative phytochemical analysis is mainly concerned with the quantity and concentration of phytochemicals in the plant. The qualitative phytochemical analysis of *S. jomyi* leaves, stem and root revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, proteins and amino acids. Similarly, phytochemical studies on *S. ciliates* callus revealed the existence of glycosides, saponin, alkaloids, tannins, flavonoids, steroids, terpenoids and phenols in methanolic extract. Whereas ethanolic extract tested negative for glycosides and saponins[1]. A phytochemical test for an ethanolic extract of *S. ciliates* leaf and stem also tested positive for alkaloids, carbohydrates, glycosides, phytosterols, terpenoids, flavonoids and tannins[7]. Recent studies on methanolic extract of the root of *Strobilanthes heyneanus* found the existence of phytochemicals like flavonoids, phenols, terpenoids, saponins, tannins, alkaloids, glycosides and carbohydrates. The report on quantitative phytochemical analysis of *Strobilanthes* species is limited. The available data on *S. heyneanus* showed the highest phenolic (126.53±2.29 μg mg^{-1} of GAE of extract) and flavonoid (32.79±0.62 μg mg^{-1} of QE of extract) content in its roots than *S. jomyi*[17]. The report on *Strobilanthes crispus* leaves also revealed the presence of TPC and TFC content in a different solvent, which was much lesser than the *S. jomyi* leaves of methanolic extract[34]. Protein and carbohydrate content in different Acanthaceae members showed remarkable variation up to the species level[35].

Based on the phytochemical analysis, a higher quantity of phenols and flavonoids can be responsible for the antioxidant activity of the plant. Some of the best naturally occurring anti-oxidant compounds contain vitamin C, vitamin A, carotenoids and vitamin E, which showed a higher amount of anti-oxidant activity[35]. Results proved that the vegetative parts of *S. jomyi* possess anti-oxidant activity in all the enzymatic and non-enzymatic assays. The previous report on the percentage of anti-oxidant activity of *S. heyneanus* methanolic root extract showed the highest amount of anti-oxidant activity in all the assays[17]. Similarly, *S. crispus* leaves revealed the highest percentage of anti-oxidant activity of ethanolic extract ie 5.44±1.76 μmoL L^{-1} in DPPH assay and 12085.7±0.006 for mmoL g^{-1} for FRAP assay[36]. Correspondingly *S. kunthiana* methanolic and ethanolic extract revealed the highest percentage of radical scavenging activities[1]. Ferric-reducing power assay is determined based on the absorbance value. Higher absorbance indicates higher antioxidant activity. Earlier studies have reported that anti-oxidant activities of *S. heyneanus* by ferric reducing power assay using methanolic extract of root showed much more anti-oxidant than *S. jomyi* root extract[17]. Among anti-oxidant studies of all the *Strobilanthes* genus concluded that *S. jomyi* also exhibits a good percentage of anti-oxidant activity in all the vegetative parts. From this study by comparing all four assays, the DPPH assay showed the highest anti-oxidant activity and is an effective protocol for determining anti-oxidant study. In the current scenario, an anti-oxidant study has a vital role in its health benefits. Anti-oxidants are comprised of a large number of chemical compounds that prevent oxidative damage caused by the free radicals in the body[35].

S. jomyi chloroform extract revealed the presence of various medicinally important bioactive compounds by GC-MS analysis. The available report on *S. kunthiana* and *S. crispus* also revealed secondary metabolites like *S. jomyi*[1,37]. Bioactive compounds like 2,4-di-tert-butylphenol have properties like anti-oxidant, insecticidal, nematicidal, antibacterial and cytotoxic activities against HeLa cell lines[38]. Phytol is another aromatic compound with antinociceptive, anti-oxidant, anti-allergic, anti-microbial and anti-inflammatory activities[39]. Squalene is a naturally present polyprenyl compound known for its properties like cholesterol synthesis, which also has anti-oxidant and anti-cancerous potential[40]. Lupeol is one of the significant components of *S. jomyi*. Lupeol is a pentacyclic triterpenoid with numerous anti-microbial, antiprotozoal, anti-cancerous, anti-diabetic, cardioprotective, anti-inflammatory, hepatoprotective, skin-protective and nephroprotective activities[41,42]. The present study is the first report on *S. jomyi*. The application of the plant is highly diverse, having immense medicinal values based on photochemical, antioxidant and GC-MS studies. The prospects of this plant find wide applications in the pharmaceutical industry and also can be used in drug development in the future. *S. jomyi* is a storehouse of several medicinal properties that still haven't been explored yet. It is highly recommended that this plant be looked at as a potential contributor to the medical field.

CONCLUSION

The present study on *S. jomyi* revealed a higher amount of phenols, flavonoids, carbohydrates, proteins, proline and chlorophyll in leaves than in roots and stems. The antioxidant activity was also higher in leaves followed by root and stem. This study concluded that *S. jomyi* can act as a source for many secondary metabolites that benefit humans. The current study claimed that lupeol was one of the significant compounds, which is responsible for many properties like anti-cancerous, cardioprotective, hepatoprotective, anti-microbial, etc. This particular plant can be used for drug development in the future medical field.

SIGNIFICANCE STATEMENT

This study provides immense information on phytochemical, anti-oxidant and GC-MS analysis of various vegetative parts of *Strobilanthes jomyi*. The current study helps future researchers to acquire knowledge on the medicinally important species of the *Strobilanthes* genus and provide insight into drug development and various preparation of ayurvedic formulations based on the quality of different plant parts by different experimental setups.

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