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A pharmacognostic approach, including phytochemical and GC-MS analysis, targeted towards the authentication of *Strobilanthes jomyi* P. Biju, Josekutty, Rekha & J.R.I.Wood

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Abstract

The genera *Strobilanthes* Blume have a rich history in therapeutic culture all over the world. Asian countries like India, China, Myanmar and Thailand still use *Strobilanthes* genus-based medicinal preparations for various diseases. *Strobilanthes jomyi* is a newly discovered species from Kerala, India. Some tribal communities of Kasaragod district still use *S. jomyi* leaf extract as a wound healing medication. The current study aims to investigate the pharmacognostic, phytochemical and GC-MS analysis of the leaves, stems and roots of *S. jomyi*. The microscopic, macroscopic, organoleptic, fluorescent, phytochemicals and GC-MS analysis of the leaves, stem, and root of *S. jomyi* were estimated using various standard protocols. The macroscopic and microscopic characters of leaves revealed the presence of non-glandular trichomes with paracytic stomata in the leaves. The transverse section of the stem and petiole showed the presence of raphides and the root showed the presence of tannin cells. Cystoliths were observed only in the petiole. Powder morphology of leaves, stems and roots revealed the presence of fibers, trichomes, palisade cells, spiral xylem vessels, bordered pit vessels and raphides. The vegetative part of *S. jomyi* powder exhibited various fluorescent coloration based on numerous chemical treatments along with different tastes, smells, colors and textures by organoleptic assays. Qualitative phytochemical analysis of different vegetative parts revealed the presence of flavonoids and other phytochemicals. GC-MS study revealed that lupeol a significant bioactive compound was present in all the vegetative parts of *S. jomyi*. The results acquired from this study can be used for the standardization, identification, quality and purity check of plant samples.

Keywords

macroscopic and microscopic study, powder morphology, organoleptic analysis, fluorescence analysis, phytochemical analysis, GC-MS analysis

Introduction

Plants have a rich history all over the world in terms of traditional medicine to treat life-threatening diseases. The plant kingdom is not fully explored to its true potential making them excellent candidates to derive new drugs and help humans to carry on age-old medical practices (1). This knowledge has been transferred by word of mouth from generation to generation. Because of this tradition, there is a massive loss of indigenous knowledge regarding the traditional system of medicine as well as the plant. However, it is crucial to conserve and document such knowledge to prevent the loss of ancient

knowledge of the plant. This also helps to restrict adulterants used in plant-based drugs and medicines that tamper with the authenticity and quality of the drugs, which can harm our health (2).

Strobilanthes Blume is the second-largest genera in the Acanthaceae family; among which, 400-422 species are widely accepted in all over the world (3). In India, around 150 species have been reported; out of that, 61 species are observed in the Western Ghats of southern India (4). The diversity of *Strobilanthes* genera is observed more in Asian countries like India, China, Pakistan, Afghanistan, Japan and Korea (3). *Strobilanthes* genera can be distinguishable from other Acanthaceae members by various floral traits, including a membranous sheath, a bifid stigma with a smaller posterior lobe and 2 clusters or rows of hairs on the inner posterior corolla wall that hold the style. However, the combination of all these characteristics may not be present in all the species (5).

The genus *Strobilanthes* has a valuable place in the traditional system of medicine (6). Various parts of the plants such as leaves, stem and root are used in several ayurvedic formulations. The medicinally important species of this genera include *S. ciliatus*, *S. heyneanus* and *S. cusia* and these species have been used for the past several years either in ayurveda or Chinese system of medicine (7-9). Ayurvedic preparations of root and leaf extracts from *S. ciliatus* (Sahachara) are commonly used to relieve inflammation and pain thus serving as an excellent anti-inflamatory and analgesic agent . They are also known to have anti-cancer, anti-diabetic, anti-microbial and hepatoprotective properties. Reports from other studies also indicate the use of *S. ciliatus* in 'kurinji kuzhambu', an ayurvedic preparation used for the treatment of health issues that arise post pregnancy (7). Similarly, extracts of *S. heyneanus* and *S. kunthiana* are commonly used in ayurvedic preparations for its anti-inflamatory, anti-cancer, anti-diabetic, anti-microbial and analgesic properties and also to treat rheumatic disorders (8, 10) . Besides the use of *Strobilanthes* plants in Ayurveda, the Chinese system of medicine has also used plants of this genus in medicinal preparations. *S. cusia* is used in traditional Chinese medicine to treat fever, hepatitis, pneumonia and meningitis (9).

S. jomyi is a newly discovered species in 2017 located in the Kasaragod district of Kerala. India. *S. jomyi* is named after Dr. Jomy Augustine, Professor of Botany at St. Thomas College Pala in Kerala, India, who spent 30 years studying *Strobilathes* species. Being a plant under a medically important genus, it is highly possible that *S. jomyi* would also share similar medical properties (11). However, in order to continue with such studies, it is necessary to standardize and authenticate them before extensively using them without scientific details, safety, authentication, or efficacy. Hence, the ultimate aim of the study was to evaluate pharmacognostic, qualitative phytochemistry and GC-MS analysis of *S. jomyi* leaves, stem and roots to fill out the research gaps that will lead to the standardization of the plant.

Morphological characters

S. jomyi is a shrub grows about 5 m tall. The leaves are simple, entire, alternate, and spirally arranged with an acuminate apex. The shape of the leaves was ovate-lanceolate, hairy, with a serrated margin, unicostate reticulate venation and a prominent midrib. The adaxial surface of the leaves showed dark green colour than the abaxial surface. The axillary spike inflorescence with numerous prominent bracts and bracteoles, along with white coloured corolla, is the characteristic feature of *S. jomyi*. The androecium is 4, didynamous and exerted with spheroidal pollen. This plant shows very close morphological similarities between *S. ixiocephala* and *S. ciliatus*, like pendulous inflorescence, exerted stamens and thinly hispid style (11).

Materials and Methods

Procurement and authentication

The leaves, stem and root of *S. jomyi* P. Biju, Josekutty, Rekha & JRI Wood were collected from Kasaragod District (latitude-12.528705, longitude-75.213831) of Kerala, India (Fig. 1, Fig. 2). The authentication of the herbarium

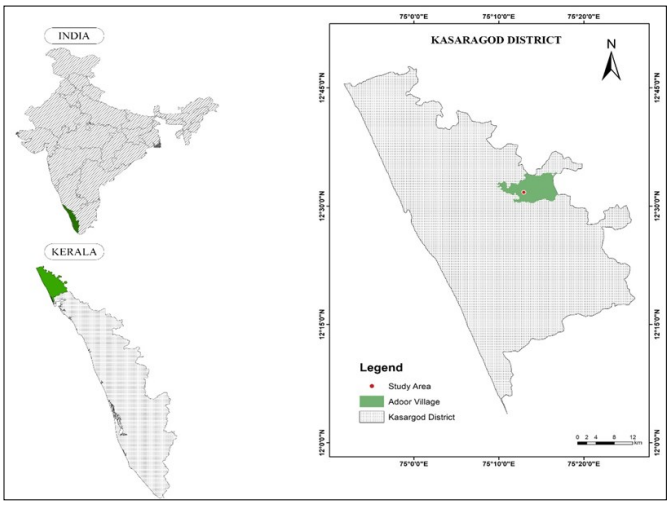


Fig. 1. Showing the map of collected area with geographical co-ordinates (Created using *ArcGis desktop.10.8*).

Fig. 2. Habit of *S. jomyi.*

specimen was done by Biju P, Assistant Professor in Botany, Government College Kasaragod (affiliated to Kannur University). The voucher specimen (Voucher No. FRLH - 123445) was submitted to Foundation for Revitalisation of Local Health Traditions (FRLHT) Bengaluru.

Preparation of plant powder

The vegetative parts of *S. jomyi,* leaves, stem and root materials from the fields were washed with regular tap water followed by distilled water and then shade-dried at room temperature for 2 weeks (in the month of January 2021). The dried samples were then powdered using a mechanical grinder and stored in an airtight container (12).

Macroscopic characteristics

Macroscopic characteristics of leaves and stems were analyzed using visual introspection and magnifying lenses. The examination of root morphology was carried out in an EPSON V850 Pro root scanner using WinRHIZO software (13, 14).

Microscopic evaluation

An anatomical study of leaves, stem, root, petiole and trichomes was carried out by the freehand sectioning method. Each of these thin specimens was then transferred into a safranin stain for 1-2 min. The excess stain was then washed off with distilled water and mounted in glycerin. The thin specimen was then observed through a LEICA DMi8 microscope (15).

Powder microscopy

The dry powder of leaves, stem and root of *S. jomyi* was treated with a few drops of chloral hydrate on the slide and then heated for about 4-5 seconds using a spirit lamp. Later, glycerine was added to the slide to avoid drying the sample. The powder microscopic characters like fibers, trichomes, palisade cells, spiral xylem vessels, bordered pit vessels and raphides were observed through the LEICA DMi8 microscope (13).

Quantitative microscopic analysis of leaves

Estimation of the stomatal index, stomatal size, vein-islet number and vein-termination number was estimated using the epidermal strip of leaf and chloral hydrate treated leaf sample as per the standard protocol using camera lucida and LEICA DMi8 microscope (16).

Organoleptic analysis

The organoleptic analysis is a vital step in identifying the adulterants mixed and assessing the purity level of the plant materials. The sensory evaluation was performed based on the color, taste, size, texture and odor of fresh and dry leaves, stems, and roots of the plant using visual interrogation, standard protocol (16).

Fluorescent analysis

A small amount of leaves, stem and root powder was treated with a few drops of freshly prepared chemical reagent Table 4. Mixtures and kept for 1-2 min. The final reaction mixture was then spread on a slide and observed through the UV-viewer chamber. Based on the visible spectrum, short (254 nm) and long (365 nm) ultraviolet radiations under the UV-viewer chamber, the coloration was noted based on different chemical treatments (16).

Preliminary phytochemical Analysis

Preparation of extract

Two g of dried leaves, stem and root powder were mixed separately with 20 mL of non-polar solvents like hexane, diethyl ether, chloroform and ethyl acetate, similarly polar solvent like ethanol was mixed with different samples and filtered through Whatman no. 1 filter paper. The final extracts were then used for the qualitative phytochemical assays (17).

Estimation of phytochemicals

Qualitative phytochemical screening of different vegetative parts of *S. jomyi* were extracted with hexane, diethyl ether, chloroform, ethyl acetate and ethanol, the final extract was then carried out using standard procedures [alkaloids- dragendroff's test (18), flavonoids- ammonia test (18), tannins- 5 % lead acetate test (8), terpenoids (8), saponins-honeycomb test (19), steroids- salkowski's test (19), phlobatannins-1% HCl test (19), resins-turbidity test (20) , proteins and amino acids- ninhydrin test (21).]

GC-MS analysis

Extract preparation

Bioactive compounds of different vegetative parts of *S. jomyi* was estimated using the Schimadzu GC-MSQP2010SE system. 1 g of leaves, stem and root powders of *S. jomyi* was mixed with 10 mL of diethyl ether and kept in a rotary shaker overnight. The extract was then filtered through filter paper and dried up using a hot-air oven. The final crude extract was then again dissolved in 0.5 mL of diethyl ether followed by 1 µL of samples were injected through GC-MS system. Schimadzu GC-MSQP2010SE contains a mass detector (quadrupole) along with a 30 m×0.25 µm and 0.25 µm thickness capillary column. Helium used as a carrier gas. Estimation of secondary bioactive compounds was done using standard MS data library (NIST 2017) (22).

Results and Discussion

Macroscopic characteristics

Macroscopic character of leaves stem and root showed the following characters. The average sized leaves (Table 1 and Fig. 3-B) were ovate to lanceolate shape ,which is 9±0.70 cm in length and 4.83±0.28 cm in width with serrated margine. The distinctive character of *S. jomyi* is the length (3.133±0.32 cm in length and 0.2±0.00 cm in width) and pale pinkish-green coloured petiole. The stem is arial, cylindrical, erect, solid and branched with fine hairs. The average length of the stem (Table 1 and Fig. 3-B) was about 33±1 cm in length and 0.36±0.057 cm in width. The entire quantitative assay of leaves, stem and petiole were carried out in triplicate and measurement was taken from 3 different parts of the same plant. The morphological study of the root was carried out using an EPSON V850 pro root scanner, which revealed a taproot system along with subsidiary roots. The report of root scanning results

Table 4. Fluorescence analysis of *S. jomyi*

		Leaves			Stem			Root		
Sl. no.	**Power(P)+ reagents**	**Visible**	**254nm**	**356nm**	**Visible**	**254nm**	**356nm**	**Visible**	**254nm**	**356nm**
1.	Plant powder	Dark Green	Dark Brown	Dark Brown	Green	Light Green	Dark Brown	Light Brown	Light Brown	Dark Brown
2	P+Ethanol	Dark Green	Fluores-cent Green	Dark Brown	Light Green	Light Green	Dark Brown	Light Brown	Light Brown	Dark Brown
3	P+Chloroform	Dark Green	Dark Green	Brown	Light Green	Light Green	Brown	Brown	Brown	Brown
4	P+Petrolium ether	Dark Green	Dark Green	Dark Brown	Light Green	Light Green	Dark Brown	Brown	Light Brown	Dark Brown
5	P+Ethyl acetate	Dark Green	Fluores-cent Green	Dark Brown	Light Green	Fluorescent Green	Dark Brown	Brown	Brown	Dark Brown
6	P+Hexne	Dark Green	Dark Green	Dark Brown	Light Green	Fluorescent Green	Dark Brown	Brown	Brown	Dark Brown
7	Glacial Acetic acid	Dark Green	Fluores-cent Green	Dark Brown	Light Green	Light Green	Dark Brown	Light Brown	Light Green	Dark Brown
8	P+Conc.H_2SO_4	Light Green	Fluores-cent Green	Brown	Light Brown	Dark Brown	Brown	Dark Brown	Dark Brown	Brown
9	P+Conc.HCl	Light Green	Dark Brown	Brown	Dark Green	Light Green	Brown	Brown	Light Green	Brown
10	P+Conc.HNO_3	Light Brown	Fluores-cent Green	Brown	Light Brown	Fluorescent Green	Brown	Brown	Dark Green	Dark Brown
11	P+NaOH(alc)	Dark Green	Fluores-cent Green	Dark Brown	Light Brown	Fluorescent Green	Dark Brown	Light Brown	Brown	Dark Brown
12	P+NaOH(aq.)	Dark Green	Fluores-cent Green	Dark Brown	Light Green	Fluorescent Green	Dark Brown	Light Brown	Fluorescent Green	Dark Brown
13	5% NaOH	Dark Green	Fluores-cent Green	Dark Brown	Light Green	Fluorescent Green	Dark Brown	Light Brown	Fluorescent Green	Dark Brown
14	5% $FeCl_3$	Dark Green	Fluores-cent Green	Dark Brown	Light Yellow	Fluorescent Green	Dark Brown	Light Yellow	Fluorescent Green	Dark Brown
15	40% NaOH+CH_3COP_b	Dark Green	Fluores-cent Green	Dark Brown	Light Green	Fluorescent Green	Dark Brown	Light Brown	Fluorescent Green	Dark Brown
16	1M H_2SO_4	Dark Green	Light Green	Dark Brown	Light Green	Light Green	Dark Brown	Light Brown	Light Green	Dark Brown
17	1M HCl	Dark Green	Light Green	Light Brown	Green	Light Green	Dark Brown	Light Brown	Light Brown	Dark Brown

exhibited main tap root, primary lateral roots (cm) and secondary lateral roots (cm^2). The length of the 2 primary laterals was 28.84 and 30.35 cm. Moreover, the secondary lateral roots mesurements were 105.69 cm^2 and 332.062 cm^2 respectively (Table 1 and Fig. 3-C).

Previous reports on *S. moylaniae* showed morphological similarities with *S. jomyi*, like the shape of leaves, arrangement of leaves and the morphology of petiole (3). A serrated leaf margin was also observed in *S. ciliatus* (23). By comparing parameters like the size of leaves, petiole and stem with *S. bolavenensis* and *S. jomyi,* vegetative structures of *S. jomyi* were larger. The characteristics features of root length, diameter and volume are used to find out soil and plant function (24).

Table 1. Macroscopic measurements of different vegetative parts of *S. jomyi*

Qualitative parameter	Measurements
Leaves	Length - 9±0.70 Cm
	Width - 4.83±0.28 Cm
Petiole	Length- 3.133±0.32 Cm
	Width - 0.2±0.00 Cm
Stem	Length- 33±1 Cm
	Width - 0.2±0.00 Cm
Root	Primary lateral Roots
	1-28.84 Cm
	2.30.35 Cm
	Secondary lateral Roots
	1. 105.69 Cm^2
	2.332.062 Cm^2

Results are means of triplicates (Mean ± SD)

cells in the adaxial and abaxial surface of the transverse section through the midrib (25).

Transverse section through the lamina

The transverse section of leaf lamina (8.04 µm thickness) was differentiated into the upper and lower epidermis. Both the epidermal layers were tightly cutinized with non-glandular trichomes present in the epidermal region. The cells in the leaves were categorized into outer single-layered palisade cells and spongy cells. The space between the spongy cells is also known as aerenchyma cells (Fig. 4). Previous studies on *S. ciliatus* and *Justicia gendarussa* (Acanthaceae family) leave lamina also showed similar characteristic features like *S. jomyi* (23, 26).

Transverse section of the petiole

The transverse section of *S. jomyi* petiole contained two lateral wings. The adaxial surface of the transverse section was slightly convex, whereas the abaxial surface was more

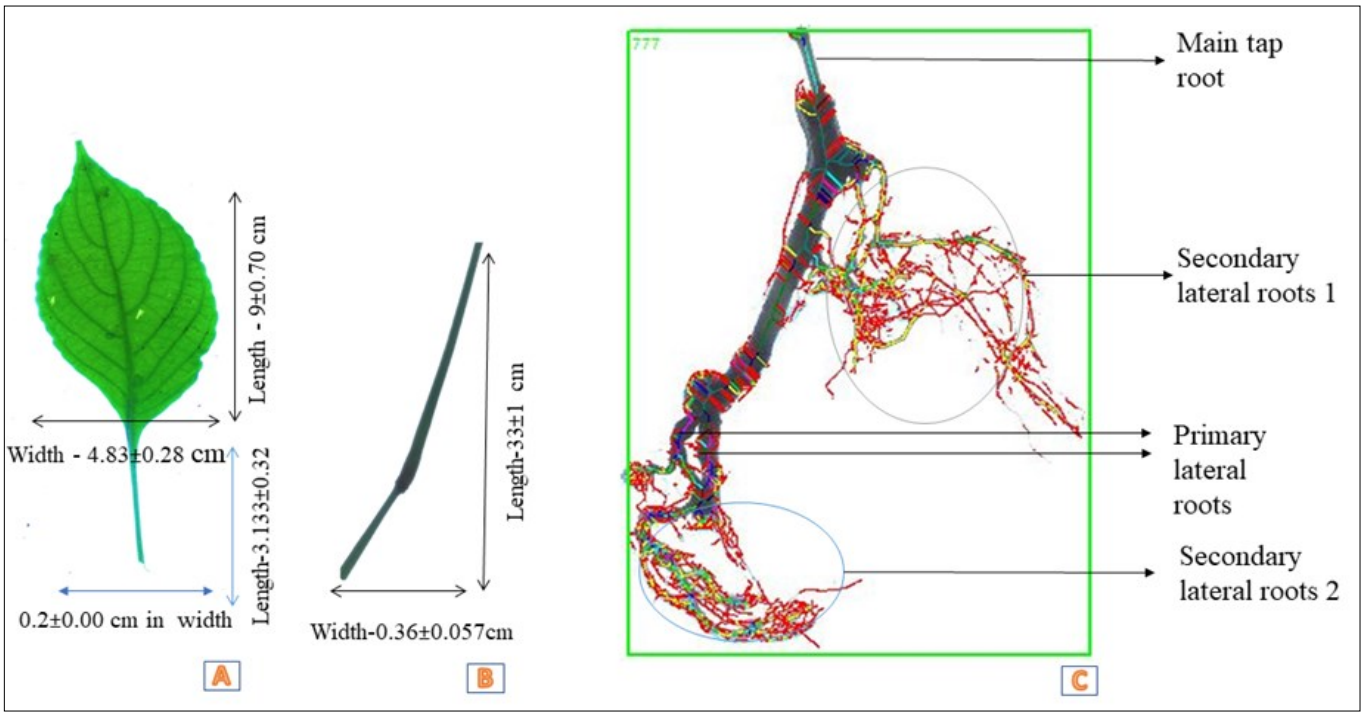


Fig. 3. (**A**). Length and and width of leaflets and petiole, (**B**) Length and thickness of stem, (**C**) Measurement of root-using root scanner.

Microscopic evaluation

Transverse section of leaves

Transverse section through the midrib

The transverse section of the midrib (25.56 µm wider) is a convex-shaped bulged structure. The outermost epidermal cells were cutinized with single-layered parenchyma cells. Inner to epidermal layers, 5 layers of large-sized collenchyma cells were present. Similar types of collenchyma cells were also found on the abaxial (2 layers) surface of the midrib. Single-layered greenish palisade cells were also seen in the upper epidermal region of the midrib. The horseshoe-shaped vascular bundles with 8 vertical rows of xylem elements surrounded by the phloem elements is the most distinguishable feature of *S. jomyi* (Fig. 4). Yesteryear studies on *S. ciliatus* showed numerous anatomical similarities except for the variation in the xylem elements (4 in number) (23). *S. sessilis* also had 5-7 layers of collenchyma

convex in shape. The transverse section (78.57 µm diameter) was differentiated into outermost single-layered cutinized cubical-shaped epidermal cells, followed by five layers of upper hypodermal collenchyma cells and four layers of hypodermal collenchyma cells in the lower epidermal region. The ground cells of the transverse section comprised numerous spherical-shaped parenchyma cells. Crescent-shaped (C-shaped) vascular bundles and phloem elements were surrounded by the xylem. Xylem and phloem cells are altogether supported by collenchyma and sclerenchyma cells. Raphides and cystoliths were also present in the parenchyma cells of the petiole (Fig. 5). A similar type of petiole study has been recorded in *S. ciliatus,* which showed anatomical similarities like the presence of collenchyma cells in the adaxial and abaxial surfaces of the section. However, the adaxial surface possessed flattened surface in *S. ciliatus* (23).

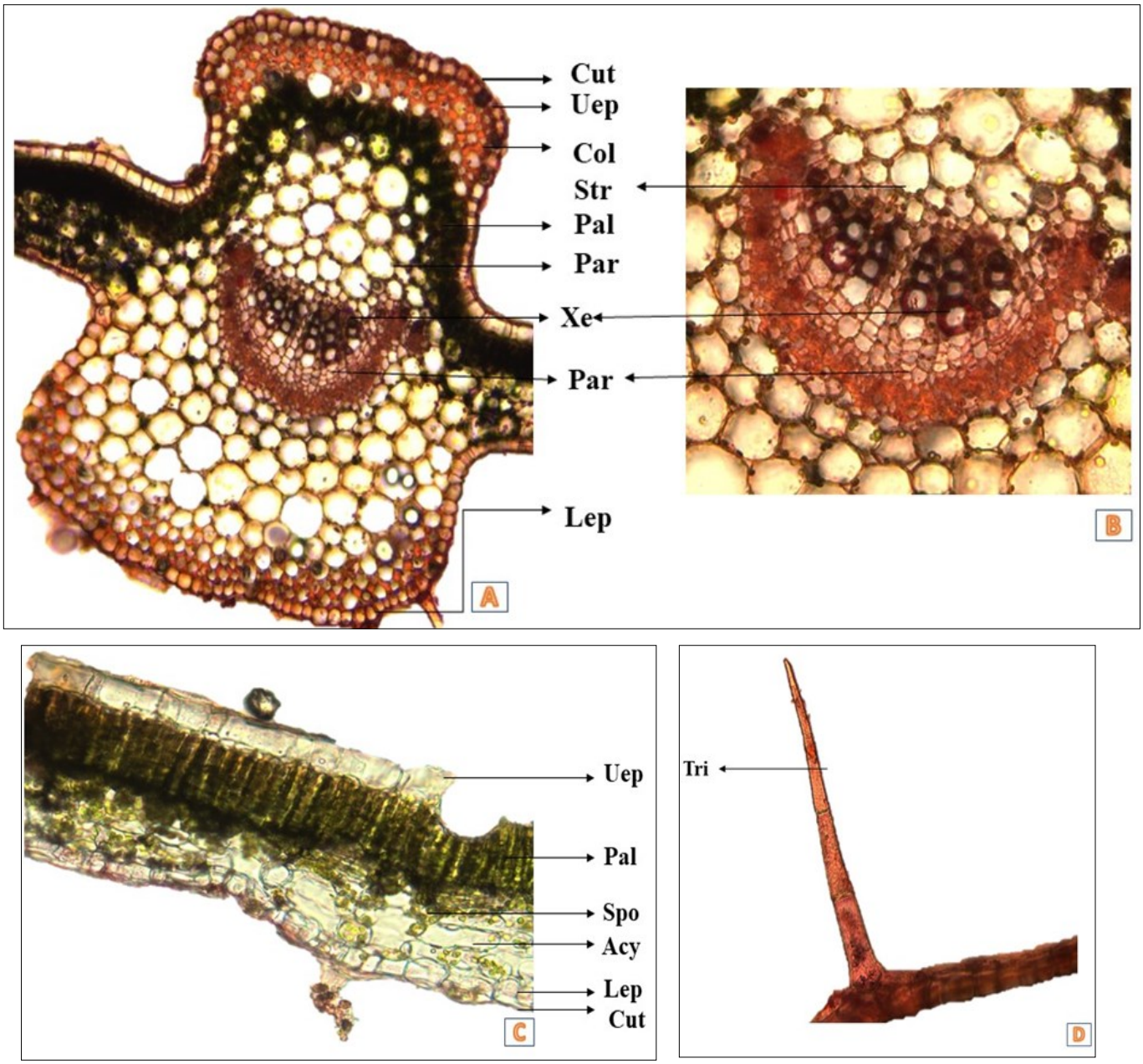
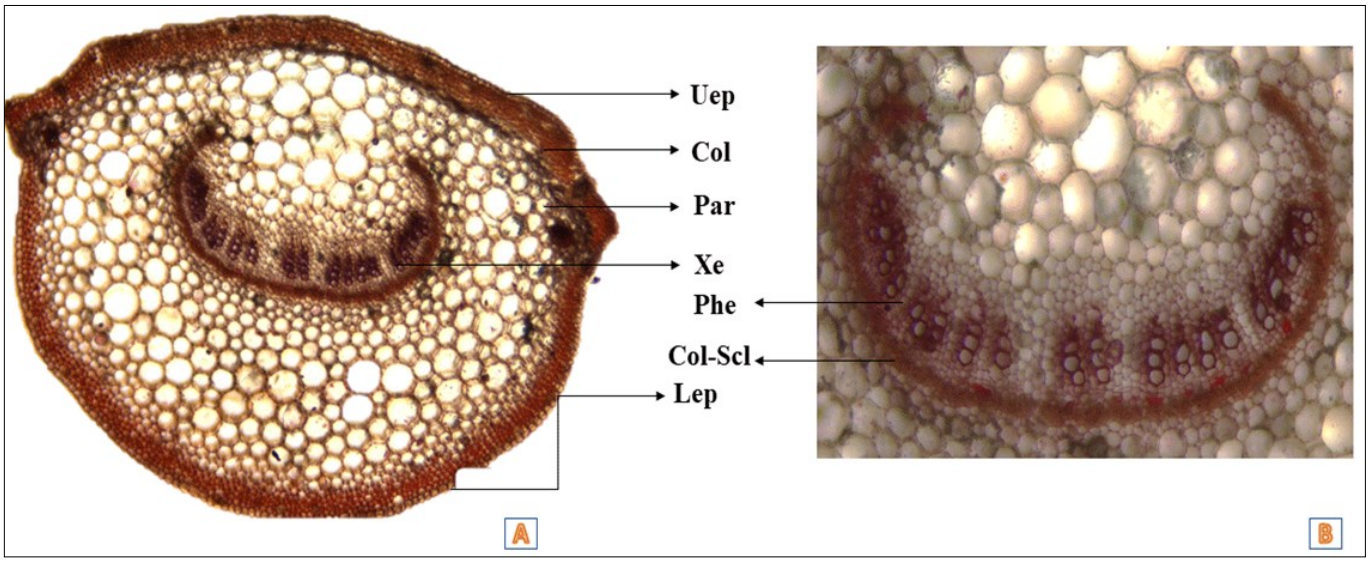


Fig. 4. (**A**) T. S of midrib of *S. jomyi* leaf under 10X magnification, (**B**) Vasculature of midrib under 45X magnification, (**C**) T.S of leaf lamina under 65X magnification, (**D**) Trichome of leaf under 45X [Cut: Cuticle; Uep: Upper epidemis; Col: Collenchyma; Str: Starch grains; Pal: Palisade cells; Par: parenchyma cells; Xe: Xylem elements; Par: Parenchyma; Lep: Lower Epidermis; Spo: Spongy cells; Acy: Aerenchyma cells; Tri: Trichome of leaf].

Transverse section of stem



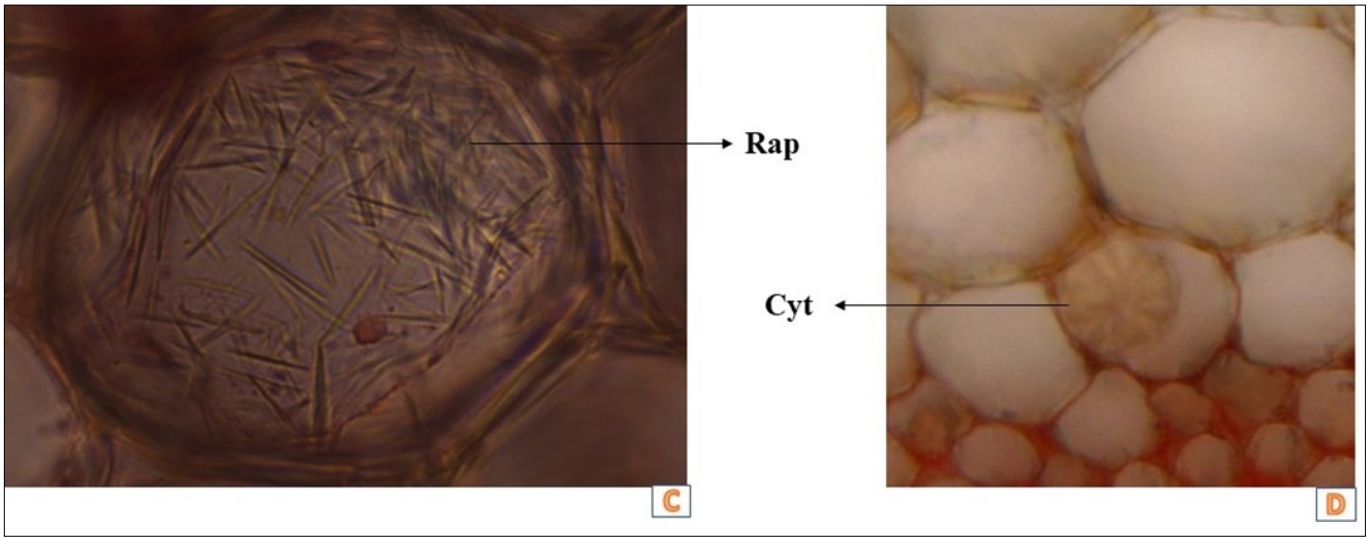


Fig. 5. (**A**) T. S of midrib of *S. jomyi* Petiole under 10X magnification, (**B**) Vasculature of Petiole under 45X magnification, (**C**) Raphides inside of leaf petiole under 100X magnification, (**D**) Cystolyth inside leaf petiole under 100X magnification. [Uep: Upper epidermis; Col: Collenchyma; Par: parenchyma cells; Xe: Xylem elements; Phe: Phloem elements; Lep: Lower epidermi; Col-Scl: Collenchyma and Schlerenchyma; Rap: Raphides; Cyt: Cystolyth].

The transverse section of the stem (82.77 µm wider) consists of the epidermis, cortex, secondary phloem, secondary xylem and pith. The outermost epidermis was slightly cutinized and made of a single-layer of elongated parenchyma cells with uniseriate trichomes. The cortical region of the stem is differentiated into hypodermis, composed of 4-5 layers of collenchyma cells followed by four layers of elliptically-shaped parenchyma cells (outer cortex) and single-layer of endodermis (inner cortex).

Below the endodermis, the phloem cells were surrounded by the xylem; the vessels were diffusely arranged in vertical rows and the secondary phloem was incorporated with sieve elements. They were thicker and broader; the companion cells were also arranged on the walls of sieve tubes. The secondary xylem presents in the stem contained thick-walled, circular and diffusely distributed vessels. The innermost layer of the stem was the pith, which was made of parenchyma cells containing numerous raphides (Fig. 6). The available report on *S. ciliatus* and *S. kunthiana* stem's transverse section also showed similar characteristic features like *S. jomyi* (23, 27).

Transverse section of root

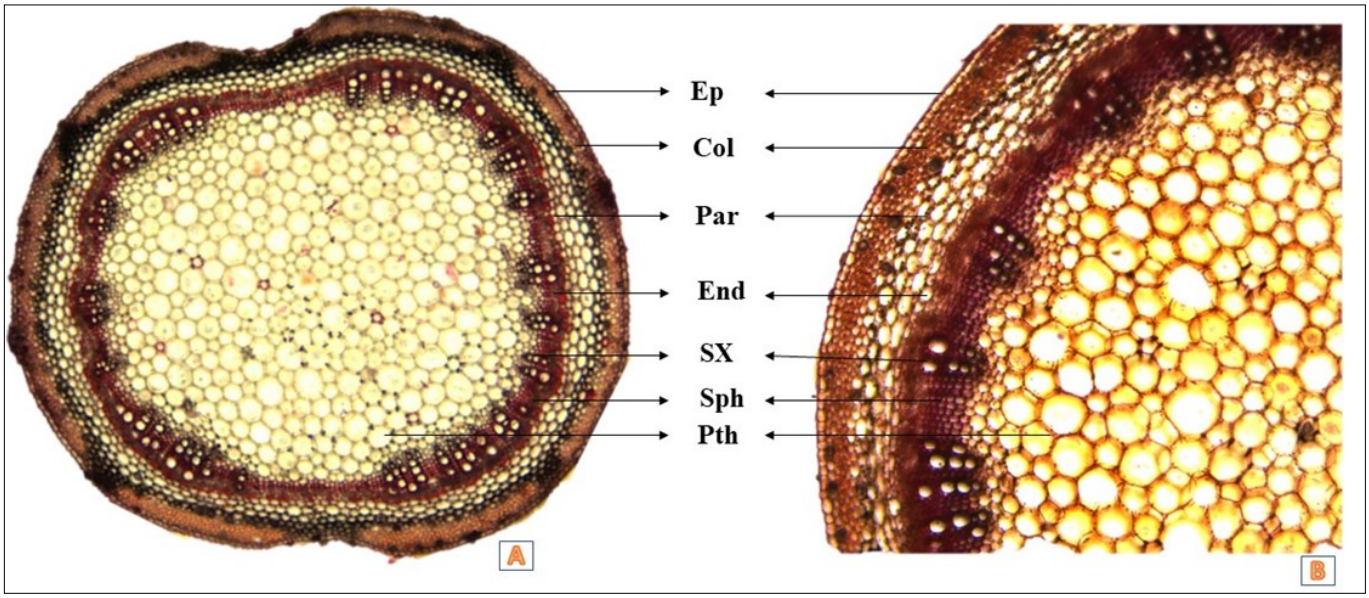
The transverse section of the root (64.99 µm diameter) was differentiated into the outermost single-layered epiblema (barrel-shaped), followed by the cortex. The cortical layer was made up of parenchyma cells, followed by each layer of endodermis and pericycle which are made up of parenchyma cells. In vascular bundles, the xylem was surrounded by the phloem. The secondary xylem has diffusely arranged vessels that also increase its size near its peripheral region towards the cortex. Pith was large and prominent (Fig. 7). Former reports on *S. kunthiana* also showed certain similar characteristics except the discontinuation of periderm (27).

Powder microscopic study

The powder morphology of leaves, stem and root showed the following characteristics.

Fibers

Fibers are elongated, pointed, narrow thick-walled structures found in the powder of leaves, stem and root. The fibers were 265.15 µm in length and 1.43 µm in thickness in leaves. Whereas for stem the length was



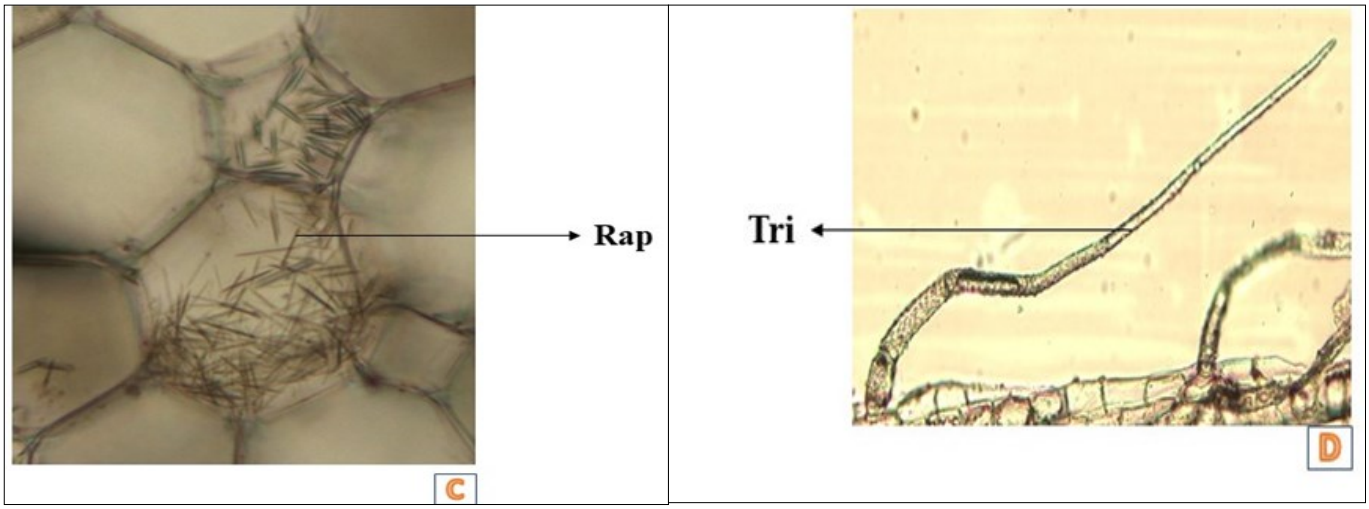


Fig. 6. (**A**) T. S of midrib of *S. jomyi* stem under 10X magnification, (**B**) Vasculature of stem under 45X magnification, (**C**) Raphides inside of stem under 100X magnification, **(D)** Trichome of stem. [Ep: Epidermis; Col: Collenchyma; Par: parenchyma cells; Sx: Secondary xylem; SPhe: Secondary Phloem; Pth; Pith; Rap: Raphides; Cyt: Cystolyth; Tri: Trichome of stem].

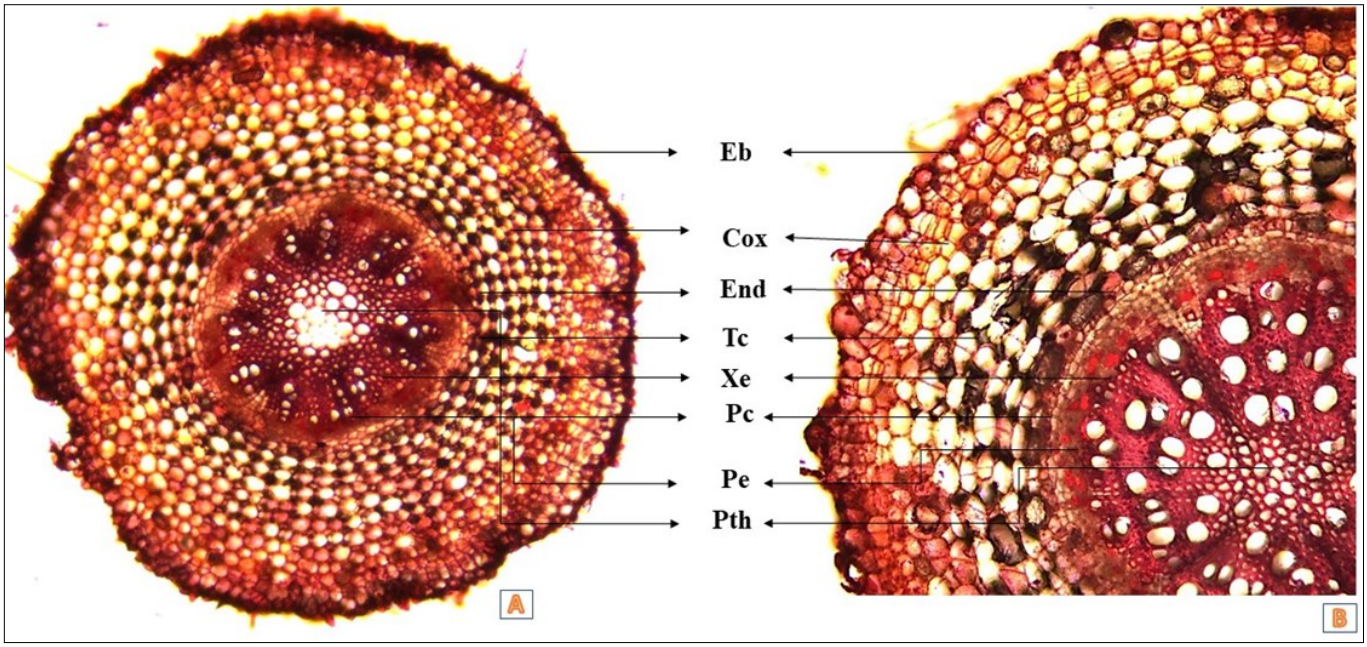
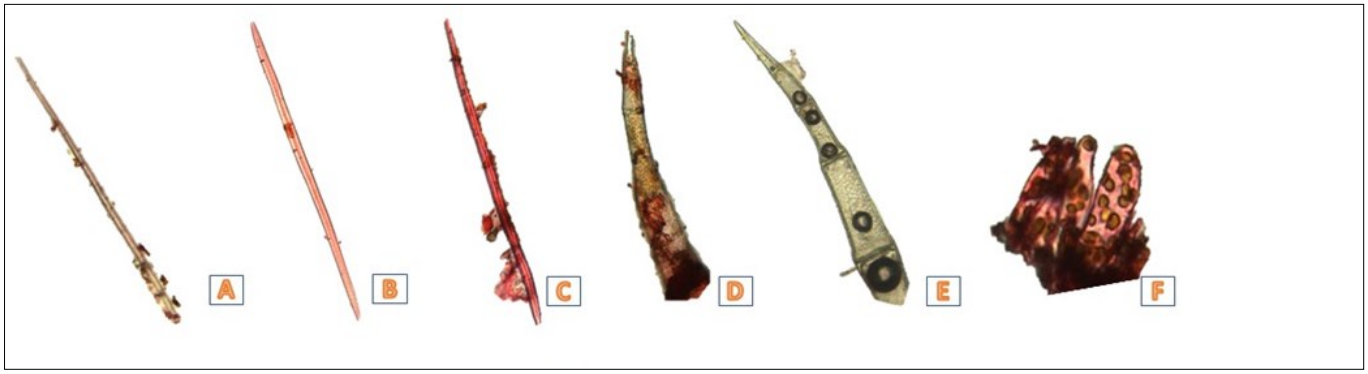


Fig. 7. (**A**) T. S of root of *S. jomyi* under 10X magnification, (**B**) Vasculature of root under 45X magnification. [Eb: Epiblema; Cox: Cortex; End: Endodermis cells; Tc: Tannin cells; Xe: Xylem elements;Pc; Pericycle; Pe: Phloem elements ; Pth : Pith].

38.76 µm and thickness 1.01 µm respectively. Root showed the highest number of fibers compared to leaves and stem. The fibers in roots were 215.79 µm in length and 3.62 µm in thickness. The walls of fibers were also highly lignified, with or without septa (Fig. 8 A, B, C). Earlier records of *S. ciliatus* fibers also showed similar characteristics, but the size of fibers of leaves and stem of *S. ciliatus* was much larger than *S. jomyi* (23).

Trichomes

The trichomes present in *S. jomyi* are mainly located in leaves and stems. They were elongated, non-glandular and septate. The size of the trichomes varied based on the vegetative part it belonged to. The non-glandular trichomes from the stem were measured at about 8×2 µm, and the leaves at 73.86 × 8.21 µm (Fig. 8 D, E). Previous reports on trichomes of the Acanthaceae family showed identical types of trichomes present in *S. jomyi.* These specific



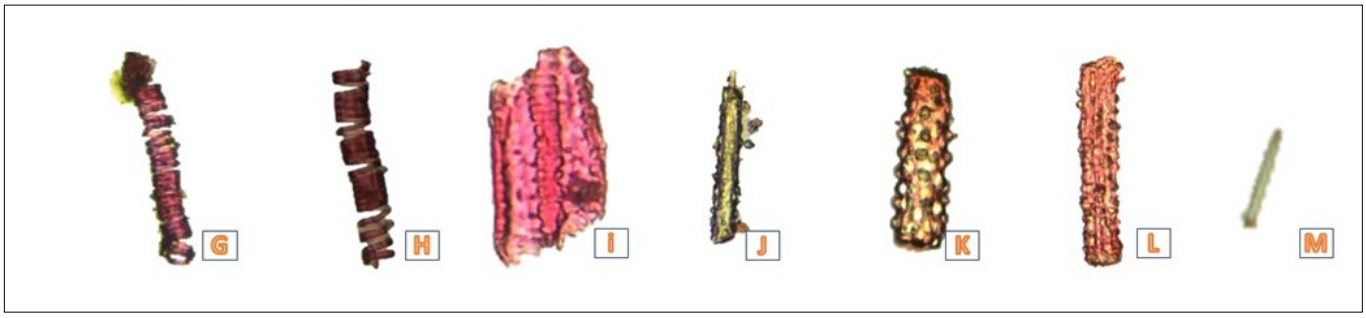


Fig. 8. The powder morphology of *S. jomyi* leaf, stem and root powders under 45x. (**A**) fibers of leaves, (**B**) Fibers of stem, (**C**) Fibers of root. (**D**) Trichomes of leaf, (**E**) Trichomes of stem. (**F**) Palisade cells of leaf. (**G**) Spiral xylem vessels of leaf, (**H**) Spiral xylem vessels of Stem, (**I**) Spiral xylem vessels of root. (**J**) Bordered pit vessels of leaf, (**K**) Bordered pit vessels of stem, (**L**) Bordered pit vessels of root. (**M**) Raphides of stem.

appendages prevent excessive transpiration, pathogen attack and ultraviolet radiation (28).

Palisade cells

The palisade cells were observed below the leaves' epidermal region, consisting of many chlorophyll pigments responsible for photosynthetic activity (29). The size of the palisade cells of *S. jomyi* leaves powder was about 3.79×3.79 µm (Fig. 8 F).

Spiral xylem vessels

The spirally arranged xylem vessels are elongated tube-like structures that function as water-conduction vessels. Spiral xylem vessels were observed in the leaves, stem and root of *S. jomyi*. The leaves (12.92×1.22 µm) possessed smaller-sized xylem vessels than the root (22.60×3.70 µm) and stem (23.50×4.02 µm) (Fig. 8 G, H, I). A similar type of spiral xylem vessel was also observed in *Trianthema portulacastrum* L. (30)

Bordered pit vessels

Bordered pit vessels are the type of cavity in the lignified cell walls of xylem channels (vessels and tracheid), which were also observed in leaves, stem and root of *S. jomyi*. The size of the vessels varied based on the vegetative part. The root (59.97× 9.98 µm) contains larger-sized bordered pit vessels than the stem (41.70× 10.35 µm) and leaves (38.12 ×9.11 µm) (Fig. 8 J, K, L) (31).

Rapides

Rapides are elongated needle-like Calcium oxalate crystals that help in calcium storage, metal detoxification and tissue support and act as a defense mechanism against insects and foraging animals (32). Among all other vegetative parts of *S. jomyi.* Calcium oxalate crystals were more prominent in the stem which was about 7.19 µm in length and 0.94 µm in thickness (Fig. 8 M).

Quantitative microscopic analysis of leaves

The quantitative microscopic characters like the stomatal index (paracyclic) of the upper epidermis of leaves, was revealed to be 21.96±4.17 % The whole quantitative assay of leaves was carried out in triplicate and measurement was taken from three different parts leaves samples. Similarly, previously documented studies on *S. sessilis* revealed a comparable % of the stomatal index (25). The size of stomata of *S. jomyi* (11.55±0.44 µm length and 5.53±0.75 µm width) was much lesser than *Barleria cristata* and *B. acanthoides* (Acanthaceae) (33). The vein islet and vein termination numbers of *S. jomyi* were 12.5±2.64 and 9.66±2.08 respectively (Fig. 9, Table 2). A similar number of vein islet and vein termination numbers were

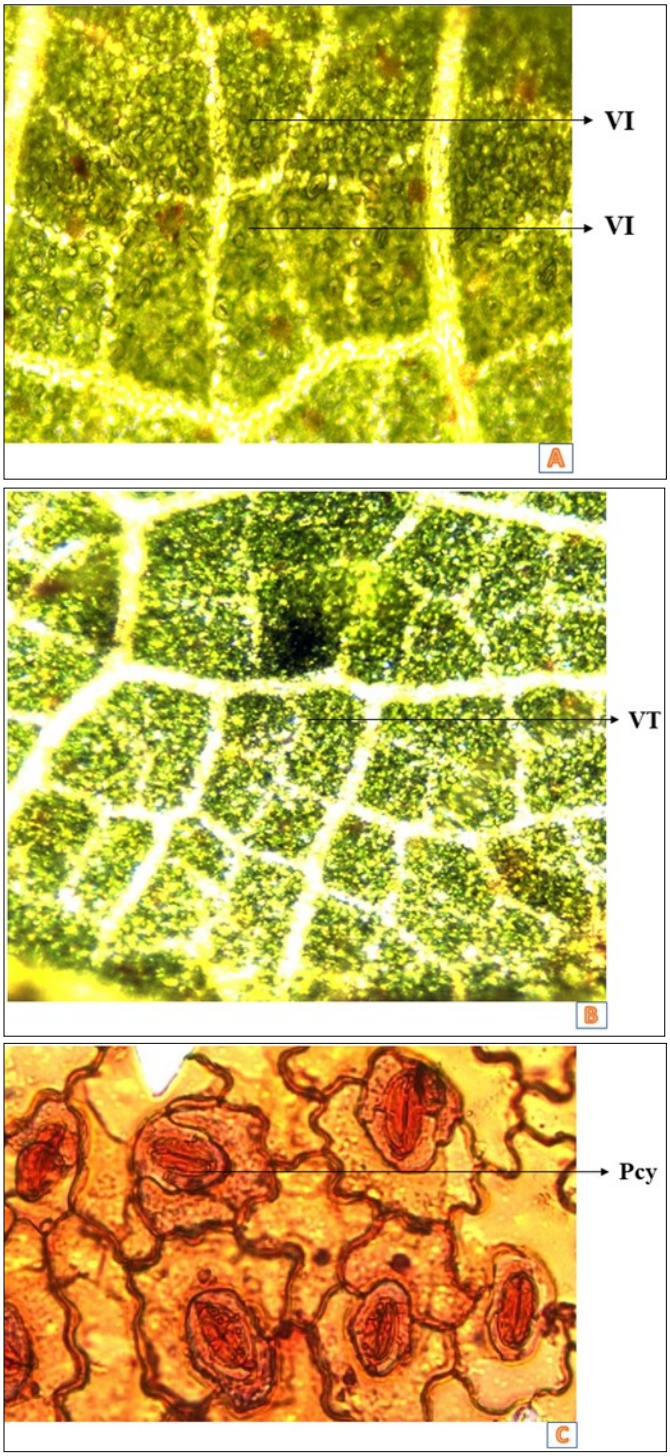


Fig. 9. Leaf surface characteristics of *S. jomyi* (**A**) vein islet (25X), (**B**) vein termination(25X) and (**C**) Paracytic stomata (45X) (label the diagrams with arrows) [VI: vein islet; VT: vein termination; Pcy: paracyclic stomata].

observed in *S. sessilis* and other Acanthaceae members showed remarkable variation up to the family level (25, 34). All these microscopic characteristics are equally important for the evaluation of the purity of the drug.

Table 2. Quantitative characters of *S. jomyi* leaves

Quantitative parameter	Values
Stomatal index	21.96±4.17 %
Vein islet number	12.5±2.64
Vein termination number	9.66±2.08
Size of stoma	Length -11.55±0.44 µm Width - 5.53±0.75 µm

Results are means of triplicates (mean ± SD)

Organoleptic analysis

The organoleptic analysis of leaves, stem, and roots of *S. jomyi,* both powdered and fresh samples, were analyzed using sensory evaluation (Table 3). The powdered and fresh samples of leaves showed dark green color. In contrast, the stem revealed light green (dry powder) and pale green for fresh samples. The powdered root and fresh samples exhibited brown and pale brown colors respectively. The olfactory analysis of leaves showed a mild smell in both fresh and dry samples. The dry samples of stem emanated an unknown smell; similarly, in fresh samples also, the smell was unfamiliar. The aroma of the root showed an unknown smell in dry samples, whereas it had no smell in the fresh root. In taste analysis, leaves showed bitterness in both dry and fresh samples. The stem's taste was sweet and bitter-sweet in dry and fresh samples respectively. Fresh and dried samples of root did not have any taste. The previous studies suggested that bitterness is because of the presence of alkaloids and sweet-bitter taste is because of the presence of saccharides and saponins (35).

Table 3. Organoleptic analysis of *S. jomyi*

Sl. no.	Organoleptic characters	Dry sample			Fresh samples		
		Leaves	Stem	Root	Leaves	Stem	Root
1	Colour	Dark green	Light green	Brown	Dark green	Pale green	Pale brown
2	Aroma	Mild smell	Unknown	Unknown	Mild smell	Unknown	No smell
3	Taste	Bitter	Sweet	No taste	Bitter	Bitter-sweet	No taste
4	Texture	Smooth	Rough	Rough	Rough	Hard	Rough

The texture variation was more prominent in leaves, stem and root of both fresh and dry samples. Leaves powder showed a smooth texture, whereas the fresh samples had a rough surface. Stem powder exhibited roughness and hardness in fresh and dry samples. Both fresh and dry samples of root texture were also rough. The already documented studies on *S. sessilis* leaves showed similar organoleptic activities of the plant (25). The previously reported studies on organoleptic analysis revealed that the taste and smell of a plant directly influences its selection for treating specific diseases. This specific analysis is one of the oldest practice to find out the medicinal properties of the plant.

Fluorescent analysis

The powders of leaves, stem and root of *S. jomyi* expressed different colouration with different chemical treatments, as shown in Table 4. The leave, stem and root powders showed dark green to dark brown coloration when they were treated with various chemical reagents. Fluorescent green coloration was observed in leaves at 254 nm when the samples were treated with ethanol, ethyl acetate, glacial acetic acid, concentrated H_2SO_4, concentrated. HNO_3 and alcoholic NaOH. Similarly, in stem ethyl acetate, hexane, concentrated. HNO_3, alcoholic NaOH, aqueous NaOH, 5% NaOH, 5% $FeCl_3$ and 40% NaOH and lead acetate treatment showed fluorescent green coloration. In the root, fluorescent green coloration was observed only in aqueous NaOH, 5% NaOH, 5% $FeCl_3$ and 40% NaOH and lead acetate treatment. Treatment with 5 % $FeCl_3$ expressed a distinctive light-yellow coloration in the stem and root in visible light.

Fluorescence study is one of the most straight forward and rapid methods to analyse the crude drug's pharmacognostic activity. This specific study can identify and authenticate adulterants present in crude drugs. Colours are produced by the samples with different chemical reagents that act as an active constituent present in the visible range in day length. Each fluorescent characteristic is different, so fluorescent analysis is one of the significant experimental setups to estimate the pharmacognostic quality of crude drugs (30). The fluorescent study of *S. jomyi* is the first report on the *Strobilathes* genus itself. Former works on Acanthaceae (*Barleria montana* Wight & Nees) also revealed fluorescent activity with different chemical treatments (36).

Qualitative phytochemical analysis

Phytochemicals are natural chemical compounds present in plants that provides defense against certain disease like cancer, diabetics and protects the plant from damages. Plant phytochemicals are mainly concentrated in leaves, stems, roots, flowers, fruit and seeds. The concentration of such phytochemicals will vary from plant to plant (37, 38).

The qualitative phytochemical analysis of leaves, stem, and root in conjunction with various non-polar and polar solvents like hexane, diethyl ether, chloroform, ethyl acetate and ethanol revealed the presence of phytochemical compounds as shown in Table 5. The hexane leaf extract of *S. jomyi* revealed the presence of flavonoids, saponins and tannins. However, remaining phytochemical compounds like terpenoids, steroids, resins,

Table 5. Qualitative phytochemical analysis of *S. jomyi* in different solvent system.

Sl no.	Phytochemicals	Solvent used														
		Hexane			Diethyl ether			Chloroform			Ethyl acetate			Ethanol		
	Part used	L		R	L	S	R	L	S	R	L	S	R	L	S	R
1	Alkaloid	—	—	+	+	—	+	+	—	+	+	—	+	+	+	+
2	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Saponin	+	—	—	—	—	—	—	+	+	+	—	+	+	+	+
4	Tannin	+	—	—	+	+	—	—	—	—	+	—	—	+	+	+
5	Terpenoids	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—
6	Steroids	—	—	—	—	—	—	—	—	—	+	—	—	+	—	—
7	Resins	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	Phlobatannins	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
9	Carbohydrates	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
10	Protein and amino acids	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+

'+'Present, '-' Absent. L-Leaves, S-Stem, R-Root

phlobatannins, proteins and amino acids tested negative. The diethyl ether and chloroform extract of leaves also revealed the presence of phytochemical constituents like alkaloids and flavonoids. Only in diethyl ether the presence of tannin was noted, whereas, in chloroform, it was absent. Similarly, ethyl acetate and ethanol leaf extract tested positive for alkaloids, flavonoids and saponin. Ethanolic leaf extract consists of steroids, phlobatannins, proteins and amino acids also tested positive. Hexane, diethyl ether, chloroform and ethyl acetate extract of *S. jomyi* stem revealed the presence of flavonoids.

On the other hand, tannin's presence was observed only in diethyl ether extract of the stem. Saponin was present in chloroform and terpenoids appeared only in ethyl acetate extract of the stem. The polar solvent- ethanol extract of stem revealed the presence of alkaloids, flavonoids, saponins, tannins, proteins and amino acids. Phytochemical compounds such as alkaloids and flavonoids were noted in hexane, diethyl ether and chloroform root extract of *S. jomyi*. Tannin's presence was observed only in chloroform extract of root. Ethyl acetate and ethanolic extract of root exhibited the presence of alkaloids, flavonoids and saponins.

Similarly, ethanolic extract of root revealed the occurrence of phytochemicals like tannins, proteins and amino acids along with other phytochemicals. Former studies on ethanolic extract of *S. ciliatus* leaf revealed the presence of terpenoids, flavonoids, tannins, alkaloids, carbohydrates, glycosides and phytosterols (23).

Former studies on *S. heyneanus* leaves methanolic extract showed the presence of phenols, glycosides, terpenoids, saponin, tannins, flavonoids and alkaloids (8). A qualitative phytochemical study among different solvents revealed that the ethanolic extract showed promising results and is an effective solvent for the phytochemical study because it has more extarctive ability than other solvents systems (39). The phytoconstituents present are reported to contribute to conventional medicinal uses because of its pharmacological effects.

GC-MS analysis

The secondary metabolites of leaves, stem and root of *S.jomyi* diethyl ether extract were evaluated by GC-MS analysis and the results are shown in Table 6. The bioactive compounds like 9,12,15-octadecatrienoic acid (9.22 %), squalene (9.14 %), lupeol (8.77%), phytol (8.68 %), gamma-

Table 6. GC-MS analysis of *S. jomyi* - Major bioactive compounds

Sl no.	RT (min)	Bioactive compounds	% of compounds	Part used
1	19.545	9,12,15-octadecatrienoic acid, (z, z,z)	9.22	leaf
2	23.695	squalene	9.14	leaf
3	18.495	lupeol	8.77	leaf
4	19.195	phytol	8.68	leaf
5	33.040	gamma-sitosterol	7.79	leaf
6	18.315	n-hexadecanoic acid	7.45	leaf
7	31.460	stigmasterol	5.97	leaf
8	25.490	tris(2,4-di-tert-butylphenyl) phosphate	22.45	stem
9	35.65	lupeol	10.36	stem
10	25.355	2,6-di-tert-butyl-4-methylphenol,	5.07	stem
11	20.225	dotriacontanal	3.91	stem
12	19.920	9,19-cyclolanostan-3-ol,24-methylene-, (3. beta)-	2.92	stem
13	13.440	2,4-di-tert-butylphenol	2.79	stem
14	19.595	octadecanoic acid	2.07	stem
15	34.450	lup-20(29)-en-3-one	13.42	root
16	31.260	stigmasterol	12.59	root
17	32.885	gamma-sitosterol	8.18	root
18	30.650	campesterol	5.65	root
19	13.430	2,4-di-tert-butylphenol	2.61	root
20	35.520	lupeol	1.51	root

sitosterol (7.79%), n-hexadecanoic acid (7.45%) and stigmasterol (5.97%) were observed in higher quantity in leaves. Followed by tris(2,4-di-tert-butylphenyl) phosphate, 2,6-di-tert-butyl-4-methylphenol, 2,4-di-tert-butylphenol and octadecanoic acid in the stem. Lup-20(29) -en-3-one, stigmasterol, gamma. -sitosterol, campesterol and 2,4-di-tert-butylphenol are chemical compounds observed in the root (Fig. 10-12). Previous reports on the bioactive compound 9,12,15-octadecatrienoic acid showed anti-plasmodic activity and squalene showed anti-tumor and anti-oxidant activities (40, 41) . Already reported studies on phytol, gamma-sitosterol, n-hexadecenoic acid and stigmasterol exhibited anti-diabetic (42), anti-cancerous (42-44), anti-tumor (44), anti-mutagenic (42) and anti-inflammatory activity (42, 45). Compounds like tris (2,4-di-tert-butylphenyl) phosphate, 2,6-di-tert-butyl-4-

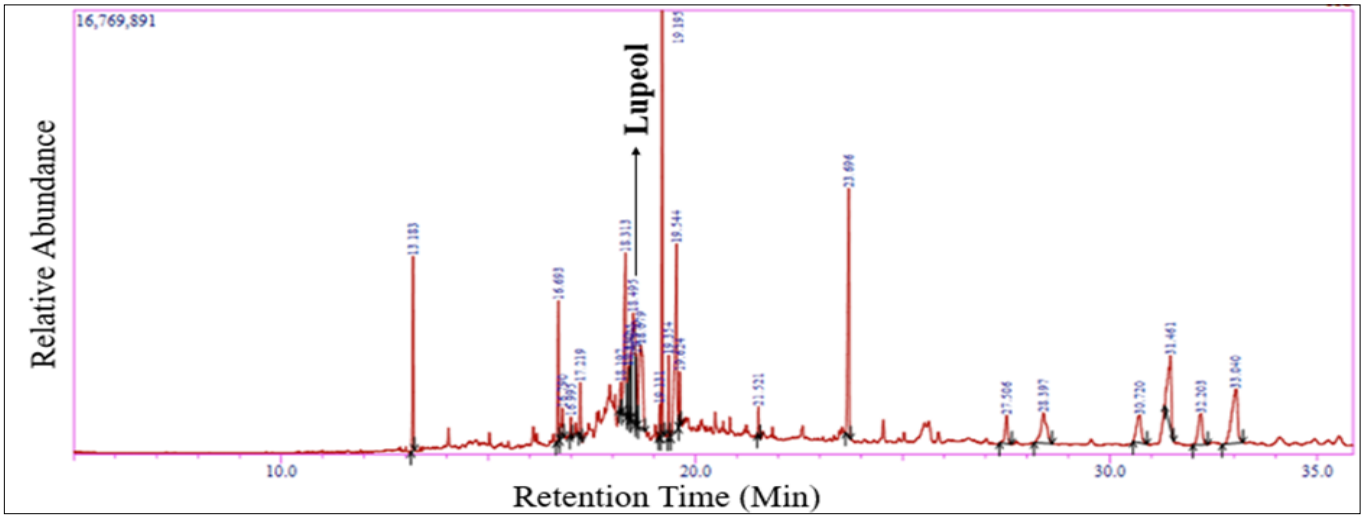


Fig. 10. GC-MS chromatogram of *S. jomyi* diethyl ether leaves extract.

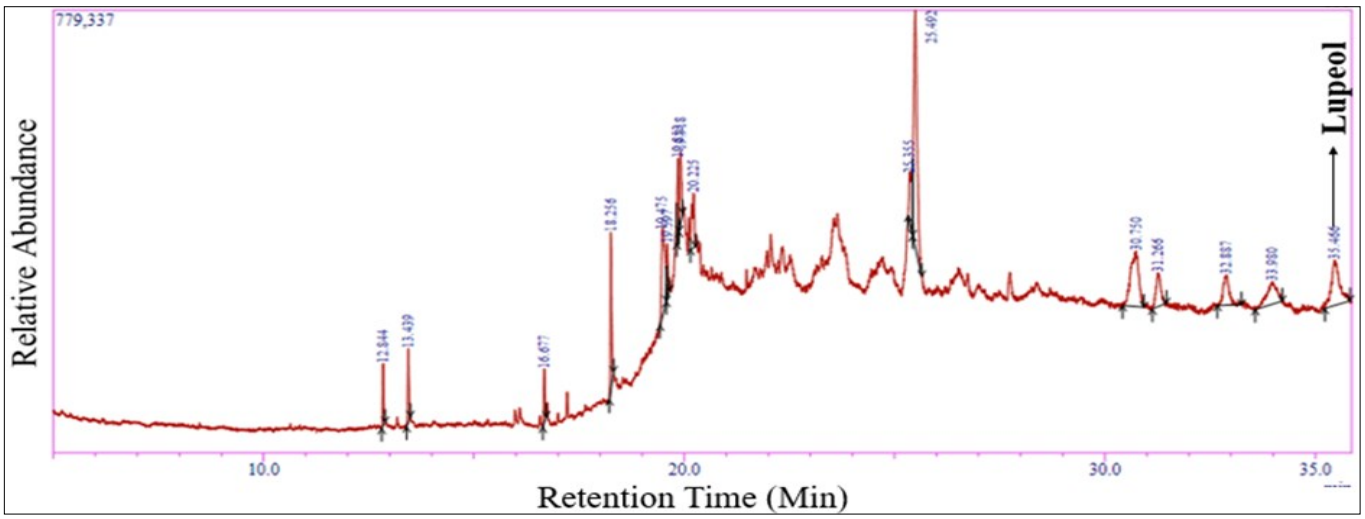


Fig. 11. GC-MS chromatogram of *S. jomyi* diethyl ether stem extract.

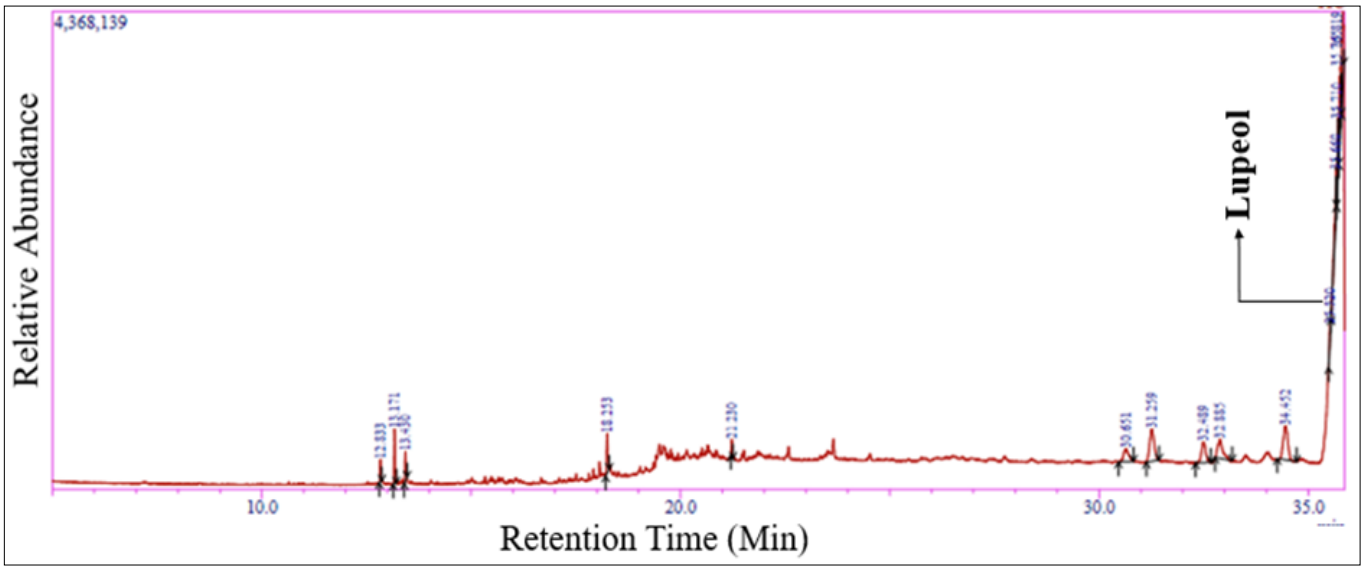


Fig. 12. GC-MS chromatogram of *S. jomyi* diethyl ether root extract.

methylphenol and 2,4-di-tert-butylphenol revealed the presence of anti-inflammatory, anti-oxidant and antifungal properties (46, 48). Lup-20(29)-en-3-one is another secondary metabolite that possesses anti-cancerous and anti-leukemia activities (49). Reports on campesterol also showed anti-cancerous properties (50). Based on the GC-MS study, lupeol was one of the significant compounds observed in all vegetative parts of *S. jomyi*, which has anti-inflammatory, anti-cancerous, anti-invasive, anti-angiogenic properties and also acts as a cholesterol-lowering agent (51, 52). Previous studies on *S. crispus* , *S. kunthiana* and *S. ciliatus* revealed similar bioactive compounds (n-hexadecanoic acid and Squalene) observed in *S. jomyi,* which carry important medicinal properties (53, 54). Based on current gas chromatography and mass spectrometry (GC-MS) is an analytical tool used to identify and quantify bioactive compounds and evaluate the quality of each secondary metabolite. Based on the quantification, the quality of a specific sample can be determined (55).

Conclusion

The present study is the first report on comprehensive pharmacognostic, phytochemistry and GC-MS analysis of leaves stem and root of *S. jomyi*. A pharmacognostic studies like macro and microscopic characters revealed the presence of paracyclic stomata and non-glandular trichomes on the leaves sample, whereas in transverse section of petiole and stem revealed the presence of raphides. Similarly root and petiole exhibited tannin and cystolyth respectively. Powder morphology of leaves, stems and roots revealed the presence of fibers, trichomes, palisade cells, spiral xylem vessels, bordered pit vessels and raphides. Fluresecence analysis showcased different colouration with different chemical treatment along with various taste, smell, colour, texture by organoleptic assay. Phytochemical and GC-MS analysis revealed presence of flavonoids and lupeol in all vegetative part of plant samples. In current scenario rural people still believe that plant-based medication is one of the safest methods and can be directly used in treating various diseases. The current study provides immense knowledge on pharmacognostic criteria for identifying plant samples. Furthermore it helps to check the purity, quality and authenticity of *S. jomyi* using pharmacognostic, qualitative phytochemistry and GC-MS analysis. The findings from this research can be used as monographs for future studies also can be utilized in medical field to identify the quality of *S. jomyi* based ayurvedic preparation in future.

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Authors contributions

AS and JX has equally contributed to this work.

Compliance with ethical standards

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

Ethical issues: None.

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