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Research Article

Morpho-anatomical and phytochemical characterization of two Apiaceae members: *Selinum vaginatum* and *Ligusticopsis wallichiana* from Himachal Pradesh

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Background: *Selinum* is an aromatic perennial herb belonging to the Apiaceae family, and the genus comprises two main species found at high altitudes in Himachal Pradesh, which are *Selinum vaginatum* (Edgew.) C.B.Clarke and *Ligusticopsis wallichiana* (DC.) Pimenov & Kljuykov. These species exhibit a high level of morphological similarity, making it challenging to distinguish between them. Therefore, a thorough study is essential to prevent misidentification and minimize the risk of adulteration. The research aims to clarify the controversies between these two species by comparing their morphology, anatomy and phytochemical profile.

Methods: A total of 31 quantitative morphological characters were selected, out of which 12 were reproductive, and 19 were vegetative. Sectional analyses and stereomicroscopic imaging were employed in the anatomical investigation. In addition, the essential oil of the entire plant of both species was analysed using gas chromatography-mass spectrometry (GC-MS) to see secondary metabolites.

Results: Results showed that the trichomes were present on the stem and mericarp of *Ligusticopsis wallichiana* but were not observed in the stem of *Selinum vaginatum*. The transverse section of the mericarp revealed differences in the number of vitae, while the stem showed variations in the number and structures of vascular bundles in both the species. Four compounds were common to both species *i.e.* Z-β-Ocimene, 3,5-Nonadiyne, cis-β-Farnesene, and α-Bisabolol, while remaining compounds were unique to each species.

Conclusion: The morphological and anatomical analysis, along with phytochemical investigations of putative chemotaxonomic marker compounds, can serve as valuable tools to support plant identification and classification, helping to prevent adulteration. This study suggests that combining structural traits and phytochemical profiling could help delineate the taxonomically challenging *Selinum* genus.

Keywords: *Morphology, anatomy, mericarp, phytochemistry, essential oil, SEM*

Introduction

The Apiaceae family include over 450 genera and 3700 species worldwide, which are rich in secondary metabolites and also economically and medically important. Plants in the Apiaceae family have characteristic features, including actinomorphic, five-lobed corollas, umbel inflorescences, an inferior ovary, and schizocarp fruits with carpophore. Morphological attributes play a crucial role in taxonomy classification. Various species are identified by their umbel,

bract, and bracteole count, flower, fruit shape, mericarp length, mericarp shape, secretory ducts, carpophore, and stylopodium (Duran et al., 2010). The genus *Selinum* is an important medicinal and aromatic plant belonging to the family Apiaceae and commonly known as "Bhutkeshi". *Selinum vaginatum* (Edgew.) C.B.Clarke and *Ligusticopsis wallichiana* (DC.) Pimenov & Kljuykov (Syns: *Selinum wallichianum* (DC.) Raizada & H. O. Saxena, *Selinum tenuifolium* Wall. ex C. B. Clarke) (Royal Botanic Gardens, Kew (2024)) are two common species found at the altitudes of 2400-4800 m in Himachal Pradesh. Genus *Selinum* is also reported from Uttarakhand, and the surrounding regions of India and its neighbouring countries (China, Nepal, Bhutan, and Pakistan) (Chauhan, 1999; Saraswat, 2020). Worldwide, there are approximately 35 species of *Selinum,* out of which some have unresolved status. These plants are widely used to treat constipation, menstrual, digestive, cardiovascular, neurological disorders (convulsion, epilepsy, insomnia), skin issues, toothache, asthma and hypertension (Saraswat, 2020). *Selinum vaginatum* is considered a threatened species and is categorised as low-risk, and least concerned. Both species are endemic to the Western Himalayas (Chawla et al., 2008), and the taxon has yet to be evaluated by the IUCN Red List, but no further *Selinum* species are listed on the IUCN (IUCN, 2024) (Joshi, 2016; Devkota et al., 2018). Initially, a study by Srivastava et al. (2018) on the morphological characteristics of the root, stem, and leaves of these two plant species showed that these species are different morphologically as well as anatomically (Srivastava et al., 2018). The Apiaceae family can be classified at different taxonomic ranks using both macro and micro-morphological traits. In a study, Schizocarp forms observed were oval, circular, triangular, flat, oblong, and globose (Kalsoom et al., 2019). SEM investigations on SV indicated that its leaves are leathery, with stretched cuticles, and few stomata on the top surface as compared to the bottom. The stem is 20 cm to 1 m in length, the base is fibrous and glabrous, stout. In LW, the length of the stem is 1-2 meters with a fibrous base, glabrous, and somewhat pubescent. In both species, the mesophyll was bifacial, and leaves were amphistomatic (Kumari et al., 2025). *Ligusticopsis wallichiana* contains bhutkesosides A and B, as well as ten other known compounds (Adhikari et al., 2016). The major sesquiterpenoids present in SV are α-copaene, germacrene-A, 14-hydroxy-δ-cadinene, khusinol, and viridiflorol (Kumar et al., 2019). It is difficult to differentiate taxa within the genus *Selinum* due to the lack of established morphological analogies, and taxonomy is further confounded by extensive terminology confusion, in which the same species has been recorded under different names in various sources (Srivastava et al., 2022). Carpological anatomical characteristics play an important role in Apiaceae classification. Their inclusion is now required in any significant taxonomic revision since they aid in the correct identification of closely associated taxa (Kljuykov et al., 2021). The identification of these species is mostly based on the leaf and mericarp. However, relatively few attempts have been made to record the morphological and anatomical aspects of their mericarps. Apart from a few basic-level research studies, there has been no published research on the fruit morphology and the presence or absence of trichomes on the fruit and stem of these species. Reproductive traits are typically less changeable, especially in their basic structure. This research focuses on the comparative study of morphological and anatomical traits of the leaf, stem, root, flower, and mericarp of two species of *Selinum,* as there is a lack of research on morphological and anatomical traits of the reproductive parts of these species. Although there is currently limited data available on the Apiaceae family, extensive SEM examination of mericarp, information on its morphology, and anatomy is lacking. As a result, the current study seeks to comprehensively evaluate and compare the morphological, anatomical, and phytochemical characteristics of these two species. The current study offers a thorough description of reproductive organs and a comparative SEM-based assessment of their mericarp and stem for the first time. This study is expected to yield reliable taxonomic markers that will aid in their proper identification and future pharmaceutical exploration. Furthermore, the results obtained from SEM imaging on the morphology of the mericarp and stem will provide insight into taxonomical significance and create a database for future studies, allowing for better precision.

Materials and methods

Collection of Sample

The plant material was collected from six locations in Himachal Pradesh. SV was collected in the wild from Solang Valley (elevation=2450 m), Kothi (elevation=2473 m), and Burwa (elevation=2997 m) from the district Kullu. LW is collected from Bakarthach (elevation=3014 m, District Kullu), Sissu (elevation=3176 m, District Lahoul & Spiti), and Dehnasar (elevation=3500 m, District Kangra) from the period between August to October. Both the plant species were authenticated at CSIR-IHBT Palampur, Himachal Pradesh, India, and voucher specimens under accession numbers 22346 and 22347 were deposited in the institutional herbarium.

Morphological studies

Morphological characteristics such as length, width, numbers, shape, size, and height of plant parts (flower, stem, and root), along with macromorphological and micromorphological features of vegetative and reproductive parts, were recorded. The macromorphological features were recorded manually using a scale and measuring tape. The

micromorphological features of each sample (fruit, leaf, stem, and root) were first investigated and photographed using a Magnus MSZ-TR stereo-microscope with Magnus camera1/2.3" (CMOS sensor, 10MP). To determine the average length and width of the mericarp, 10 mature mericarps for each species were measured. The dorsal and ventral surfaces of the mericarp and the outer surface of the stem were examined by scanning electron microscopy (SEM). SEM investigations were carried out with a CamScan S-2 (accelerating voltage 20.00 kV and working distance 20.5 mm), at a magnification of 200 and 20 µm. To conduct SEM, the dry mericarp and a segment of the stem were placed on Al stubs using double-sided adhesive tape and sputter-coated with gold. To ascertain their surface ornamentation, they were examined and photographed on camera.

Character selection

Based on the morphological variation in the selected and measured material, 31 Quantitative morphological characters, of which 12 reproductive and 19 vegetative, were selected. The measured morphological parameters were length, thickness and width of plant shoot, leaf, pinnula, petiole, stem, root, inflorescence, peduncle, mericarp, stylopodium, pedicel, and umbellet. The total characters measured in cm were PLL, CLL, CAW, CMW, CBW, CFL, CFW, PIL, PEL, SAW, SMW, SBW, PIW, PTW, PTL, ROL, RAW, RMW, RBW, INW, INL, PDL, ML(+S), ML(-S), MEW, MET, STP, LRL, PCL, UML, and UMW. The other 10 quantitative characters were selected based on numbers such as the number of nodes per shoot, leaves per shoot, SCL per leaf, PL per leaf, IN per shoot, rays per IN, IF per umbellet, seeds per umbellet, VV per mericarp, and CV per mericarp for each species. The measured anatomical parameters were the width and thickness of VB, ground tissues, endosperm, vitae, pericarp, secretary ducts, pith, xylem rays, secondary phloem, cortex, and the distance between vascular bundles. For each morphological trait, three replicates were randomly recorded. The mean values of morphological and anatomical parameters were computed for specimens that differed in length, width, number, and quantity. The qualitative characteristics selected were shape, color, texture, size, presence or absence, arrangement, and aroma for each character of plant species. The abbreviations used in this study are mentioned in Table 1.

Table 1. The table lists the codes used and their abbreviations

Codes used	Full name
SV	*Selinum vaginatum*
LW	*Ligusticopsis wallichiana*
PLL	Plant Length
CLL	Compound leaf length
CAW	Compound leaf apical Width
CMW	Compound leaf middle width
CBW	Compound leaf basal Width
CFL	Compound leaflet length
CFW	Compound leaflet width
PIL	Pinnula length
PEL	Petiole length
SAW	Stem apical width
SMW	Stem middle width
SBW	Stem basal width
PIW	Pinnula width
PTW	Petiole width
PTL	Petiolule length
ROL	Root length
RAW	Root apical width
RMW	Root middle width
RBW	Root basal width
INW	Inflorescence width
INL	Inflorescence length
PDL	Peduncle length
ML(+S)	Mericarp length with seed
ML(-S)	Mericarp length without seed
MEW	Mericarp width
MET	Mericarp thickness
STP	Stylopodium length

LRL	Lateral rib length
PCL	Pedicel length
UML	Umbellet length
UMW	Umbellet width
SCL	Secondary leaflet
PL	Primary leaflet
IN	Inflorescence
IF	Immature fruit
VV	Vallecular vittae
CV	Commissure vittae
VB	Vascular bundles

Anatomical studies

Fresh plant components (stem, petiole, root, and fruit) of SV and LW were fixed in 50 % ethanol. The freehand sections of the mericarp, stem, petiole, and root were cut with the help of a razor blade and are made as thin as possible. Safranin and cotton blue were used to stain the various sections. After staining, the sections were transferred to a petri plate containing distilled water and washed gently to remove extra stain. A small drop of glycerol was placed on a clean microscope slide and the sections were carefully transferred to the slide. A coverslip is gently placed over the slide to avoid any bubbles. The stained or unstained slides were imaged using a stereo microscope and Kyowa getner microvision XLP-35 trinocular microscope (magnification 10x to 40x) with a camera (10MP).

Extraction of essential oils

The fresh, complete plant material is thoroughly washed with distilled water. After being shade-dried, the essential oil was extracted for 6-7 hours using a Clevenger apparatus. 700 mL of distilled water was added to 100 grams of plant material. The extracted oil was then collected in glass vials, and the moisture was removed using Sodium sulfate.

Gas chromatography-mass spectrometry (GC–MS) analysis

A GC (Agilent Technologies) fitted with a ZB-5MS (nonpolar) column (30 m x 0.25 mm i.d., 0.25 µm) was used to perform the analysis. Helium was used as the carrier gas, and the linear velocity was 1.05 mL/min. The oven's temperature was set to 70 °C for five minutes, then raised by 4 °C every minute until it reached 220 °C, and maintained for five minutes. The MS parameters are as follows: The ionisation energy and mass range was 70 eV and 40–800 m/z, respectively. The injector temperature was kept at 240 °C. The interface temperature was 250 °C. The sample was prepared in DCM at 10 mg/1.5 mL, with 2 µL utilized for injection at split mode (10:1). The compounds were tentatively identified by comparing their mass spectrum and retention times with the ADAM, NIST (NIST2020.L), and WILEY libraries, along with a comparison of mass spectral data with previously reported literature, which was used for confirmation.

Statistical analysis

The collected data were organised in a Microsoft Excel spreadsheet for additional analysis. An ANOVA for each variable was performed to evaluate whether there were variations in the means among groups, followed by post hoc comparisons using Tukey's HSD test. Correlation and principal component analysis (PCA) were used to determine significant differences in quantitative morphological characteristics among populations under study. The statistical analysis of the quantitative data and the design of the chord diagram, alluvial diagram, box plot, and dendrogram were carried out using the statistical software Origin 2023.

Results

Quantitative character

Morphology of the vegetative parts

The morphological observations of the vegetative part revealed that SV typically range in length from 66 to 95 cm, and LW ranges from 57 to 61 cm in average length. The SV collected from Kothi has the greatest plant length, petiolule length, and compound leaf length, wide compound leaf width, long petiole, wide stem width and pinnula, long root, and

wide root width as compared to other locations. LW collected from Sissu has long plant length, compound leaf length, wide leaf width, long petiolule, and peduncle length. LW from Sissu and Bakarthach have wider widths and longer petioles. LW from Bakarthach has the longest pinnula length, a wide stem, root and petiole width, the longest roots. The average number of nodes in SV is 4 to 5, while in LW, they vary from 3 to 4 for all locations. Overall observations indicate that the SV specimens collected from the Kothi location and LW from the Sissu location display the longest and widest macromorphological characteristics compared to those from other places. The results indicate that when comparing SV and LW, SV is larger (Figure 8).

Morphology of the reproductive parts

In SV inflorescence width and length were 5 to 6.5 cm and 5.7 to 6.5 cm, respectively. Peduncle length was 10 to 11 cm and 16 to 17 cm in LW and SV, respectively. The mericarp length with the stylopodium was between 0.45 to 0.46 cm and 0.47 to 0.50 cm, and in LW and SV, respectively. Overall observation indicates that the SV from Kothi and Solang Valley showed the largest measurements. SV from Kothi had the longest inflorescence, lateral rib, pedicel length and mericarp, and the thickest mericarp. LW from Dehnasar showed the lowest measurements for each character.

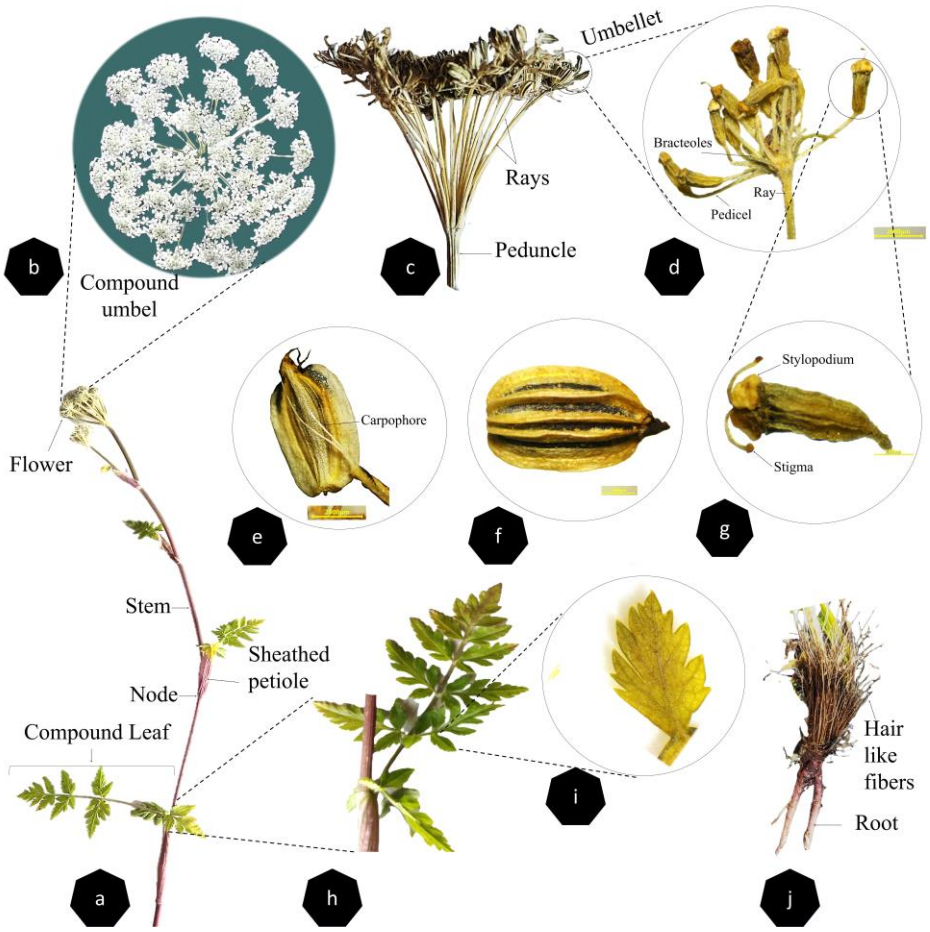

Figure 1. Morphological characters of *S. vaginatum* a) Whole plant showing compound leaf, nodes, stem, sheathed petiole, and flower, b) Inflorescence (Compound umbel), c) Mature flower showing peduncle and rays, d) Umbellet showing immature fruit, pedicel, bracteoles, and ray, e) Mature fruit showing carpophore, f) Mericarp dorsal view, g) Immature fruit, h) Compound leaf, i) Enlarged image of pinnulet, j) Root covered with hairlike fibers.

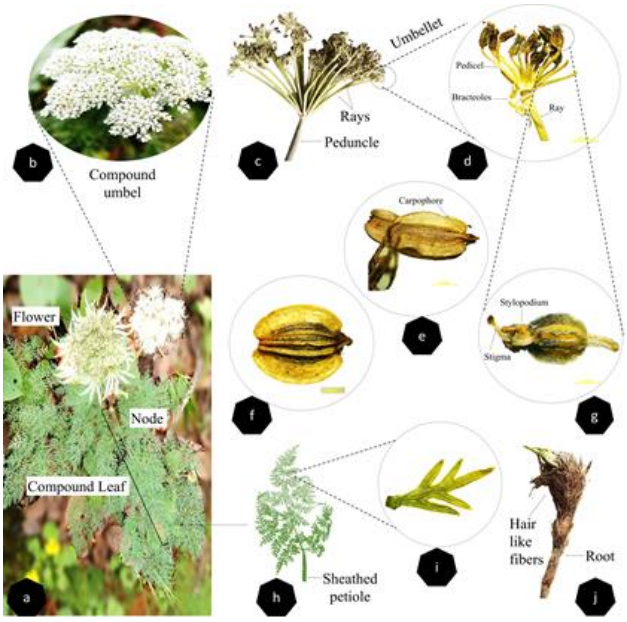

Figure 2. Morphological characters of *L. wallichiana* a) Whole plant showing compound leaf, node, and flower, b) Inflorescence (Compound umbel), c) Mature flower showing peduncle and rays, d) Umbellet showing immature fruit, pedicel, bracteoles, and ray, e) Mature fruit showing carpophore, f) Mericarp dorsal view, g) Immature fruit, h) Compound leaf, i) Enlarged image of pinnulet, j) Root covered with hairlike fibers.

LW from Dehnasar had a small inflorescence width and length, a short peduncle, short and thin mericarp, and the shortest stylopodium. Analysis of SEM images showed that the surface of the mericarp in both species is irregularly reticulate with stomata present. In LW, trichomes were also observed on the mericarp surface (Figure 7). A mericarp was emarginated at the base in LW, while a non-emarginated base was present in SV (Figures 1 and 2). When both the species were compared, the SV showed slightly wider and longer inflorescence, longer peduncles, and mericarp, while LW had wider mericarps and longer and wider umbellets. LW from Sissu showed the lowest values for each measurement (Figure 8).

Qualitative characteristics

A comparative analysis of qualitative, quantitative (Figure 8), and anatomical characters is provided in Table 2.

Anatomical analysis

Figure 3. Morphology and anatomy of stem. *S. vaginatum* a) Transverse section of stem showing VB arranged in ring, b) Enlarged image of VBs, c) enlarged image of single VB. *L. wallichiana* d) Transverse section of stem showing VB

arranged in ring, e) Enlarged image of VBs, f) enlarged image of single VB. g) *L. wallichiana* stem surface showing trichomes, h) *S. vaginatum* stem surface showing no trichomes. PT=Pith, VB=Vascular Bundle, GR=Groove, RD=Ridge, PE=Parenchyma tissue, SC=Sclerenchyma cells, XL=Xylem, PR=Pericycle, HD=Hypodermis, EP=Epidermis, CB=Cambium, PH=Phloem, SD=Secretary duct, PC=Parenchyma cell, TR=Trichome, TA=Trichome absent.

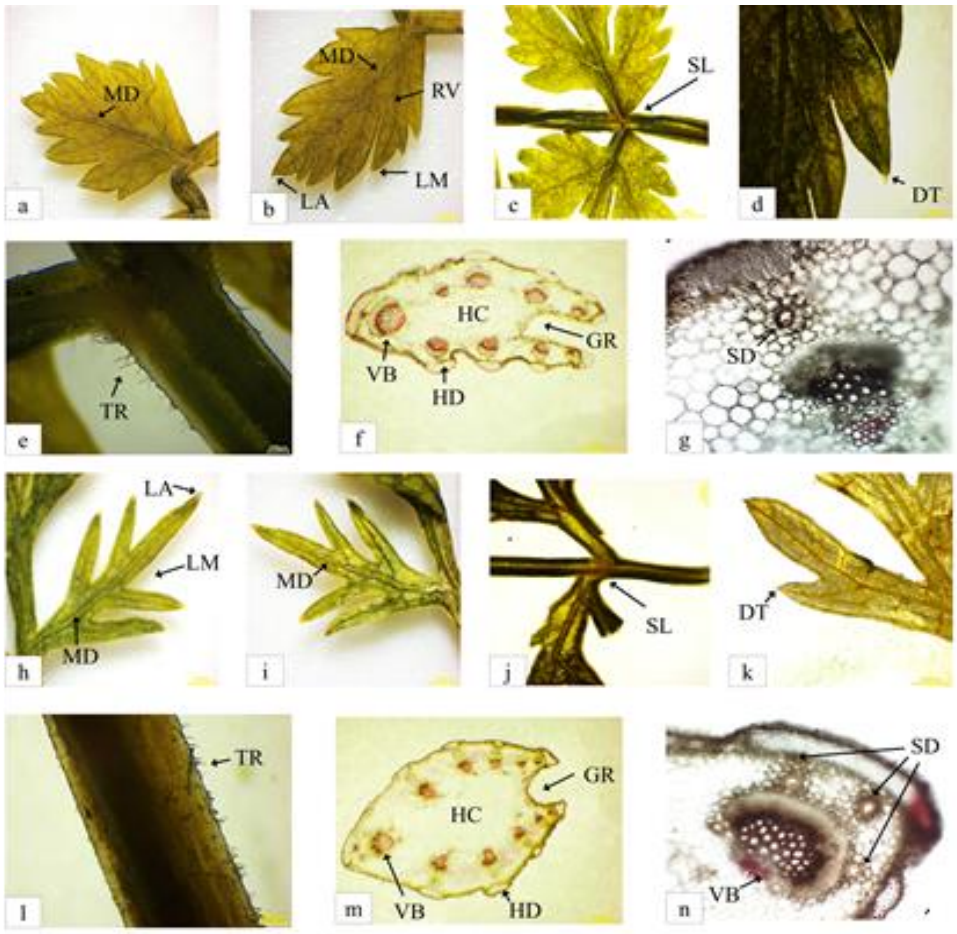

Figure 4. Morphology of leaflet. *S. vaginatum* a) Adaxial surface of leaflet, b) Abaxial surface of leaflet, c) Sessile leaflet, d) leaflet margin is dentine, *L. wallichiana* h) Adaxial surface of leaflet, i) Abaxial surface of leaflet, j) Sessile leaflet, k) leaflet margin is dentine. Morphology and anatomy of petiole. *S. vaginatum* e) Petiole surface showing trichomes, f) Transverse section of petiole showing arrangement of VB, g) Enlarged picture of single large VB showing single secretary duct. *L. wallichiana* l) Petiole surface showing trichomes, m) Transverse section of petiole showing arrangement of VB, n) Enlarged picture of single large VB showing 3 secretary ducts. MD=Midrib, LA=Leaflet apex, RV=Reticulate venation, LM=Leaflet margin, RV=Reticulate venation, SL=Sessile leaflet, DT=Dentate, TR=Trichome, HC=Hollow cavity, VB=Vascular bundle, HD=Hypodermis, GR=Groove, SD=Secretary ducts

Stem

The VBs were surrounded by sclerenchyma. The Horizontal Length of the hypodermis was larger in LW (0.69 mm) compared to SV (0.40 mm). The width of the hypodermis was slightly larger in LW (0.15 mm) compared to SV (0.14 mm). The width of VB was slightly larger in SV (0.39 mm) compared to LW (0.37 mm). The secretary ducts in the stem were significantly larger in LW (0.14 mm) compared to SV (0.07 mm). The distance between two VB was larger in LW (0.50 mm) than in SV (0.47 mm) (Figure 3).

Petiole

Each VB had a bundle cap. The width of parenchymatous cells was wider in SV (0.23 mm) compared to LW (0.18 mm) (Figure 4).

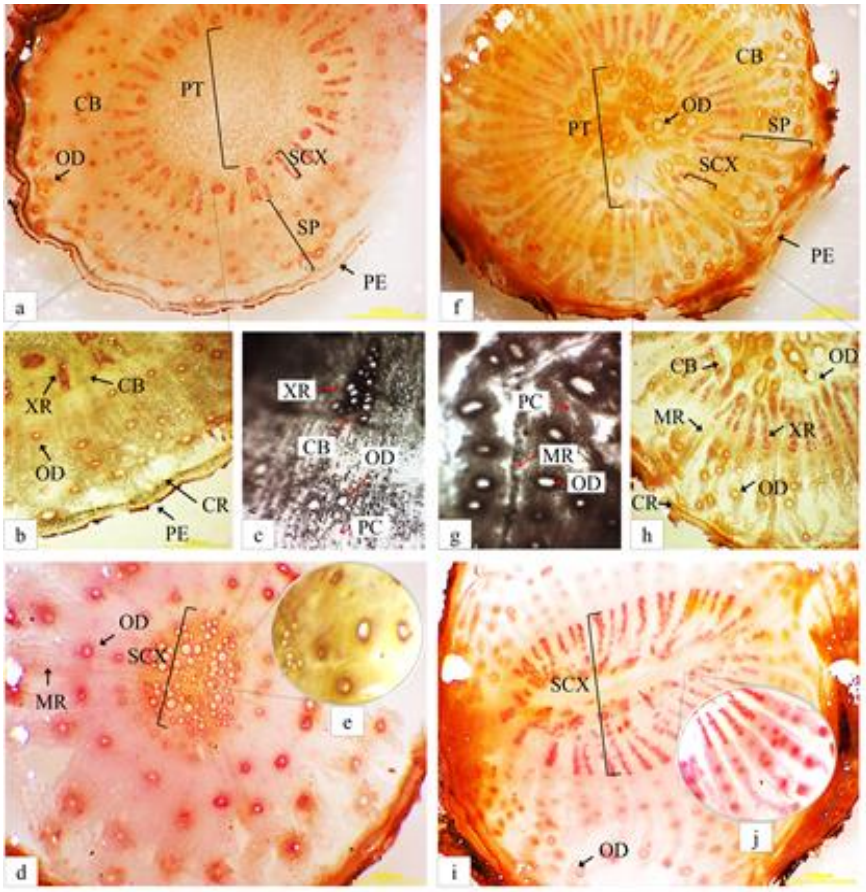

Figure 5. Anatomy of Root. *S. vaginatum* a) Transverse section of mature stem showing pith, xylem rays, secondary phloem, and cambium, b) Enlarged picture of a portion of transverse section of stem showing peridermis and cortex portion, c) Close up of transverse section of root showing xylem ray and secondary phloem with many oil ducts, e) Magnified image showing xylem vessels and oil ducts. *L. wallichiana* f) Transverse section of mature stem showing pith, xylem rays, secondary phloem, and cambium, g) Close up of transverse section of root showing xylem ray and secondary phloem with many oil ducts, h) Enlarged picture of a portion of transverse section of stem showing peridermis and cortex portion, i) Transverse section of Immature root, j) Magnified image showing xylem rays and oil ducts. PT=Pith, CB=Cambium, OD=Oil duct, SCX=Secondary xylem, SP=Secondary phloem, PE=Periderm, XR=Xylem ray, CR=Cortex, MR=Medullary ray.

Root

The peridermis was thicker in LW (2.13 mm) compared to SV (1.63 mm). The width of the secretary ducts was significantly larger in LW (0.18 mm) compared to SV (0.07 mm). The width of the secondary phloem was slightly larger in LW (1.56 mm) compared to SV (1.38 mm). The width of the pith in the mature root was larger in LW (2.62 mm) compared to SV (2.04 mm) (Figure 5).

Mericarp (Carpological analysis)

The outermost layer of the mericarp was the pericarp, which was divided into the exocarp, mesocarp, and endocarp. The cells were thin-walled in exocarp, while the mesocarp was multilayered and made up of large parenchymatous cells. Sclerenchymatic tissues were present in the marginal wings, and VB can be seen in the dorsal and marginal ribs. The endosperm was copious. The width of the endosperm in LW (2.40 mm) was wider than in SV (2.01 mm). The endosperm in SV (0.68 mm) was thicker than in LW (0.57 mm). Overall result indicates that LW had greater measures for several metrics, including commissure width, hypodermis horizontal length in stem and petiole, peridermis thickness in roots, and VB width in many regions. SV had higher values for several parameters, such as endosperm thickness, stem structure width, and root xylem vessel width (Figure 8).

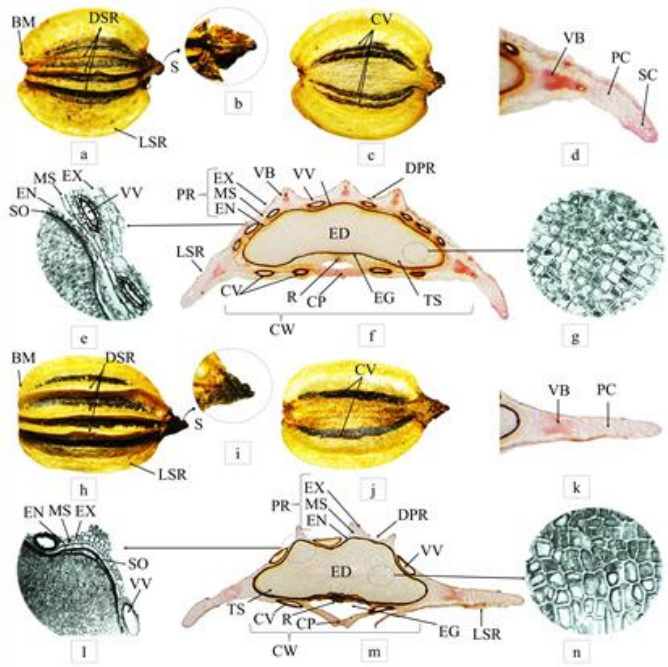

Figure 6. Morphology of mericarp *L. wallichiana* a) Dorsal surface of mericarp showing dorsal ribs and lateral ribs, b) Stylopodium, c) Ventral surface of mericarp. Anatomy of mericarp *L. wallichiana* d) lateral rib showing parenchymatous and sclerenchymatous cells, e) Pericarp showing different layers, f) Transverse section of fruit, g) endosperm showing irregular cells. Morphology of mericarp *S. vaginatum* a) Dorsal surface of mericarp showing dorsal ribs and lateral ribs, b) Stylopodium, c) Ventral surface of mericarp. BM=Base of mericarp, DSR=Dorsal secondary rib, LSR=Lateral secondary rib, S=Stylopodium, CV=Commissural vitae, VB=Vascular bundle, PC=Parenchyma, SC=Sclerenchyma, SO=Seed coat, EN=Endocarp, MS=Mesocarp, EX=Exocarp, VV=Vallecular vittae, PR=Pericarp, DPR=Dorsal primary rib, CW=Commissure width, R=Raphae, CP=Carpophore, EG=Endosperm groove, TS=Testa, ED=Endosperm

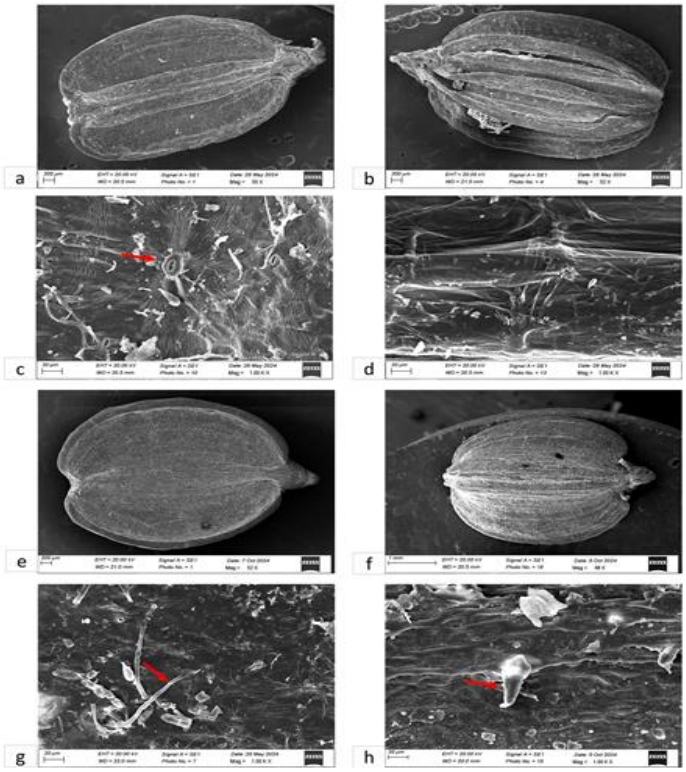

Figure 7. SEM images of *S. vaginatum* a) Mericarp in ventral view, b) Mericarp in dorsal view, c) Mericarp surface showing stomata, d) Outer surface of the stem. *L. wallichiana* e) Mericarp in ventral view, f) Mericarp in dorsal view, g) Mericarp surface showing trichomes, h) Outer surface of the stem showing trichomes

Statistical analysis

Correlation between morphological characteristics:

There was a strong positive correlation between PLL and CLL, CMW, CBW, CFL, CFW, PIL, and ML(-S). CLL positively correlates with CMW, CBW, CFL, and CFW. CAW showed a positive correlation with SAW, PW, and RBW. ROL positively correlated with RAW and INL. INW positively correlated with ML(+S). PDL positively correlated with ML(+S) and ML(-S). PIL negatively correlated with RMW. CMW, PIL, PDL, ML(+S) and RMW negatively correlated with ML(-S) (Figure 8).

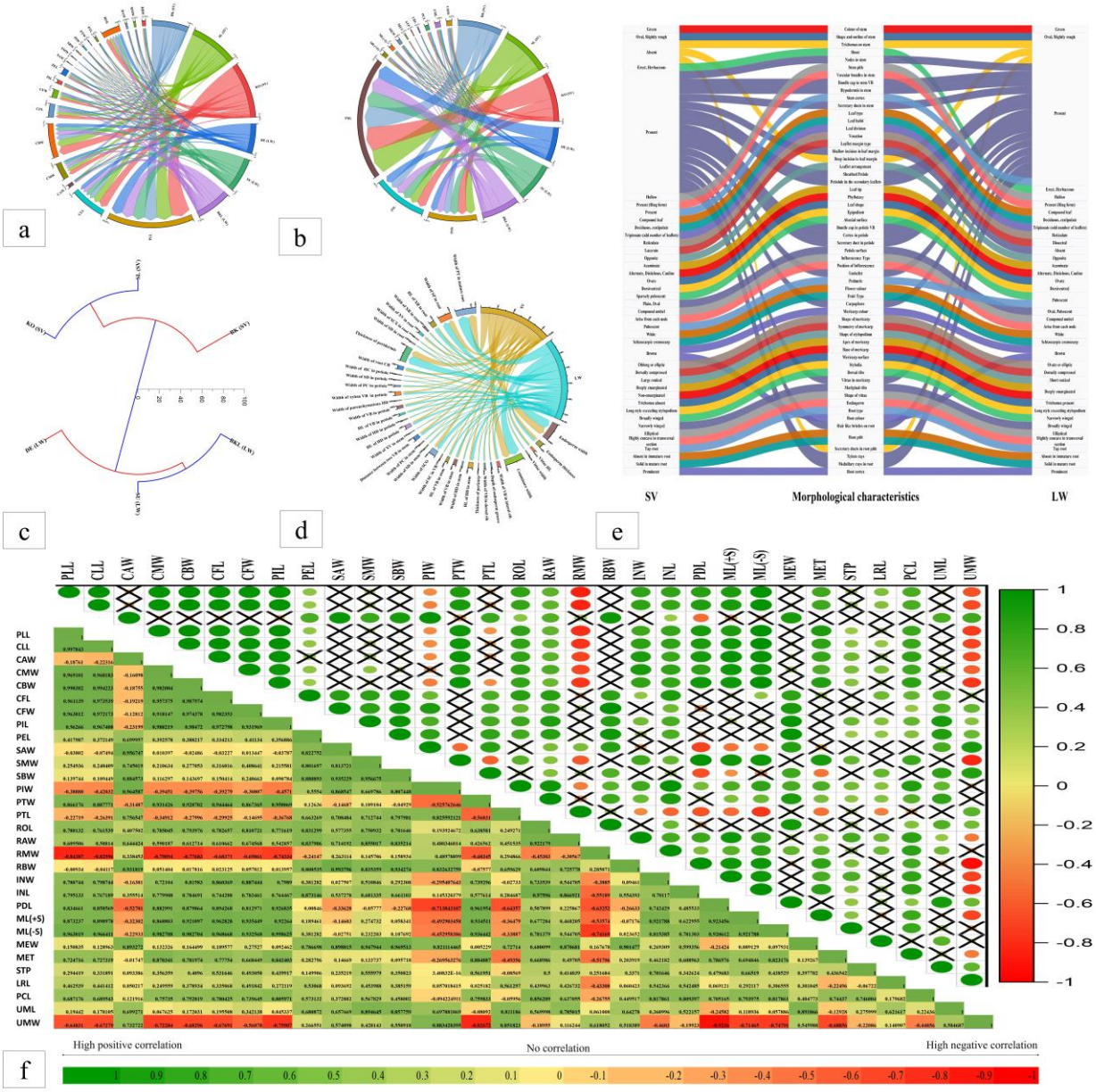

Figure 8 (Find the HD image in supplementary). a) Chord diagram showing comparative analysis of vegetative characters of *S. vaginatum* and *L. wallichiana* collected from different locations, b) Chord diagram showing comparative analysis of reproductive characters of *S. vaginatum* and *L. wallichiana* collected from different locations, c) Cluster dendrogram shows the hierarchical clustering of the plants collected from different locations d) Chord diagram showing comparative analysis of anatomical characters of *S. vaginatum* and *L. wallichiana* collected from different locations, e) Alluvial plot showing comparative analysis of morphological characters of *S. vaginatum* and *L. wallichiana* f) Pearson's correlations matrix between morphological characters of plants collected from different locations. Dark green colour = positively strong correlation (+0.90 to +1), Light green colour = Moderate positive correlation (+0.361 to +0.899), Yellow colour = nonsignificant correlation (-0.267 to +0.360), Red colour = negatively correlation (-0.361 to -1).

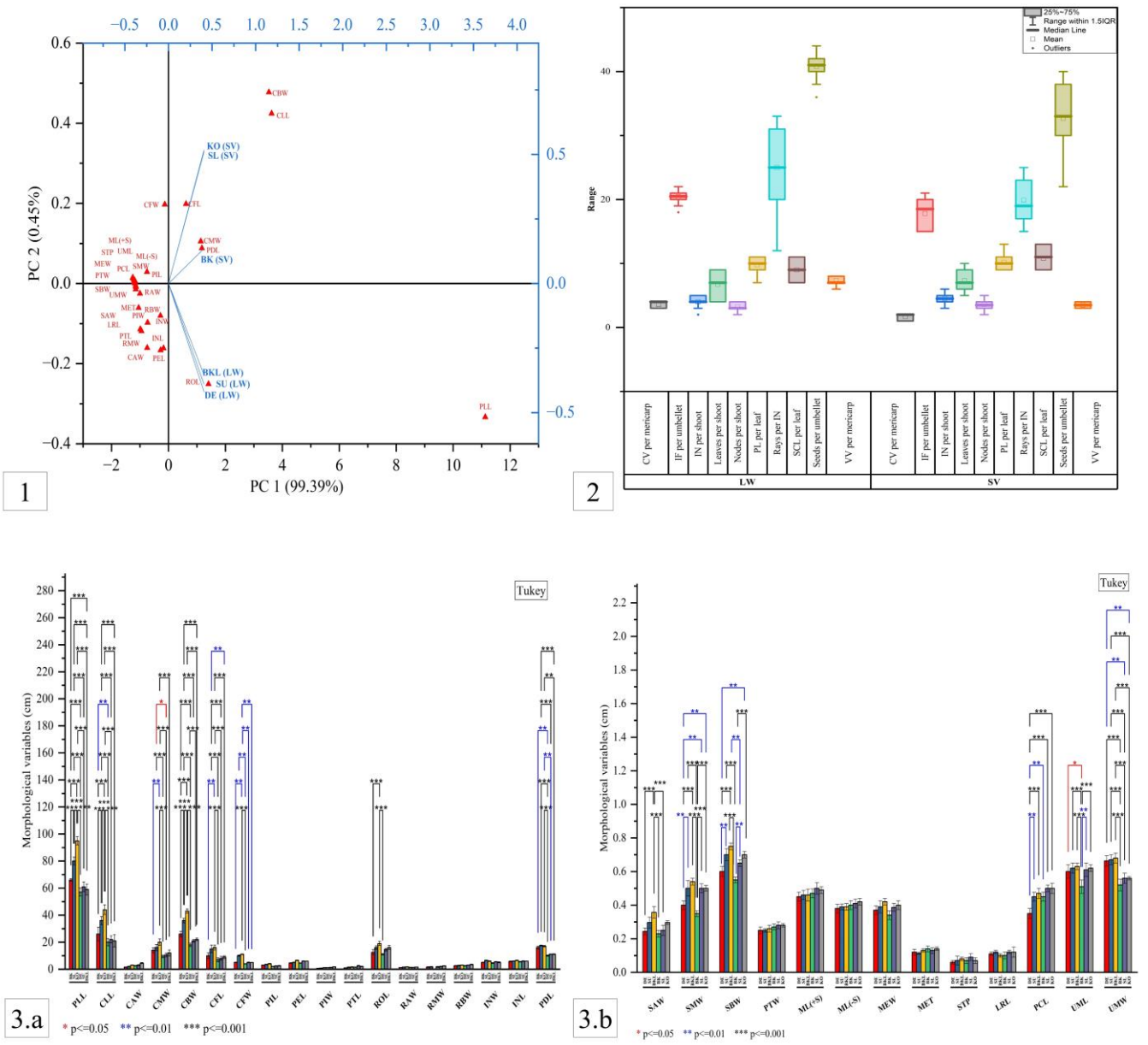

Figure 9 (Find the HD image in supplementary). 1) PCA biplot showing the relationship among morphological characters of plants collected from different locations 2) Boxplots of the morphological characters of *S. vaginatum* and *L. wallichiana*. The black diamond markers represent outliers. 3. a) Analysis of variance (ANOVA) for 18 macromorphological characters 3. b) Analysis of variance (ANOVA) for 13 micromorphological characters. SCL=Secondary leaflet, PL=Primary leaflet, IN=Inflorescence, IF=Immature fruit, VV=Vallecular vittae, CV=Commissure vittae.

PCA

The PCA was carried out to determine which variable contributed more with its variance to the total variance observed in the plant species collected from different locations. 31 morphological characters were measured in cm to conduct PCA. The first two components of PCA analysis contributed 99.84% of the variability, with eigenvalues 5.963 for PC1 (contributed 99.39%) and 0.026 for PC2 (contributed 0.45%) of total variability. SV collected from Burwa, Solang Valley, and Kothi were plotted on the same right portion of PCA, showed positive values of eigenvectors to both PC1 (0.408, 0.407, 0.407) and PC2 (0.136, 0.510, 0.517), indicating the plants from Kothi and Solang Valley were closely related as compared to Burwa. LW from Sissu and Bakarthach were closely related as compared to Dehnasar (Figure 9), also shown in the dendrogram, refer to Figure 8.

Box and Whisker plot analysis

For box plot analysis, 10 characters were taken into consideration. There were differences in the quantitative morphological characters of both species, as shown in the boxplot analysis (Figure 9). CV per mericarp showed low

variation in both species. IN per shoot, showed a larger spread in the case of LW. Greater variability was observed in the case of LW for the character leaves per shoot. Nodes per shoot were moderate in both species. PL per leaf showed more variation in the case of LW. Rays per IN were larger incase of LW. SCL per leaf was the same in both species. LW showed higher values for WV per mericarp. LW showed greater variability in all the traits, which indicated its stronger adaptability. There can be possible significance of traits for taxonomic importance, such as CV per mericarp and seeds per umbellet, which are highly conserved in both species.

ANOVA

An ANOVA for each variable was performed with a $p \leq 0.05$ (*=significant), $p \leq 0.01$ (**=highly significant), and $p \leq 0.001$ (***=very highly significant) to evaluate whether there were variations in the means among groups, followed by post hoc comparisons using Tukey's HSD test. Analysis of variance (ANOVA) was used to test for differences between the plants collected from different locations and morphological parameters. The phenotypic variance for 31 traits of LW and SV was estimated. Significant differences were observed among the macromorphological and micromorphological characters, such as PLL, CLL, CMW, CBW, CFL, CFW, ROL, PDL, and SAW, SMW, SBW, PCL, UML, and UMW, respectively of plant species from different locations (Figure 9).

Table 2. Comparative morphological and anatomical data on shoot, leaves, petiole, root, inflorescence, and mericarp of SV and LW. (✓) indicate Present, (-) indicate Absent.

Plant characters	Quantitative and qualitative traits	SV	LW
Shoot Morphology	Erect, herbaceous, nodes are present, green, oval, outline with ridges and furrows, surface slightly rough	✓	✓
	Trichomes on the stem surface	-	✓
Shoot Anatomy	Hollow pith, VB (ring form), 17 to 26, presence of thick fiber bundle, secretary canals	✓	✓
Leaves Morphology	Compound leaf, deciduous, ovate, exstipulate, acuminate, tripinnate (odd number of leaflets), pinnule 09-11, pinnulets 07-11, alternate, distichous, cauline, venation reticulate, abaxial surface is sparsely pubescent, sessile secondary leaflet, dentate	✓	✓
	Leaflet margin lacerate, shallow incision	✓	-
	Leaflet margin dissected, deep incision	-	✓
Petiole Morphology	Sheathed petiole, sheaths inflated, grooves on adaxial side, pubescent	✓	✓
Petiole Anatomy	09 to 10 small VBs, a single secretary canal is present above a single large VB, and at each VB	✓	-
	07 to 10 small VBs, three secretary canals are present above a single large VB, and a single secretary canal is present above each VB	-	✓
Root Morphology	Tap root, sometimes divided, brown, covered with long fibre like bristles present	✓	✓
Root Anatomy	Solid pith in mature root and absent in immature root, oil ducts not observed in pith of mature root, xylem is surrounded by phloem and pith is absent in early stages but present in the mature root, xylem rays are present, xylem is followed by secondary phloem, medullary rays are prominent in secondary phloem, cortex is prominent and covers a major portion of the root	✓	✓
	Few secretary ducts are present in phloem	✓	-
	Oil ducts were observed in the pith of the mature root, numerous secretary ducts are present in the phloem	-	✓
Inflorescence Morphology	Arise from each node, compound umbel, peduncle and pedicel pubescent, flower white, five (petals, sepals, stamens) epigynous, bisexual, two mericarps per flower dissected, bracteole exceeding umbellule	✓	✓
	Rays 12-24, number of immature fruits, and mericarp per umbellet 15-20 and 30-40, respectively	✓	-
	Rays 17-33, number of immature fruits, and mericarp per umbellet 18-22 and 36-44, respectively	-	✓
Mericarp Morphology	Schizocarpic cremocarp, brown, homomorphic, dry, mature fruit is attached to carpophore, dorsally flattened, three secondary dorsal ribs, two secondary lateral ribs, and two primary dorsal ribs in each mericarp	✓	✓

	Oblong or elliptic shape, large conical stylopodium, non-emarginated base, dorsal ribs and marginal ribs are broadly winged, trichomes not observed	✓	-
	Ovate-oblong shape, short conical stylopodium, deeply emarginated base, dorsal ribs are slightly thickened and marginal ribs are broadly winged, trichomes are present	-	✓
Mericarp Anatomy	Vittae shape: elliptical, endosperm highly concave in transversal section, copious, presence of minute embryo, small thin-walled and irregularly shaped endosperm cells, oil ducts are present in the endosperm, VB present in the dorsal and lateral rib	✓	✓
	Number of vallecular vittae=03-04, commissural vitae= 01-02	✓	-
	Number of commissural vitae=05-08, commissural vitae=02	-	✓

Phytochemicals screening of the essential oils

The phytochemical screening of the essential oil of whole plants from the two species of *Selinum* was conducted and analyzed. Forty-one phytochemicals were found through phytochemical screening of the essential oil of both species. The list of compounds, along with their percent area, is provided in Table 3. Four compounds were common to both species, such as Z-β-Ocimene, 3,5-Nonadiyne, cis-β-Farnesene, and Bisabolol. Bornyl acetate, Spathulenol, epi-alpha-Muurolol, and δ-Cadinene were present in high percentages in SV and can be considered as putative chemotaxonomic markers compounds for these species. Oxacyclotetradecan-2-one 14-methyl, E-Nerolidol, Humulene epoxide II, and Z-β-Ocimene were detected in LW with area sum percentage of 9.25 %, 9.21 %, 6.17 %, 5.86 % respectively. Thus, it may be used as a key putative marker for LW. 21 distinguishable compounds are restricted to both SV and LW (Table 3).

Table 3. GC-MS phytochemicals profiling of essential oil for the two studied *Selinum* species

No.	Compound Name	RT (SV/LW)	Area sum %age	
			SV	LW
1.	Z-β-Ocimene	7.6169	3.03	-
2.	3,5-Nonadiyne	9.3093/9.3093	1.33	2.5
3.	Bornyl acetate	16.0467	8.24	-
4.	δ-Elemene	16.5429	2.11	-
5.	*α*-Copaene	18.9796	1.31	-
6.	Caryophyllene	20.3729	1.16	-
7.	cis-*β*-Farnesene	21.4862/21.4799	3.18	3.78
8.	Germacrene D	22.2879	2.47	-
9.	Bicyclogermacrene	22.7523	1.69	-
10.	γ-Cadinene	23.274	0.93	-
11.	*δ*-Cadinene	23.5476	3.75	-
12.	Selina-3,7(11)-diene	24.0883	1.6	-
13.	Germacrene B	24.5464	1.67	-
14.	Nerolidol (E)	24.7118	3.03	-
15.	Spathulenol	25.1762	5.76	-
16.	Caryophyllene oxide	25.3353	1.08	-
17.	(E)-Sesquilavandulol	26.6395	1.07	-
18.	epi-*α*-Cadinol	26.9513	1.48	-
19.	*β*-Eudesmol	27.2376	2.33	-
20.	epi-alpha-Muurolol	27.3457	5.61	-
21.	α-Bisabolol	28.1855/28.1728	3.68	3.76
22.	((8R,8aS)-8-Isopropyl-5-methyl-3,4,6,7,8,8a-hexahydronaphthalen-2-yl)methanol	31.2902	1.1	-
23.	m-Camphorene	35.0247	3.12	-
24.	*p*-Camphorene	35.8454	1.15	-
25.	*α*-Pinene	5.1167	-	1.29
26.	Z-*β*-Ocimene	7.617	-	5.86
27.	Cryptone	12.7066	-	1.07
28.	p-Methyl acetophenone	13.0756	-	1.13
29.	7-epi-1,2-Dehydro sesquicineole	21.9061	-	2.11
30.	ϒ-Muurolene	22.1288	-	1.8

31.	α-Curcumene	22.3006	-	1.91
32.	Phenyl ethyl 2-methylbutanoate	22.4342	-	1.53
33.	Phenyl ethyl 3-methyl butanoate	22.5614	-	3.49
34.	α-Muurolene	22.8414	-	1.09
35.	E-Nerolidol	24.6991	-	9.21
36.	Humulene epoxide II	26.0797	-	6.17
37.	Oxacyclotetradecan-2-one, 14-methyl	27.2376	-	9.25
38.	α-Cadinol	27.333	-	1.09
39.	Isofuranodienone	31.3538	-	2.85
40.	n-Hexadecanol	32.5053	-	2.5

Note: RT: Retention time

Discussion

Visual conflicts can arise due to similarities in the physical appearance of plants, potentially leading to adulteration and a decline in drug quality. To prevent such adulteration, accurate identification of plants is essential. Compared to the plants from Burwa, a higher-altitude region, the Solang Valley plants were taller and more vigorous when the traits of SV collected from three distinct locations were examined. In the case of LW, the plants collected from Dehnasar are smaller, likely due to variations in altitude and environmental factors that influence plant growth, meanwhile, the plants from Bakarthach and Sissu show greater similarities in size and growth. SV is taller and broader compared to LW, which is shorter and more compact. The most significant differences are seen in the leaves of SV and LW. In SV, the leaf margins have shallow incisions, whereas in LW, the leaf margins exhibit deep incisions and both plants have sheathed petioles. However, in both species, the leaves are pinnately compound. Srivastava et al. (2018) noted a similar observation in both plant species. Leaves in both species are dentate and dissected, according to Mahajan et al. 2020 this feature indicates their adaptation to shaded and cooler environments (Mahajan et al. 2020), but it was not observed in the study conducted by Srivastava et al. (2018). LW has a higher number of oil ducts and the presence of oil ducts is recorded in the root pith of LW. Primary secretory ducts are found in the cortex and pith of *Kielmeyera appariciana,* a member of the family callophyllacease (Costa et al., 2021). The dorsal ribs are more prominently winged in SV compared to LW. A study conducted on other species of this genus, i.e. *Selinum carvifolia* showed SEM analysis of the mericarps (Ostroumova et al., 2019) showed the presence of these traits. The trichomes were observed on the leaflets, immature fruit, and inflorescences of both species, but in LW, they were also present on the fruit and stem. A study on the genera *Mackinlaya* and *Schoenolaena* of the Apiaceae family examined and described the presence of trichomes (Liu et al. 2016). The use of Gas Chromatography (GC-MS) as an integrated analytical technique supplements traditional systematic botany, remarkably improving recognition and identification of plant species by analysing the similarities and differences in their secondary metabolites (Olivia et al., 2021). Specifically, members of the Apiaceae family were characterized by aromatic nature and rich in essential oil with a wide range of bioactive secondary metabolites (Thiviya et al. 2021). Additionally, by comparing chemical profiles, GC-MS makes it easier to comprehend evolutionary linkages, investigates novel trends in plant taxonomy research, and makes a substantial contribution to the area of plant taxonomy (Abdelfattah et al., 2024). The GC-MS detects unique compounds in plants, which aids in distinguishing species, subspecies, or genus that may appear morphologically similar. These secondary metabolites may serve as chemotaxonomic markers for this genus. On the other hand, the oil of the root of LW is rich in 3,5-nonadiyne (90.5%) (Padalia et al., 2012), which can serve as a key marker for identifying this species and aid in avoiding adulteration. As seen in other groups, essential oils were a useful tool for characterising and distinguishing *D. carota* subspecies. The quantity of phenylpropanoids distinguishes *D. carota* subsp. *maximus* and *subsp. halophilus'* essential oils differ from other subspecies (Tavares et al. 2014). The variations observed in this study may result from differences in altitude, climatic stress, or the adaptive responses of plants to shifting environmental conditions. The identification of morphological traits is the first step in correctly identifying a plant, and this study offered a thorough understanding of the plant morphology and anatomy of both species. However, since environmental plasticity might alter these characteristics, identification on a morphological basis is challenging; therefore, we also incorporated the phytochemical profiles of both species. This divergent information can aid in the chemotaxonomy of plants, where accurate marker compound identification can contribute to accurate species identification and prevent taxonomic confusion between *Selinum* species.

Conclusion

This comparative analysis reveals that while SV and LW share many similarities in their morphological and anatomical features, there are significant differences in the presence of trichomes, the structure of sclerenchyma layers, leaf margins, secretory canals, inflorescence characteristics, fruit structure, and root anatomy. The leaves of both species exhibit

significant differences and can play an important role in the delimitation two species. However, the shape and size of the leaf can change according to the changes in environmental conditions; therefore, it is important to investigate the mericarp of these plants because the mericarp shows significant differences. The morphology of the roots is quite similar, but the anatomical analysis of both roots shows certain differences that can be utilised to avoid adulteration and contribute to the authenticity. This is the first study using SEM imaging for the morphology of the mericarp in SV and LW. We also reported the presence of trichomes on the mericarp and stem of LW through SEM analysis. A correlation study of morphological characteristics facilitates trait correlations, species identification, and breeding approaches. These findings can help guide future restoration and phytochemical studies on medicinal plants. In contrast, the chemotaxonomy approach involved analysing the essential oils of the studied species using GC-MS to assess the similarity of their metabolites. The chemical composition of essential oils clearly distinguishes the two species. The findings from both chemotaxonomy and classical taxonomy showed remarkable consistency in distinguishing between the two studied species. In conclusion, combining morphological features with phytochemical data revealed discriminating characteristics that differentiated the plant species. This approach can be applied across various plant species for precise identification and classification. The current study found trichomes on the surface of LW leaves and fruits, which may be investigated further to add additional characteristics to LW taxonomic keys. Since the reproductive characteristics are less susceptible to shifting environmental conditions, differences in fruit morphology and anatomy may aid in accurate species identification. The identification of marker compounds aids in the accurate authentication of species, which can be used in pharmaceutical research, ethnobotanical studies, and the preparation of taxonomic keys. Reliable identification offers an adequate basis for selecting authentic plant sources for pharmacological assessments and quality control of herbal formulations, helps prevent adulteration and substitution, and assures consistency in raw medicinal plant material.

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Author contributions

Ruchika Kumari: Writing-review & editing, Writing-original draft, Visualization, Software, Resources, Methodology, Data curation, and Funding acquisition.

Ashun Chaudhary: Writing-review & editing, Software, Formal analysis, Conceptualization, Supervision, Investigation, and Validation.

Conflict of interests

Authors declare no conflict of interest.

Ethics approval

Not applicable.

AI tool usage declaration

No AI and associated tools are used for writing scientific content in the article.

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