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COMPARATIVE ANALYSIS OF LEAF MORPHOLOGICAL CHARACTERIZATION AND HPLC-BASED PHYTOCHEMICAL PROFILING OF VITEX NEGUNDO GENOTYPES FROM DIFFERENT GEOGRAPHICAL REGIONS

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ABSTRACT
Vitex negundo L. (Lamiaceae) is a medicinally important shrub valued for its flavonoid-rich phytochemical profile, particularly vitexin, which contributes to its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The present study aimed to characterize morphological diversity and phytochemical potential among 30 V. negundo genotypes collected from different geographical regions of South India (Tamil Nadu, Kerala, Karnataka and Andhra Pradesh) through integrated leaf morphometric and HPLC-based phytochemical analysis. Seven quantitative leaf traits, namely petiole length, leaf length, mid leaflet width, larger leaflet length and width, and smaller leaflet length and width, were measured and subjected to descriptive statistics, correlation, path coefficient, hierarchical cluster, and principal component analyses. Considerable variability was observed among genotypes, with coefficients of variation ranging from 0.10 to 0.20. Correlation analysis revealed strong positive associations among leaflet width traits, while path analysis identified larger leaflet width as the principal direct determinant of petiole length. Cluster analysis grouped the genotypes into eight clusters, indicating that morphological similarity did not strictly follow geographic origin. Principal component analysis showed that mid leaflet width, leaf length, and petiole length were the major contributors to overall variation. HPLC fingerprinting of vitexin revealed pronounced differences in phytochemical accumulation among genotypes. Genotype VN13 (Wayanad, Kerala) exhibited superior leaf morphology and the highest chromatographic response (total peak area 106,671,402; major peak 71.34%), identifying it as the most promising genotype for phytochemical exploitation. In contrast, genotype VN9 (Thoothukudi) recorded the smallest leaf dimensions and the lowest chromatographic yield (total peak area 33,737,182), marking it as the least promising accession. These findings highlight the combined utility of morphological and chemical profiling for selecting elite V. negundo germplasm for cultivation, conservation, and pharmaceutical applications.

KEYWORDS: *Vitex Negundo*, HPLC, Leaf Morphology, Phytochemical profiling, Cluster analysis

1. INTRODUCTION
Vitex negundo L. (Lamiaceae) is a perennial medicinal shrub widely distributed throughout tropical and subtropical Asia. The species has long been utilized in traditional systems of medicine for the treatment of inflammation, arthritis, respiratory disorders, wounds, microbial infections, and fever owing to its rich content of biologically active secondary metabolites, including flavonoids, iridoids, lignans, terpenoids, and phenolic compounds [1,2]. Among these phytochemicals, vitexin has attracted considerable attention because of its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties and is widely used as a marker compound for quality evaluation of *V. negundo* leaf extracts [3].
Considerable morphological variation exists among *V. negundo* populations collected from different geographical regions, making germplasm identification and classification challenging. Leaf traits such as petiole length, leaflet length, leaflet width, mid-leaf dimensions, and leaflet size have been recognized as valuable taxonomic characters for evaluating genetic diversity and distinguishing morphotypes [4]. Environmental factors including climate, altitude, and soil conditions also influence morphological expression and phytochemical accumulation, resulting in variation in medicinal quality among geographical populations.
High-Performance Liquid Chromatography (HPLC) is a reliable analytical technique for qualitative and quantitative assessment of phytochemicals in medicinal plants. HPLC fingerprinting enables authentication, quality control, and identification of elite chemotypes based on marker compounds. [3] demonstrated significant geographical variation in vitexin content among *V. negundo* populations while maintaining conserved chromatographic fingerprints, indicating the usefulness of vitexin as a phytochemical marker. Therefore, integrating leaf morphological characterization with HPLC-based phytochemical profiling provides an effective approach for identifying superior *V. negundo* genotypes for conservation, breeding, and pharmaceutical applications.

MATERIALS AND METHODS

Plant Materials

Thirty *Vitex negundo* genotypes were collected from different geographical regions of South India, comprising twenty-six genotypes from Tamil Nadu, two from Kerala, one from Pondicherry and one from Andhra Pradesh. Healthy, mature, and fully expanded leaves were collected from each genotype during the same growth stage to minimize environmental variation. Plant identity was confirmed using standard taxonomic descriptions, and voucher specimens were maintained for future reference.

Leaf Morphological Characterization

Morphological observations were recorded using ten mature leaves randomly collected from each genotype following standard descriptors described by [4]. The quantitative characters evaluated included:

- Petiole length (cm)
- Leaf length (cm)
- Mid leaflet length (cm)
- Mid leaflet width (cm)
- Larger leaflet length (cm)
- Larger leaflet width (cm)
- Smaller leaflet length (cm)
- Smaller leaflet width (cm)

Measurements were recorded using a digital Vernier caliper and a measuring scale, and the mean values were subjected to statistical analysis for comparison among genotypes.

Preparation of Leaf Extract

Fresh leaves were shade dried, powdered, and extracted using methanol in a Soxhlet apparatus for 18 h. The extracts were filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The dried extracts were stored at 4 °C until HPLC analysis [3].

HPLC Analysis of Vitexin

Vitexin in *Vitex negundo* leaf extracts were analyzed by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) using a C18 column coupled with a PDA detector. The mobile phase consisted of methanol and water at a flow rate of 1.0 mL min^{-1}, and chromatograms were recorded at 254 nm. A 20 µL volume of the vitexin standard and each methanolic sample extract was injected into the HPLC system. Vitexin in the samples was identified by comparing the retention time of the sample peaks with that of the authentic vitexin standard, and the relative vitexin content was determined from the corresponding peak area under identical chromatographic conditions [3].

Statistical Analysis

Morphological data were analyzed using descriptive statistics, analysis of variance (ANOVA) [5], correlation analysis, path analysis, Hierarchical cluster analysis and principal component analysis (PCA) to determine variability among genotypes. HPLC data were subjected to analysis of variance, and mean comparisons were performed to identify significant differences in vitexin content among geographical populations. The statistical analysis was done by adopting the standard procedures [6].

RESULTS

Mean performance of vitex genotypes

Considerable variation was observed among the 30 *Vitex negundo* genotypes for all seven leaf morphological traits (Table 1). Petiole length ranged from 3.0 cm (VN9) to 6.5 cm (VN13), with a mean of 4.9 ± 0.9 cm. Leaf length varied between 9.0 cm (VN9) and 15.0 cm (VN13), averaging 11.6 ± 1.3 cm. Mid leaflet width ranged from 3.0 cm (VN2, VN4, VN9, VN12, VN16, VN20 and VN22) to 5.0 cm (VN13), while larger leaflet length varied from 7.5 cm (VN9) to 12.0 cm (VN13). Larger leaflet width ranged from 3.0 cm to 5.5 cm (VN13), smaller leaflet length from 4.5 cm (VN9) to 8.0 cm (VN13), and smaller leaflet width from 2.0 cm to 4.0 cm (VN13). Among all genotypes, VN13 (Wayanad, Kerala) recorded the highest values for almost all leaf traits, whereas VN9 (Thoothukudi) exhibited the lowest values. The coefficients of variation ranged from 0.10 to 0.20, indicating moderate morphological variability among the *V. negundo* genotypes collected from different geographical regions.

Table 1. performance of *Vitex negundo* genotypes

Genotypes	Location	Petiole Length	Leaf Length	Mid Leaflet width	Larger leaflet length	Larger leaflet width	Smaller Leaflet length	Smaller leaflet width
VN1	Anaikatti	5.4	11.2	4	9	4	6	3
VN2	Coimbatore	6	12.1	3	10	5	7	2
VN3	Pollachi	4.9	12.5	4	11	3	5	3
VN4	Erode	4.3	11.4	3	8	4	6	2
VN5	Dharmapuri	5.3	10.3	4	9	5	7	3
VN6	Salem	5.1	11.4	3	10	3	5	2

VN7	Villupuram	4	11	4	9	3	5	3
VN8	Tirunelveli	4.7	10.1	4	10	4	5	3
VN9	Thoothukudi	3	9	3	7.5	3	4.5	2
VN10	Nagerkovil	5	12	3.5	10.5	4	6	2.5
VN11	Pechiparai, Kaniyakumar	6	13	4.5	11.5	5	7.5	3
VN12	Peechi, KFRI, Thrissur	4	12	3	8	3	5	2
VN13	Wayanad, Kerala	6.5	15	5	12	5.5	8	4
VN14	Nadugani, Gudalur	5.5	13.5	4	11.5	4	6	3
VN15	Andra Pradesh	4	10	4	11	3	5	3
VN16	Karanataka	5	11	3	8	4	6	2
VN17	Tiruchirappalli	6	12	4	9	5	7	3
VN18	Pondicherry	4.2	12.2	3	10	3	5	2
VN19	Ariyalur	5	12	4	11	4	6	3
VN20	Thanjavur	5.5	10	3	8	5	7	2
VN21	Dindugal	4	11	4	9	3	5	3
VN22	vellore	5	12	3	10	4	6	2
VN23	Chengam, Tiruvannamalai	6	13	4	10	5	5	3
VN24	Madurai	6	14	3	9	3	6.5	2.5
VN25	Theni	4	11.5	4	11	4	6.5	2.3
VN26	Chengalpet	3.5	10.5	3.4	9.8	3.5	6.8	2.8
VN27	Tenkasi	4.9	10.4	4.1	9.8	4.5	6	3.1
VN28	Krishnagiri	5.2	10.2	3.8	8.8	4.7	7	3.2
VN29	Kanchipuram	3.6	10.7	3.5	10	3.4	6.8	3
VN30	Trivandram, Kerala	5.5	12.5	4.3	11.3	5.1	7.3	3.1
	Mean	4.9	11.6	3.7	9.8	4.0	6.1	2.7
	Standard deviation	0.9	1.3	0.6	1.2	0.8	0.9	0.5
	Variance	0.8	1.7	0.3	1.5	0.7	0.9	0.3
	CV	0.2	0.1	0.2	0.1	0.2	0.2	0.2

Correlation analysis

The correlation analysis revealed significant positive associations among most of the leaf morphological traits of *Vitex negundo* genotypes (Table 2; Figure 1). Petiole length exhibited a strong positive correlation with larger leaflet width ($r = 0.713$, $p < 0.001$) and leaf length ($r = 0.643$, $p < 0.001$), followed by smaller leaflet length ($r = 0.544$, $p < 0.001$). Moderate positive correlations were also observed with mid leaflet width ($r = 0.327$, $p < 0.05$), larger leaflet length ($r = 0.320$, $p < 0.05$), and smaller leaflet width ($r = 0.301$, $p < 0.05$). Leaf length showed a significant positive relationship with larger leaflet length ($r = 0.548$, $p < 0.001$) but weak positive correlations with mid leaflet width ($r = 0.290$, $p < 0.05$) and smaller leaflet length ($r = 0.314$, $p < 0.05$), while its association with larger leaflet width ($r = 0.243$) and smaller leaflet width ($r = 0.254$) was not significant.

Among all the traits, the strongest positive correlation was recorded between mid-leaflet width and smaller leaflet width ($r = 0.908$, $p < 0.001$), indicating a high degree of association between leaflet width measurements. Mid leaflet width also showed significant positive correlations with larger leaflet length ($r = 0.631$, $p < 0.001$), larger leaflet width ($r = 0.430$, $p < 0.001$), and smaller leaflet length ($r = 0.308$, $p < 0.05$). Larger leaflet length was positively correlated with smaller leaflet width ($r = 0.521$, $p < 0.001$) and smaller leaflet length ($r = 0.265$, $p < 0.05$), whereas its correlation with larger leaflet width ($r = 0.225$) was not significant. A strong positive relationship was also observed between larger leaflet width and smaller leaflet length ($r = 0.741$, $p < 0.001$), while moderate correlations existed with smaller leaflet width ($r = 0.340$, $p < 0.01$). Overall, the correlation matrix indicated that leaf size-related traits were positively associated, suggesting coordinated growth among different leaf components in *V. negundo* genotypes collected from different geographical regions.

Table 2. Correlation Matrix for *Vitex negundo* leaf parameters

	Petiole Length	**Leaf Length**	**Mid Leaflet width**	**Larger leaflet length**	**Larger leaflet width**	**Smaller Leaflet length**	**Smaller leaflet width**
Petiole Length	1	0.643***	0.327*	0.32*	0.713***	0.544***	0.301*

Leaf Length	0.643***	1	0.29*	0.548***	0.243	0.314*	0.254
Mid Leaflet width	0.327*	0.29*	1	0.631***	0.43***	0.308*	0.908***
Larger leaflet length	0.32*	0.548***	0.631***	1	0.225	0.265*	0.521***
Larger leaflet width	0.713***	0.243	0.43***	0.225	1	0.741***	0.34**
Smaller Leaflet length	0.544***	0.314*	0.308*	0.265*	0.741***	1	0.323*
Smaller leaflet width	0.301*	0.254	0.908***	0.521***	0.34**	0.323*	1

*** *Correlation is significant at 0.001 level (two tailed)*
** *Correlation is significant at 0.01 level (two tailed)*
* *Correlation is significant at 0.05 level (two tailed)*

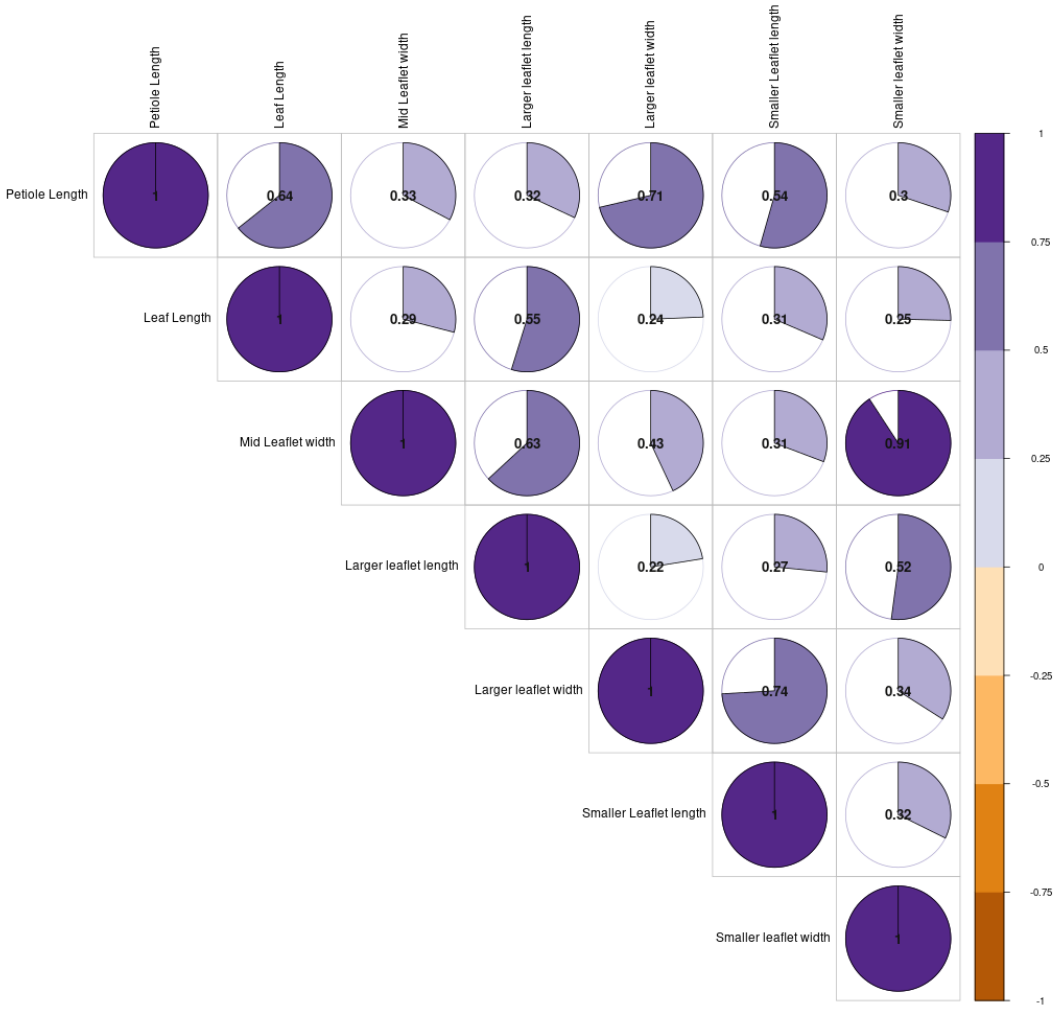


Figure 1. Correlation analysis diagram

Path analysis

The path coefficient analysis (Table 3; Figure 2) revealed that larger leaflet width exerted the highest positive direct effect on petiole length (0.773), followed by leaf length (0.564) and smaller leaflet width (0.312). In contrast, mid leaflet width (-0.368) and smaller leaflet length (-0.182) showed negative direct effects, indicating their relatively lower direct contribution to petiole length. Larger leaflet length exhibited only a small negative direct effect (-0.045).

Among the indirect effects, leaf length had a notable positive indirect influence through larger leaflet width (0.188), while larger leaflet length contributed positively via leaf length (0.309) and smaller leaflet width (0.162). Similarly, smaller

leaflet length exerted a strong positive indirect effect through larger leaflet width (0.573) despite its negative direct effect. The highest genotypic correlation with petiole length was observed for larger leaflet width (r = 0.713), followed by leaf length (r = 0.643), smaller leaflet length (r = 0.544), mid leaflet width (r = 0.327), larger leaflet length (r = 0.320), and smaller leaflet width (r = 0.301). Overall, the path analysis indicated that larger leaflet width was the most influential trait determining petiole length, while the positive associations of the remaining characters were largely mediated through their indirect effects via larger leaflet width.

Table 3. Direct (diagonal bold) and Indirect effects of component characters on character of interest

	Leaf.Length	**Mid.Leaflet.width**	**Larger.leaflet.length**	**Larger.leaflet.width**	**Smaller.Leaflet.length**	**Smaller.leaflet.width**	**gen_corr with main variable**
Leaf.Length	**0.564**	-0.107	-0.025	0.188	-0.057	0.079	0.643
Mid.Leaflet.width	0.164	**-0.368**	-0.028	0.333	-0.056	0.283	0.327
Larger.leaflet.length	0.309	-0.232	**-0.045**	0.174	-0.048	0.162	0.32
Larger.leaflet.width	0.137	-0.158	-0.01	**0.773**	-0.135	0.106	0.713
Smaller.Leaflet.length	0.177	-0.113	-0.012	0.573	**-0.182**	0.101	0.544
Smaller.leaflet.width	0.143	-0.334	-0.023	0.263	-0.059	**0.312**	0.301

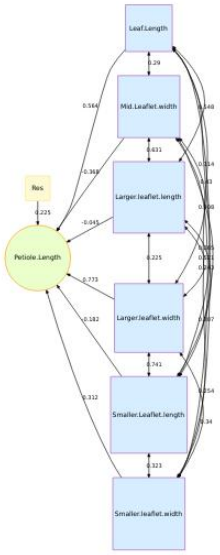


Figure 2. Path analysis diagram

Cluster analysis

The hierarchical cluster analysis grouped the 30 *Vitex negundo* genotypes into eight distinct clusters based on leaf morphological traits (Figure 3). Cluster I consisted of VN5, VN28, VN25, VN19, VN8, and VN27, indicating high morphological similarity among these genotypes. Cluster II included VN20, VN2, and VN17, while Cluster III comprised VN13, VN11, and VN30. Cluster IV contained VN9, VN7, and VN21, and Cluster V consisted of VN15, VN26, and VN29. Cluster VI included only VN12 and VN24, indicating that these two genotypes were closely related but distinct from the remaining groups. VN23 formed a separate Cluster VII, suggesting that it possesses unique morphological characteristics. The largest Cluster VIII comprised VN14, VN22, VN1, VN10, VN4, VN16, VN6, VN3, and VN18,

indicating considerable similarity among these genotypes. The dendrogram further revealed that Clusters I–III formed one major group, whereas Clusters IV–VIII formed another major group at a higher linkage distance, demonstrating substantial morphological diversity among the *V. negundo* genotypes collected from different geographical regions.

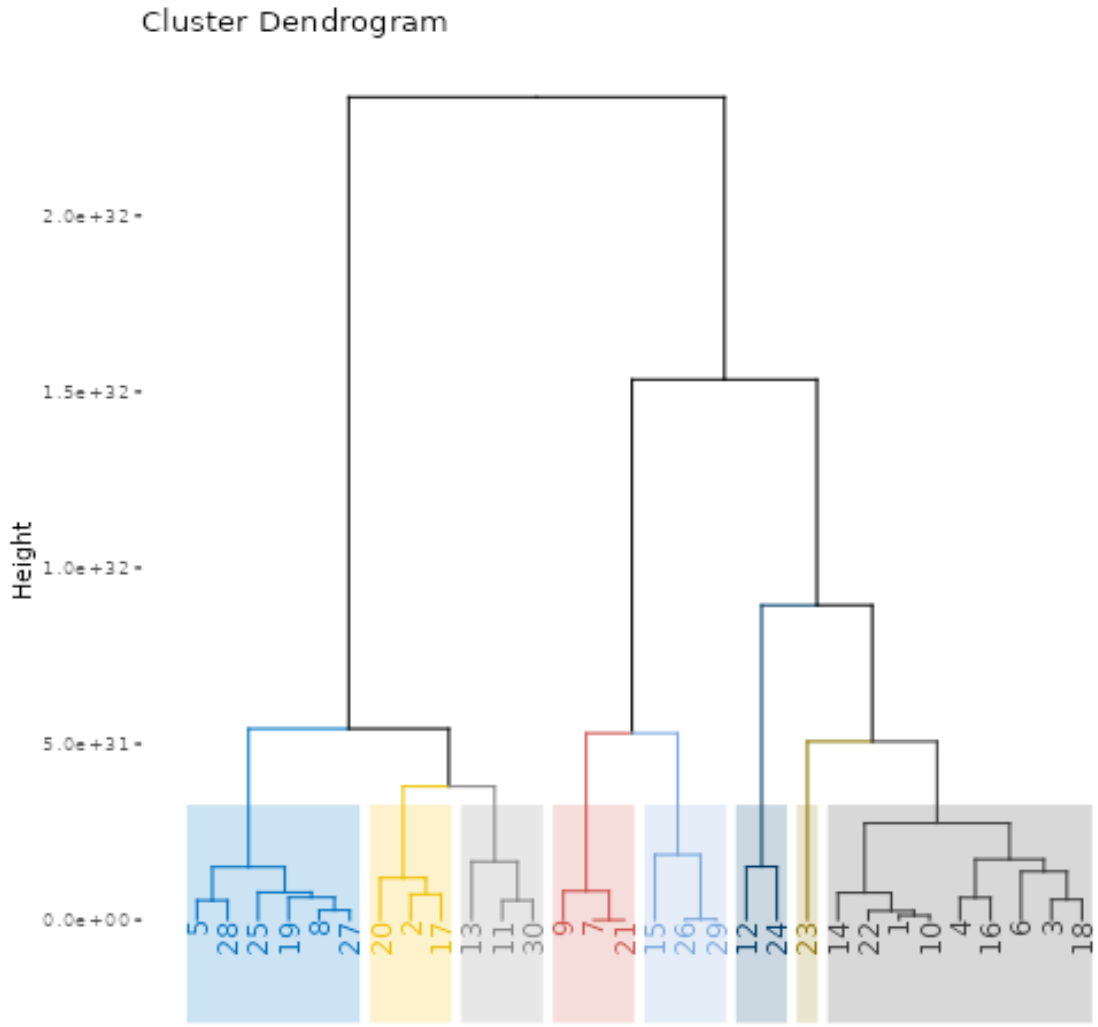


Figure 3. Hierarchical cluster analysis for 30 *Vitex negundo* genotypes

Principal Component analysis

The principal component analysis (PCA) showed that all seven leaf morphological traits contributed differently to the observed variation (Table 4). PC1 was mainly influenced by mid leaflet width (16.854%) and petiole length (15.966%), while PC2 was dominated by smaller leaflet width (20.034%) and mid leaflet width (19.757%). Leaf length contributed the highest to PC3 (50.225%), indicating it as the major source of variation in this component. PC4 was primarily associated with smaller leaflet length (33.552%) and larger leaflet length (32.209%), whereas PC5 was mainly explained by smaller leaflet length (23.892%) and larger leaflet length (23.372%). Petiole length (31.735%) showed the highest contribution to PC6, while mid leaflet width (43.176%) and smaller leaflet width (29.022%) contributed most to PC7. Overall, mid leaflet width, leaf length, petiole length, and leaflet dimensions were the principal traits contributing to morphological variation among the *Vitex negundo* genotypes.

Table 4. Principal Component analysis for vitex genotype

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Petiole Length	15.966	15.279	4.131	23.352	4	31.735	5.538
Leaf Length	11.124	0.736	50.225	1.036	15.035	20.463	1.381
Mid Leaflet width	16.854	19.757	5.656	2.852	0.342	11.363	43.176
Larger leaflet length	13.058	12.624	11.99	32.209	23.372	5.314	1.433

Larger leaflet width	15.055	16.209	12.668	0.229	19.477	21.345	15.017
Smaller Leaflet length	13.139	15.361	7.338	33.552	23.892	2.284	4.433
Smaller leaflet width	14.804	20.034	7.992	6.769	13.882	7.496	29.022

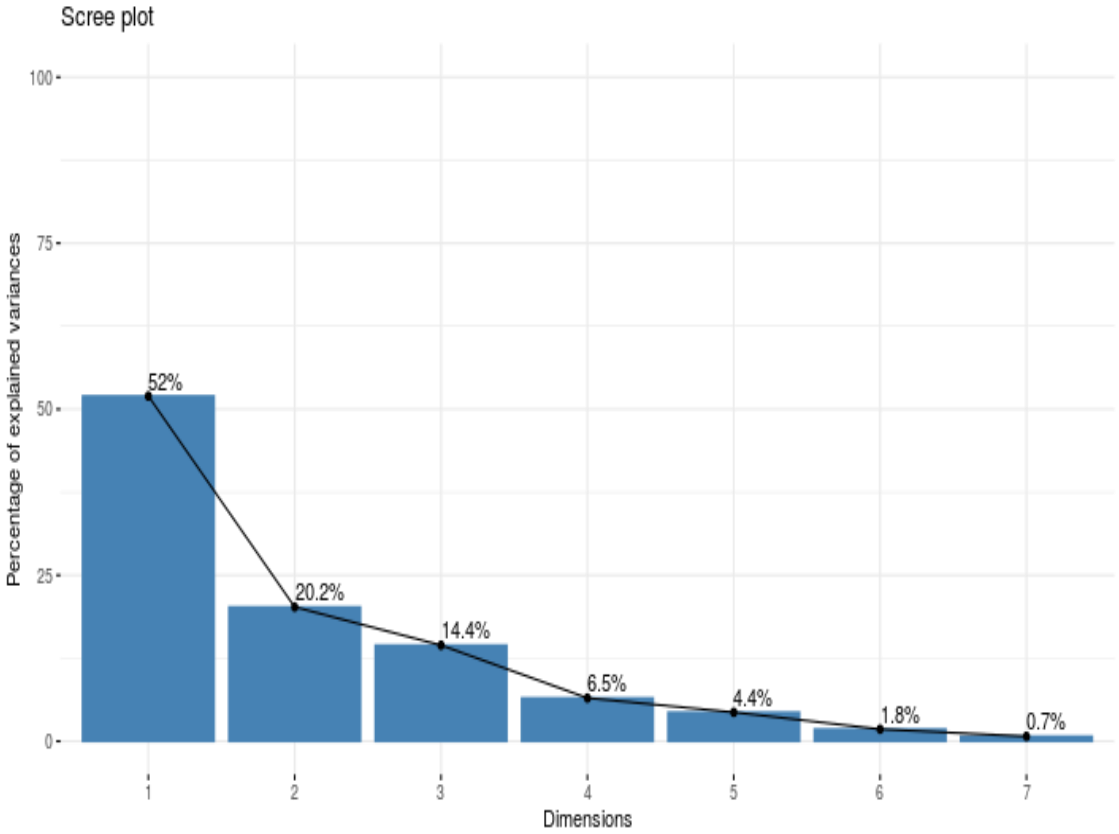


Figure 4, 5, 6and 7. Scree plot, PCA Variables, Individual PCA and Biplots for vitex genotypes.

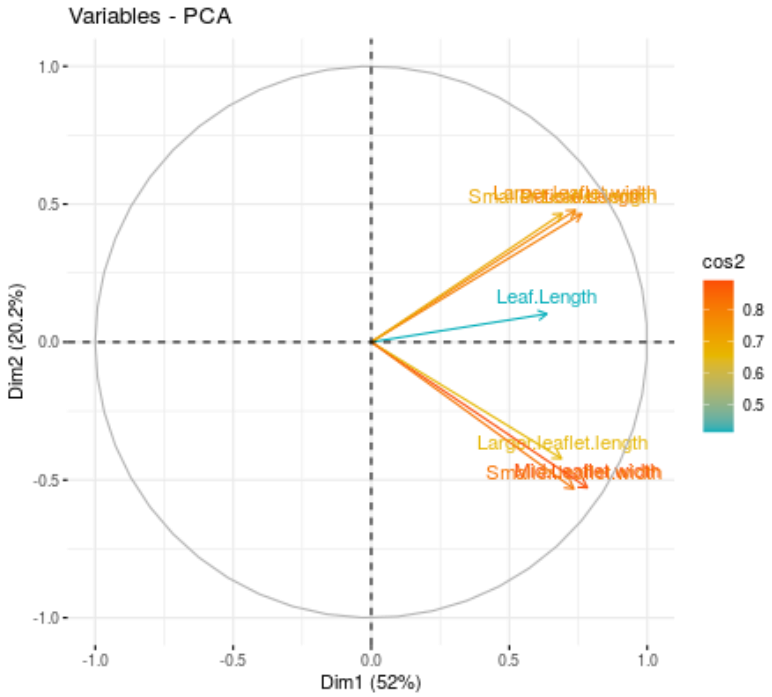
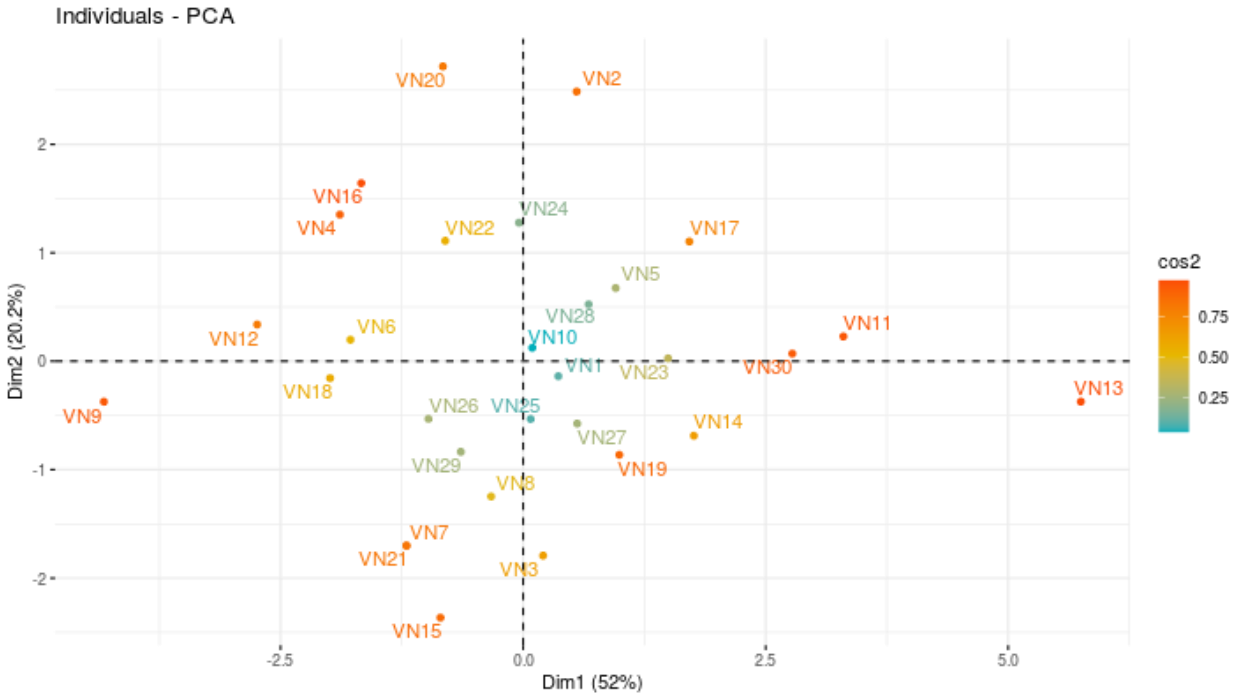
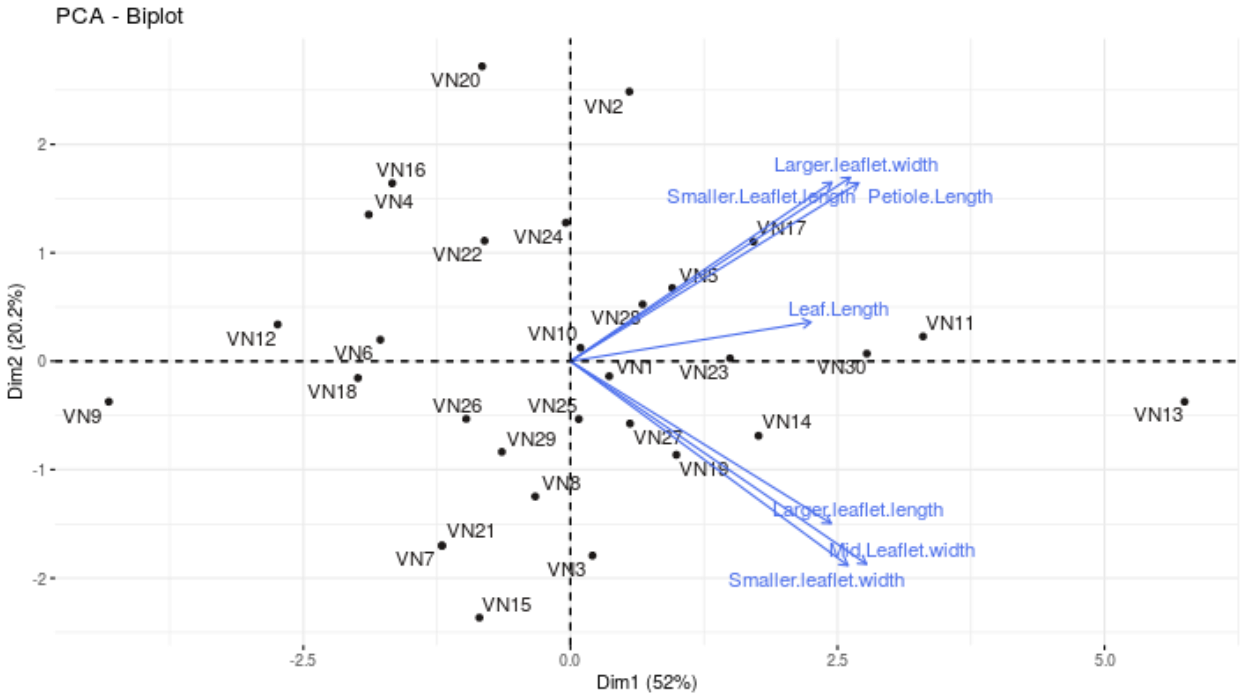


Figure 4, 5, 6and 7. Scree plot, PCA Variables, Individual PCA and Biplots for vitex genotypes.





HPLC

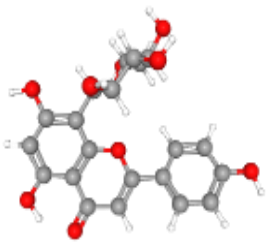


Figure 8. Structure of vitexin

Vitexin standard exhibited a distinct and well-resolved chromatographic peak at its characteristic retention time under the optimized HPLC conditions. The chromatographic profile confirmed the purity and identity of the standard, providing a reliable reference for the identification and comparison of vitexin in the *Vitex negundo* genotype samples. **Vitexin (49.65 ppm) Concentration.**

Promising genotype

Based on the HPLC chromatogram provided, Genotype 2 (VN13 – Wayanad) exhibited the highest chromatographic response among the reported peaks. The major peak was observed at a retention time (Rt) of 6.159 min with a peak area of 76,098,838, accounting for 71.339% of the total chromatographic area. The other peaks were detected at Rt 5.598 min (peak area 24,415,341; 22.888%) and Rt 7.911 min (peak area 6,157,223; 5.772%). The total peak area was 106,671,402, indicating a high abundance of the major compound in this genotype. These findings suggest that VN13 (Wayanad) is one of the most promising genotypes among the 30 *Vitex negundo* accessions for accumulation of the target phytochemical (e.g., vitexin depending on the standard used), making it a valuable candidate for further phytochemical characterization and breeding programs.

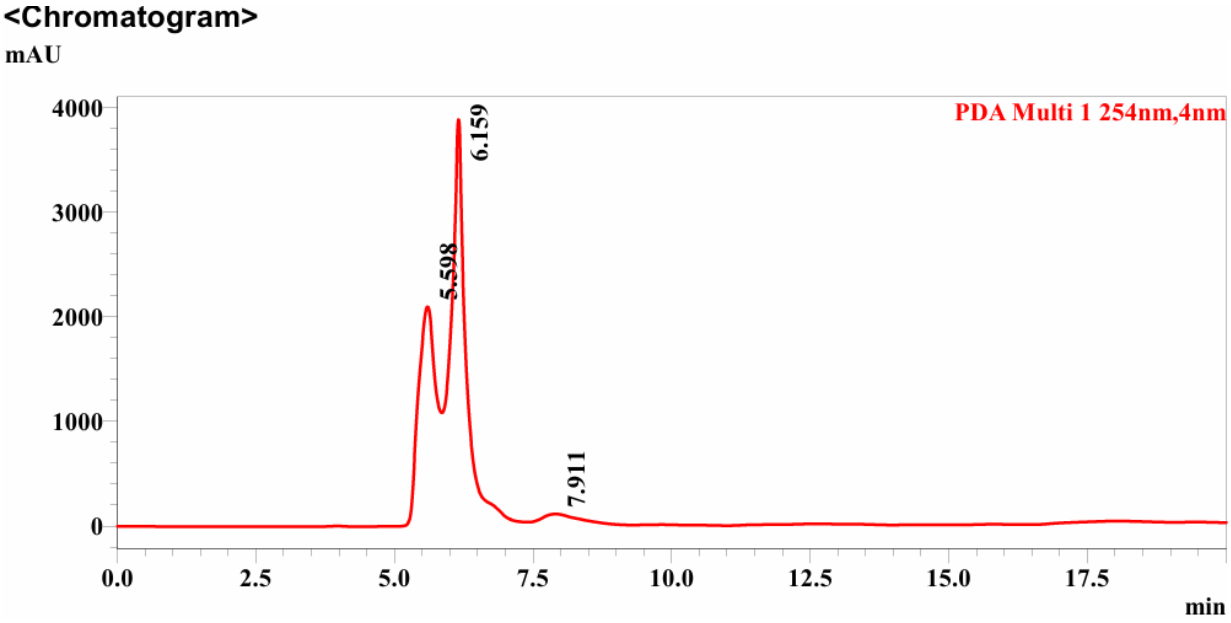


Figure 9. HPLC chromatogram of the highest-performing *Vitex negundo* genotype (VN13, Wayanad)

Table 5. HPLC peak profile of the highest-performing *Vitex negundo* genotype (VN13) collected from Wayanad.

Peak No.	Retention Time (min)	Peak Area	Area %	Height
1	5.598	24,415,341	22.888%	1,552,640
2	**6.159**	**76,098,838**	**71.339%**	**3,886,534**
3	7.911	6,157,223	5.772%	114,807
Total	**-**	**106,671,402**	**100%**	5,553,980

Least genotypes

Based on the HPLC chromatogram, Genotype VN9 (Thoothukudi) exhibited the lowest chromatographic response among the *Vitex negundo* genotypes evaluated. The major peak was observed at a retention time (Rt) of 6.126 min with a peak area of 18,376,246, contributing 54.469% of the total chromatographic area. The remaining peaks were detected at Rt 5.535 min (peak area 11,481,066; 34.031%), Rt 8.372 min (peak area 3,468,093; 10.280%), and Rt 14.545 min (peak area 411,777; 1.221%). The total peak area was 33,737,182, which was considerably lower than that of the other genotypes, indicating a comparatively lower accumulation of the target phytochemical in this accession. These findings suggest that VN9 (Thoothukudi) is the least promising genotype among the 30 *Vitex negundo* accessions for the accumulation of the target bioactive compound (e.g., vitexin, depending on the standard used), and therefore may have limited potential for phytochemical improvement and commercial utilization.

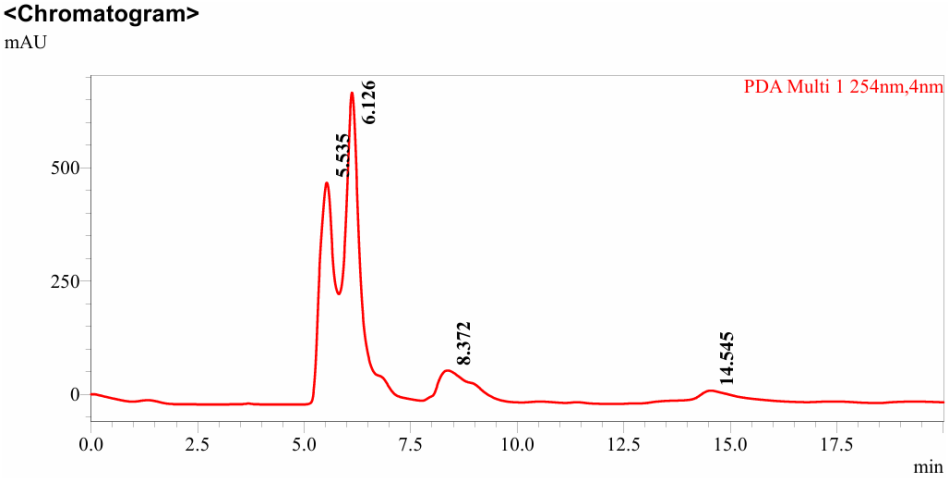


Fig 10. HPLC chromatogram of the lowest-performing *Vitex negundo* genotype (VN9, Thoothukudi) showing

Table 5. HPLC peak profile of the lowest-performing *Vitex negundo* genotype (VN9) collected from Thoothukudi

Peak No.	Retention Time (min)	Peak Area	Area %	Height
1	5.535	11,481,066	34.031	489,518
2	6.126	18,376,246	54.469	688,064
3	8.372	3,468,093	10.280	66,975
4	14.545	411,777	1.221	14,036
Total	-	33,737,182	100.000	1,258,594

DISCUSSION

The present study revealed considerable phenotypic variability among the 30 *Vitex negundo* genotypes collected from diverse geographical regions of Tamil Nadu, Kerala, Andhra Pradesh, Karnataka and Pondicherry, with respect to petiole length, leaf length and leaflet dimensions. The coefficients of variation, ranging from 0.10 to 0.20 across the seven traits, indicate moderate but consistent morphological diversity, which is in agreement with earlier reports on intraspecific leaf variation in other genera being linked to adaptation along environmental and aridity gradients [7]. Genotype VN13 from Wayanad, Kerala, consistently recorded the highest values for nearly all leaf traits, while VN9 from Thoothukudi recorded the lowest, suggesting that geographic and possibly climatic differences between the high-rainfall Western Ghats region and the semi-arid coastal tract of Thoothukudi may have shaped the observed morphological divergence. Such region-linked variation in leaf architecture has previously been demonstrated to carry taxonomic and ecological significance in other Lamiaceae and related taxa [8, 9], reinforcing the utility of leaf morphometrics as a tool for distinguishing intraspecific variants within Vitex.

The correlation analysis demonstrated that most leaf traits were positively and significantly associated with one another, particularly the very strong relationship between mid leaflet width and smaller leaflet width ($r = 0.908$), and the strong association between larger leaflet width and smaller leaflet length ($r = 0.741$). This pattern of coordinated trait expression mirrors findings in other comparative leaf anatomical and morphometric studies, where width- and length-related traits of compound or palmate leaves tend to scale proportionally, reflecting shared developmental and genetic control [10, 11, 12]. The strong positive correlation of petiole length with larger leaflet width and overall leaf length further suggests that petiole elongation in V. negundo is functionally linked to leaflet expansion, consistent with the general principle that vegetative organ dimensions co-vary to maintain efficient mechanical support and photosynthetic surface area [13, 14].

Path coefficient analysis clarified the nature of these associations by partitioning correlations into direct and indirect components. Larger leaflet width emerged as the trait exerting the highest positive direct effect on petiole length (0.773), confirming it as the principal determinant of petiole elongation, while traits such as mid leaflet width and smaller leaflet length showed negative direct effects despite positive overall correlations, indicating that their influence on petiole length is mediated indirectly, largely through larger leaflet width. This is consistent with the classical interpretation of path analysis, wherein genotypic correlations may mask antagonistic direct and indirect components [15], and underscores that selection based on larger leaflet width alone could be an effective surrogate trait for improving overall leaf vigor in breeding or selection programs targeting V. negundo.

The hierarchical cluster analysis grouped the genotypes into eight clusters, broadly resolved into two major groups at higher linkage distance, indicating that morphological diversity among the accessions does not strictly follow geographic proximity. For instance, Cluster III combined VN13 (Wayanad), VN11 (Kanniyakumari) and VN30 (Trivandrum), all

from Kerala, supporting a degree of regional clustering, whereas Cluster VIII, the largest group, comprised genotypes from geographically distant locations such as Nadugani, Pondicherry and Karnataka, suggesting that environmental plasticity or convergent morphology can override strict geographic patterning. Similar non-monophyletic clustering based on leaf architecture has been reported in numerical taxonomic studies of other genera, where morphological similarity reflected ecological convergence rather than phylogenetic relatedness [16, 17, 18]. The distinctiveness of VN23, forming its own single-genotype cluster, further highlights the presence of a morphologically unique accession that may warrant closer phytochemical and genetic scrutiny.

Principal component analysis corroborated the cluster and correlation findings by showing that mid leaflet width, leaf length and petiole length were the major contributors to the early principal components, collectively explaining a substantial proportion of the total morphological variance. The dominance of leaf length in PC3 (50.225%) and the substantial loadings of leaflet length and width traits in PC4–PC7 indicate that variation among the genotypes is multidimensional, with no single trait accounting for the bulk of diversity. This is consistent with previous applications of PCA in leaf morphometric studies of woody and herbaceous taxa, where multivariate ordination effectively separated genotypes or species based on combinations of size and shape traits rather than single characters [19, 20, 21, 22].

The HPLC analysis provided a phytochemical dimension complementing the morphological findings. The vitexin standard produced a sharp, well-resolved peak, validating the chromatographic method used for quantification, in line with established protocols for fingerprinting bioactive flavonoid glycosides in medicinal plant extracts [23, 24, 25]. Notably, genotype VN13 (Wayanad), which also exhibited the most vigorous leaf morphology, showed the highest chromatographic response, with a dominant peak contributing over 71% of the total peak area, suggesting a possible positive association between leaf size/vigor and vitexin accumulation in this accession. Conversely, VN9 (Thoothukudi), the genotype with the smallest leaf dimensions, also displayed the lowest total peak area and a more complex four-peak profile with comparatively diminished proportions of the major compound. This concordance between morphological vigor and phytochemical richness in VN13 versus VN9 lends support to the hypothesis that favorable growing conditions in the Western Ghats region of Kerala may simultaneously promote larger leaf development and enhanced secondary metabolite biosynthesis, a relationship that has also been noted in other Vitex-based phytochemical investigations [26, 27].

The differences in retention time and peak number between VN13 and VN9 further point to qualitative as well as quantitative variation in the phenolic profile across genotypes, consistent with reports that geographic origin and environmental factors can substantially influence the chromatographic fingerprint of medicinal plant extracts [28, 29, 30].

Taken together, the integration of leaf morphometric analysis (correlation, path coefficient, cluster and PCA) with HPLC-based phytochemical profiling provides a robust multidimensional characterization of the 30 V. negundo genotypes. The consistent identification of VN13 (Wayanad) as the genotype combining superior leaf morphology with the highest vitexin-related chromatographic response, and VN9 (Thoothukudi) as consistently inferior on both fronts, strengthens the reliability of leaf morphological traits as indirect indicators of phytochemical potential in this species. These findings hold practical relevance for the selection of elite *Vitex negundo* accessions for cultivation, conservation and pharmaceutical exploitation, and are consistent with the broader literature emphasizing the combined value of morphometric and phytochemical approaches in resolving intraspecific variation in medicinally important taxa [31, 32].

CONCLUSION

The integrated morphometric and HPLC-based phytochemical evaluation of 30 *Vitex negundo* genotypes revealed substantial intraspecific diversity in leaf characters and vitexin accumulation, with leaflet width traits emerging as key contributors to overall variation. Cluster and principal component analyses confirmed that morphological similarity did not strictly follow geographic origin, reflecting environmental influence alongside genetic variation. Genotype VN13 (Wayanad, Kerala) combined superior leaf morphology with the highest vitexin-related chromatographic response, identifying it as the most promising accession, whereas VN9 (Thoothukudi) was least promising on both fronts. These findings support the use of leaf morphological traits as reliable indirect indicators for selecting elite, high-yielding V. negundo germplasm for conservation and pharmaceutical development.

Future Perspectives

Future studies should integrate molecular markers and genome-wide diversity analysis with morphological and phytochemical data to validate genotype classification. Multi-environment trials assessing seasonal and edaphic influences on vitexin accumulation, along with bioactivity-guided pharmacological screening of elite genotypes like VN13, would further support germplasm conservation, large-scale cultivation, and pharmaceutical product development of *Vitex negundo*.

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