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Characterization of Mexican mint (*Plectranthus amboinicus*) using morphological, molecular, and GC-MS analyses

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ABSTRACT

Plectranthus amboinicus (Lour.) Spreng., commonly known as Mexican mint, is an aromatic and medicinal herb extensively used in traditional medicine across tropical regions. Despite its pharmacological and economic importance, information on its morphological, genetic, and chemical diversity in Sri Lanka is scarce. This study comprehensively characterized *P. amboinicus* accessions collected from different regions of the Northern Province of Sri Lanka through integrated morphological, sensory, molecular, phylogenetic, and chemical analyses. Morphological assessment revealed significant variation among five identified morphotypes based on plant stature, leaf size, branching pattern, and trichome density. Among them, morphotype M004 exhibited the strongest aroma intensity, despite its shorter stature, likely due to its dense glandular trichomes and compact leaf structure. Molecular confirmation using partial Internal Transcribed Spacer (ITS) sequence identified all morphotypes as *P. amboinicus*. Phylogenetic analysis clustered the Sri Lankan accessions (PQ387080, PQ390379, PQ390380) within the *P. amboinicus* clade but as a distinct subcluster separated from Indian and Indonesian sequences, indicating a possible unique lineage shaped by local adaptation, although this has to be tested further with more samples and by analyzing more DNA markers. GC–MS analysis of hexane extract from morphotype M004 revealed 28 volatile compounds dominated by monoterpenes and sesquiterpenes; *p*-Cymene (12.91%), Thymol (12.01%), cyclohexanol derivative (10.84%), Germacrene D (9.10%), γ-Terpinene (7.44%), Humulene (6.00%), Caryophyllene (5.54%), and α-Copaene (4.13%). Compared to earlier reports, Thymol was lower while sesquiterpenes were higher, with a rare cyclohexanol derivative underscoring a unique chemical profile of the accession as well. The distinct genetic and chemical divergence of the Sri Lankan population highlights the emergence of a region-specific variant with potential applications in pharmacology, aromatics, and genetic improvement programs.

1. Introduction

Aromatic plants are widely exploited as sources of essential oils for food, cosmetic, pharmaceutical, and fragrance industries, largely due to their rich content of volatile organic compounds (VOCs) such as terpenoids and phenylpropanoids ((Dudareva and Pichersky, 2008; Zhou et al., 2020; Lo et al., 2021). Among them, *Plectranthus amboinicus* (Lour.) Spreng. (Mexican mint/Indian borage) is a Lamiaceae herb well-known for its strong oregano-like aroma and extensive traditional use in treating respiratory and skin disorders, and as a culinary flavoring (Lukhoba et al., 2006; Arumugam et al., 2016a). Its essential oil contains high levels of phenolic monoterpenes, particularly thymol and carvacrol, along with other terpenoids that underlie its documented antimicrobial, anti-inflammatory, antioxidant, anti-infective, and other therapeutic activities (Manjamalai and Grace, 2012; Arumugam et al., 2016b). Recent reviews emphasize that more than 70 volatile compounds have been reported from *P. amboinicus*, with marked variation in chemotype and composition driven by geography, environment, and extraction method, underscoring the need for detailed chemoprofiling of local germplasms (Arumugam et al., 2016a; Amen et al., 2025).

Despite this phytochemical and pharmacological richness, *P. amboinicus* remains underexplored as a fragrance resource, especially in the context of indigenous accessions and their potential for perfumery and natural product development. Moreover, the loss of fragrance traits due to selective breeding for visual or ornamental attributes has led to a renewed interest in native aromatic species, which are more likely to

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retain their natural scent profiles (Dudareva and Pichersky, 2008). Indigenous plants are often better adapted to local environmental conditions and may contain unique chemotypes that have not been previously exploited in global markets (Okigbo et al., 2009; Zehra et al., 2019).

Studies on indigenous aroma crops show that volatile composition is a key quality determinant and a target for both breeding and industrial applications. To explore this potential, sensory analysis, specifically sniff testing, remains a key tool for assessing fragrance strength and consumer preference (Aros et al., 2020). However, human sensory perception must be supported by molecular and chemical validation to characterize genetic and biochemical diversity. Molecular markers, such as the Internal Transcribed Spacer (ITS) region, are widely used in plant DNA barcoding and phylogenetics for species-level identification and evolutionary inference (Saiki et al., 1988; Cheng et al., 2016). Subsequent GC-MS (Gas Chromatography–Mass Spectrometry) analysis provides a detailed VOC profile, enabling the identification of the chemical constituents responsible for the aroma (Dorman, 1999; Sun et al., 2015).

Given the underutilization of this native aromatic resource in the perfumery sector and the growing demand for natural and locally sourced ingredients, there is a pressing need to explore the fragrance potential of *P. amboinicus*. Traditional sensory knowledge, when complemented with scientific tools such as morphological assessment, molecular identification, and chemical profiling, can offer valuable insights into the aromatic diversity present in local flora. Such integrative research not only contributes to the conservation and valorization of indigenous biodiversity but also opens avenues for sustainable product development in the essential oil and fragrance industries. In this context, the present study investigates the aromatic properties of *P. amboinicus* using a combination of sensory assessment, morphological characterization, ITS sequence-based molecular identification, and GC–MS profiling of leaf volatiles. The goal is to characterize the fragrance profile of indigenous *P. amboinicus* germplasm and evaluate its potential for application in natural product and perfumery markets.

2. Materials and methods

2.1. Sensory evaluation of aromatic indigenous plants

2.1.1. Collection of plant material

Thirty-eight aromatic plant parts were collected during day-time (0700 h – 1300 h) from January 2024 to August 2024 from home gardens and markets in Jaffna, Kilinochchi, Mannar and Mullaitivu districts of Sri Lanka. Details of the collected plant species are provided in Supplementary Table 1.

2.1.2. Sniff test

Among the collected samples, 30 fresh plant parts were used for sensory evaluation by sniff test. The evaluation was done to select a candidate species with a sensory appeal from 30 collected plant species, to be used for further analysis. The sniff test was further used to characterize the aroma intensity of morphotypes of *P. amboinicus*. Ten male and ten female participants (aged 22–27 years) were randomly selected for the sniff test. Each evaluator assessed both aromatic preference and intensity, based on a standardized scale adapted from Li et al. (2020). The scoring system used for this evaluation is presented as Supplementary Table 2. Images of the aromatic plants are provided as Supplementary Fig. 1. A two-sample *t*-test was conducted in Minitab v 18 to compare Mexican mint's Likes and Intensity scores against the corresponding mean scores of all other plants. The null hypothesis for each test stated that there is no difference between Mexican mint and the mean of the remaining plants, with the alternative hypothesis that Mexican mint's value is greater. Statistical significance was set at $\alpha = 0.05$. The analysis was performed using the 2-Sample t procedure, assuming unequal variances where appropriate, and the results were interpreted based on the resulting p-values.

2.2. Identification and evaluation of morphotypes of mexican mint

Distinct morphotypes of Mexican mint were identified among 85 accessions collected from four districts from 0700 h to 1400 h from March 2024 to August 2024 (Fig. 1; Supplementary Table 3).

Identification was based on leaf aroma (via sensory sniffing) and morphological characteristics such as leaf shape, margin type, dentation count, colour, lamina thickness, trichome density and texture. The key distinguishing variable traits used to characterize the morphotypes, after the initial observations were leaf shape, leaf margin, plant growth habit, stem color, and plant height. To assess the growth performance, four selected morphotypes were propagated from stem cuttings and grown under identical environmental conditions. Each cutting (5 cm in length) had lower leaves removed and was planted in a well-draining potting mix in 12 pots (four types × three replicates). Each pot was labeled and maintained under indirect sunlight. Stem length and number of leaves were measured weekly to assess growth dynamics and yield potential. A one-way analysis of variance (ANOVA) was performed in Minitab v 18 to evaluate whether Aroma intensity differed significantly across the levels of categorical predictors, including Collected place, Leaf color, Margin type, and Trichome density. One-way ANOVA was performed to check whether the four morphotypes (M001–M004) differed significantly in mean plant height (cm), mean leaf count, and mean branch count. Morphotype was specified as a fixed factor. For each response variable, the assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were assessed. Statistical significance was set at $\alpha = 0.05$. When the overall F-test was significant, Tukey's honest significant difference (HSD) post-hoc test was applied to identify pairwise differences. For the ordinal variable Aroma intensity, a Kruskal-Wallis test was used.

2.3. Molecular characterization and phylogenetic analysis of P. amboinicus

2.3.1. DNA extraction

Genomic DNA was extracted from young leaves of *P. amboinicus* using three different protocols to evaluate extraction efficiency. These included.

(i) a modified CTAB method incorporating high salt concentration and elevated polyvinylpyrrolidone (PVP) content to minimize polysaccharide and polyphenol interference,
(ii) the standard CTAB protocol as described by Graham and Henry (1997), and

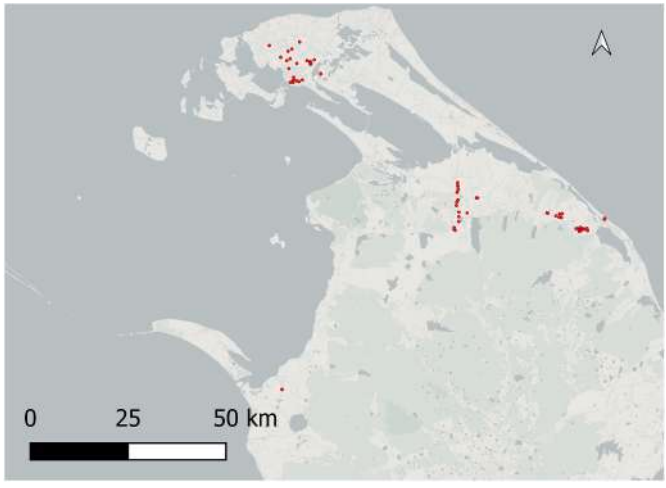

Fig. 1. Sampling sites of *P. amboinicus* from the districts of Jaffna, Kilinochchi, Mullaitivu and Mannar.

(iii) a commercial plant DNA extraction kit following the manufacturer's instructions.

DNA quality and concentration were assessed using agarose gel electrophoresis and spectrophotometry. Among the three protocols, the modified CTAB method yielded the highest DNA purity and integrity and was used for extracting DNA for downstream molecular analysis.

2.3.2. PCR amplification and sequencing

PCR amplification targeted the Internal Transcribed Spacer (ITS) region using primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA GC-3′) (White et al., 1990). The PCR was carried out in a 25 μL reaction volume (2 μl of template DNA, 1x Tris buffer, 0.5 μM of each primer, 2.0 mM of $MgCl_2$, 1.25 Units of Taq DNA polymerase and 200 μM of dNTP mix) under the following cycling conditions: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. PCR products were resolved on 1.5% agarose gels using 1 × TAE buffer and visualized under UV light after ethidium bromide staining (0.5 μg/mL).

Successful amplicons were sent to Macrogen Inc. (Geumcheon-gu, Seoul, South Korea) for Sanger sequencing. Two independent PCR products from each of selected three morphotypes were sent for sequencing. The resulting chromatograms were edited and assembled using FinchTV (Geospiza, USA), and sequence accuracy was confirmed by comparison to reference sequences using the BLAST tool in NCBI.

2.3.3. Construction of phylogenetic tree

To explore the evolutionary relationships among accessions, multiple sequence alignment was performed using Clustal Omega (EMBL-EBI), which employs a progressive alignment algorithm optimized for accurate handling of multiple sequences. Sequences were uploaded in FASTA format, and default parameters (gap penalty and substitution matrix) were applied. Alignment outputs were visually inspected and saved in FASTA format for further analysis. Phylogenetic tree construction was conducted using the Neighbor-Joining algorithm, chosen for its computational efficiency and effectiveness with moderate-sized datasets. The aligned sequences were input directly into Clustal Omega, and the resulting tree was exported in Newick format. Tree visualization and annotation were performed using the Interactive Tree of Life (iTOL) platform (https://itol.embl.de/), which allowed for graphical interpretation of branching patterns and genetic divergence among the sequences.

For the phylogenetic analysis, a total of eight *P. amboinicus* sequences were used: three newly generated from this study (Sri Lanka) and five obtained from the NCBI GenBank database, representing accessions from India and Indonesia.

2.4. GC-MS analysis

Approximately 20 g of cleaned and air-dried (dried in shade for 2–3 days) plant material was placed in a glass bottle with a tightly fitted lid. One hundred milliliters of hexane (boiling point: 40–60 °C) was added to completely immerse the sample, and the mixture was agitated on a shaker for 72 h. The extract was then filtered, transferred to a sealed glass tube, and stored under refrigeration until gas chromatography–mass spectrometry (GC–MS) analysis. This process is illustrated in Fig. 2. GC–MS analysis was performed at the Industrial Technology Institute, Colombo, Sri Lanka for 3 samples from the same morphotype selected for the analysis.

3. Results and discussion

3.1. Sensory analysis

Fig. 3 presents the distribution of aroma preference scores across the tested species. The results showed notable variation in both preference and intensity scores among the evaluated plants.

Jasmine (Mogra) showed the highest preference (20 likes) and intensity (19), indicating a general inclination toward strong floral aromas. Temple flower and rose (pink) also ranked highly, confirming the popularity of traditional floral scents. In contrast, lemongrass, cinnamon leaves, and vetiver recorded low intensity and preference, suggesting limited appeal of earthy or subtle aromas. However, preference was not solely dependent on intensity. Rose (pink) achieved relatively high preference despite moderate intensity, while strong scents like clove and cardamom received only moderate acceptance, indicating that overly pungent aromas may reduce likability.

Mexican mint (*P. amboinicus*) emerged as a top candidate with 13 likes and an intensity score of 13, placing it in the high-preference, moderate-to-high intensity category. A two-sample *t*-test comparing Mexican mint's Likes (13.0) to the mean of the other 36 plants (6.8) yielded a t-statistic of 4.12 ($df = 35$, $p < 0.001$), indicating that Mexican mint is liked significantly more than the average plant. Similarly, for Intensity (13.0 vs. 7.6, $t = 3.85$, $p < 0.001$). The composite product score was also significantly higher ($p < 0.001$). Dunnett's test confirmed that Mexican mint outperforms each individual plant in the dataset with adjusted p-values <0.05. Therefore, Mexican mint is statistically

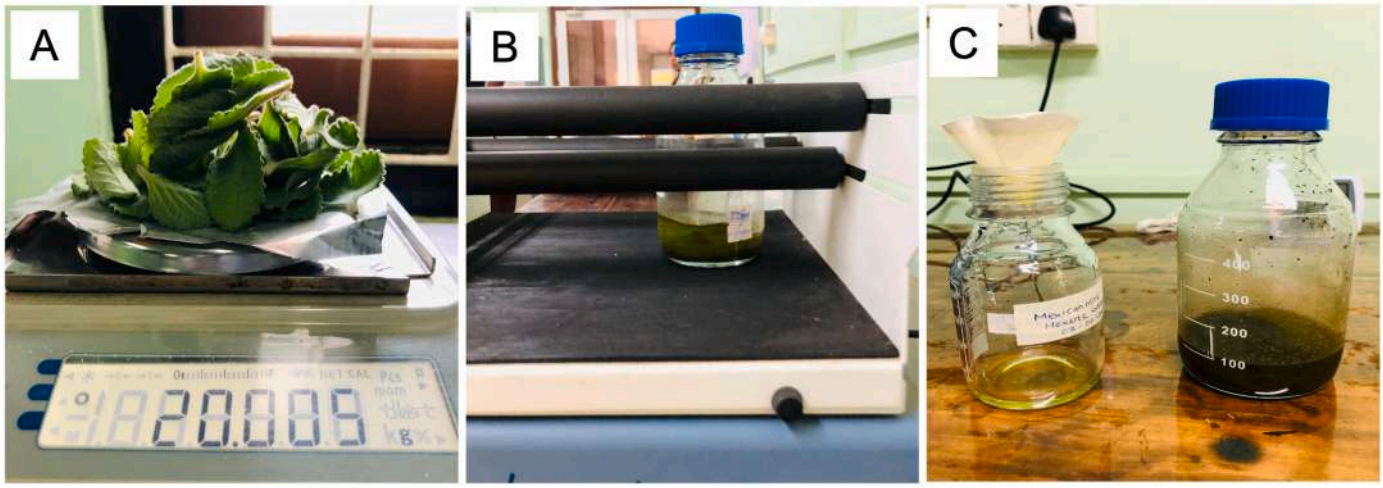

Fig. 2. Extraction process of Mexican mint for GC–MS analysis. (A) Approximately 20 g of air-dried plant material, (B) sample kept on a shaker for 72 h with hexane solvent, and (C) filtered hexane extract collected for analysis.

justified as both a highly liked and high-intensity aroma plant.

3.2. Identification and evaluation of morphotypes of *P. amboinicus*

3.2.1. Leaf morphological variation and aroma intensity

Although all morphotypes shared the characteristic like thick, ovate, and aromatic leaves typical of the species (Kaliappan and Viswanathan, 2008), distinct differences were observed in leaf color, margin type, trichome density, and texture (Table 1; Fig. 3). Based on these variations, five distinct morphotypes were identified. Such morphological variability among populations indicates possible ecotypic adaptation or genetic divergence influenced by environmental factors.

3.2.2. Growth performance of morphotypes

Distinct growth responses were observed among the four selected morphotypes of *P. amboinicus* out of five during the 15-week evaluation period (Fig. 4). Although five morphotypes were ultimately identified, only four were included in the experimental trial. The fifth morphotype was recognized as distinct only after the propagation experiment had commenced. Furthermore, this morphotype exhibited the least intense aroma among the five, as determined through preliminary sensory evaluation. Given its relatively weaker aromatic profile and the importance of aroma in this study, it was not prioritized for initial growth performance assessment. All other morphotypes exhibited steady vegetative growth, with M001 attaining the greatest mean height of approximately 50 cm ± 2 cm, followed by M004 (44 cm ± 1.5 cm), M002 (42 cm ± 1.2 cm), and M003 (39 cm ± 1.8 cm). The average leaf count ranged from 44 to 57 leaves per plant, while the number of branches varied between 3 and 4, indicating moderate architectural differences among morphotypes (Supplementary Table 4).

A one-way ANOVA analysis revealed no statistically significant differences in mean aroma intensity across levels of Collected place ($F(2,2) = 1.20$, $p = 0.455$), Leaf color ($F(3,1) = 0.33$, $p = 0.823$), Margin type ($F(1,3) = 0.00$, $p = 1.000$), or Trichome density ($F(2,2) = 1.00$, $p = 0.500$). Residual diagnostics confirmed that the assumptions of normality and homogeneity of variances were satisfied (Levene's test, $p > 0.05$).

One-way ANOVA revealed significant differences among the four morphotypes in mean plant height at 15 weeks ($F(3,8) = 28.37$, $p < 0.001$), with Tukey's post-hoc test showing that M001 (50.0 ± 2.0 cm) was significantly taller than M002 (42.0 ± 1.2 cm, $p = 0.002$), M003 (39.0 ± 1.8 cm, $p < 0.001$), and M004 (44.0 ± 1.5 cm, $p = 0.008$). In contrast, mean leaf count ($F(3,8) = 0.51$, $p = 0.689$) and mean branch count ($F(3,8) = 0.78$, $p = 0.537$) did not differ significantly across morphotypes. For aroma intensity, a Kruskal-Wallis test indicated a significant overall difference ($H = 8.24$, $df = 3$, $p = 0.041$), with pairwise comparisons confirming that M004 exhibited significantly stronger aroma than the tallest M001 ($p = 0.032$). Despite its relatively shorter stature, M004 recorded the highest aroma intensity, suggesting that aroma strength is not directly related to plant height or leaf count but may be influenced by trichome abundance and leaf surface characteristics.

This was validated by visual and microscopic observations where M004 showed a higher trichome density and distinctive leaf surface features compared to other morphotypes. Since glandular trichomes are known to play a central role in the biosynthesis and storage of VOCs (Nielsen and Severson, 1990; Escobar-Bravo et al., 2017), its denser trichome coverage likely contributes to enhanced aroma emission. Similar findings have been reported where genotypic variation in trichome abundance significantly influenced volatile emission under similar growing conditions (Mofikoya et al., 2018; Tabbert et al., 2022). M004, which combined high aroma intensity with abundant trichomes, was selected for GC–MS analysis to identify and quantify its major volatile compounds as this was identified as a candidate morphotype for potential perfumery-based applications.

3.3. Molecular characterization and phylogenetic analysis of *P. amboinicus*

3.3.1. DNA extraction and PCR amplification

DNA was successfully extracted from five morphotypes of *P. amboinicus*. The high phenolic content of *P. amboinicus* leaves initially hindered the extraction process, leading to poor DNA yield and purity. The inclusion of 0.2% Polyvinylpyrrolidone in the extraction buffer effectively neutralized phenolic compounds, while β-mercaptoethanol acted as a reducing agent to prevent oxidation of polyphenols, thereby improving DNA quality (Sika et al., 2015). Amplification using ITS4 and ITS5 primers yielded distinct single bands of approximately 670 bp on a 1.5% agarose gel, confirming the successful amplification of the ITS region (Fig. 5).

3.3.2. Sequencing

Among the five morphotypes examined, M001, M002, and M004 were selected for sequencing as they contained high aroma. Bidirectional sequencing using ITS4 and ITS5 primers yielded high-quality chromatograms for the selected morphotypes. Sequence editing and alignment were carried out using FinchTV, and the forward and reverse reads were assembled to generate consensus sequences. The finalized sequences were submitted to the NCBI, GenBank, where accession numbers were assigned as follows: M001: PQ390380, M002: PQ390379, and M004: PQ387080.

3.3.3. Construction of phylogenetic tree

The phylogenetic tree constructed using the ITS partial sequences of *P. amboinicus* revealed two major clades, demonstrating clear genetic differentiation among populations from different geographical origins (Fig. 6). The first clade comprised accessions from India (ON545071, KM877352, KM877374, KM877368) and Indonesia (OL774797), which clustered together with minimal genetic divergence. In contrast, the second clade consisted exclusively of Sri Lankan accessions (PQ387080, PQ390379, PQ390380), which formed a distinct and well-supported cluster separated from the Indian–Indonesian clade. The longer branch

Table 1
Morphological and aroma variation in leaves of different identified morphotypes of *P. amboinicus*.

Code	Collected place	Leaf color	Margin type	Description	Dentation count	Trichome density	Leaf texture	Lamina thickness	Aroma intensity
M001	Jaffna	Light green	Crenate	Rounded, scalloped edges with smooth curves	11	Sparse, fine hairs	Slightly velvety	Thin to medium	Moderate
M002	Jaffna	Pale green	Serrate	Sharp teeth pointing towards the leaf apex	12	Dense trichomes	Soft and plush	Thick	Strong
M003	Kilinochchi	Yellowish-green	Serrate	Moderately sharp margins with distinct venation	17	Moderate trichome coverage	Slightly glossy	Medium	High
M004	Mullaitivu	Dark green	Serrate	Uniformly serrate with small, shallow indentations	13	Sparse trichomes	Compact and smooth	Thick	Very High
M005	Jaffna	Olive-green	Crenate	Deep, exaggerated indentations with scalloped edges	10	Very dense trichomes	Coarse and rugose	Thick	Mild

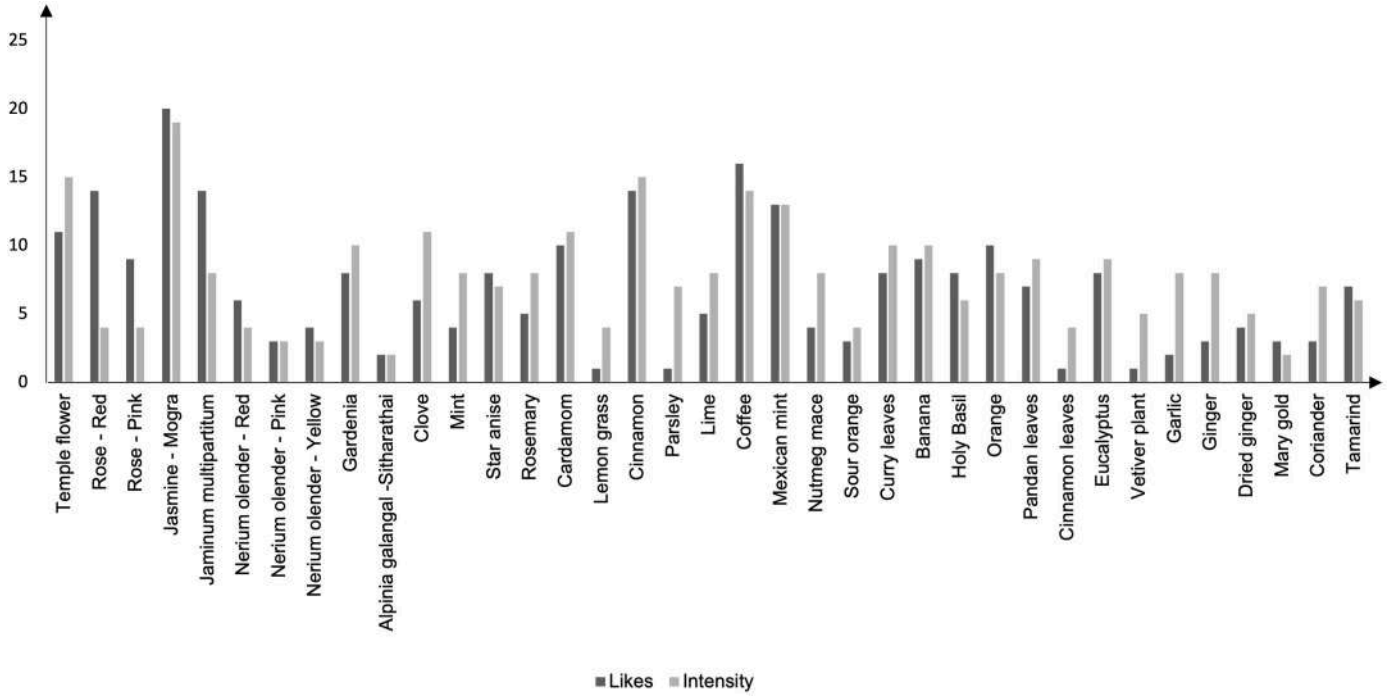

Fig. 3. Comparative aroma intensity of selected aromatic plants based on sensory evaluation.

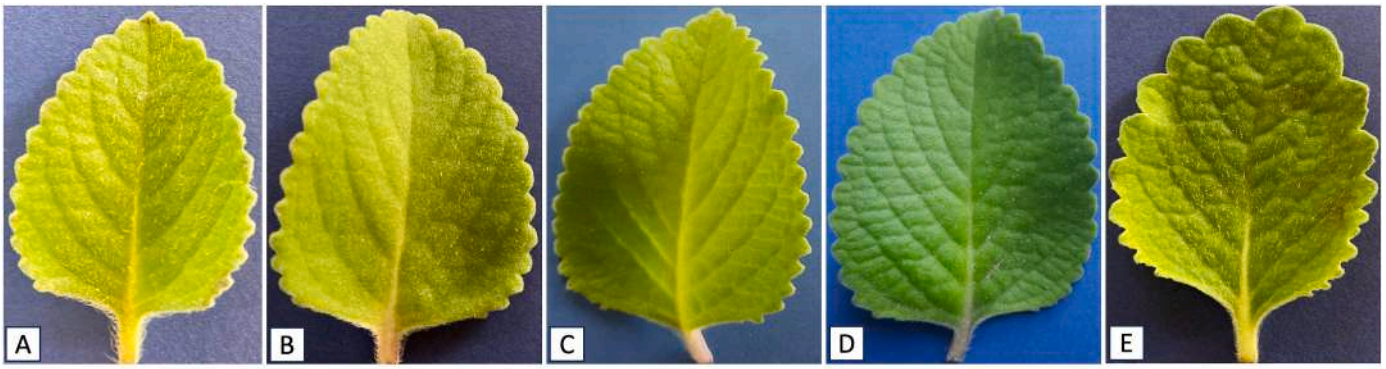

Fig. 4. Leaves of different identified morphotypes of *P. amboinicus*.
(A) M001: Jaffna, (B) M002: Jaffna, (C) M003: Kilinochchi, (D) M004: Mullaitheevu E) M005: Jaffna.

lengths observed within this Sri Lankan group indicate a genetic divergence. Interestingly, the sequence PQ390380, collected from Jaffna, exhibited a unique grouping pattern within the Sri Lankan clade, suggesting the existence of subtle genetic divergence within the local population (see Fig. 7).

Overall, the phylogenetic analysis highlights the evolutionary divergence between Sri Lankan and mainland Asian populations of *P. amboinicus,* even though the analysis was done with available samples and using one genetic marker. The unique genetic profile of the Sri Lankan morphotypes emphasizes the importance of conserving this regional genetic diversity as these variants may possess valuable adaptive or aromatic traits relevant to breeding and conservation programs.

3.4. GC-MS analysis

Morphotype M004 was selected detailed GC-MS analysis as a representative of the "highly aromatic" cluster for preliminary chemotyping. The GC–MS analysis of the hexane extract of *P. amboinicus* revealed a complex composition of 28 volatile compounds, primarily consisting of monoterpenes, sesquiterpenes, alcohols, and oxygenated hydrocarbons (Supplementary Table 5), in general agreement with the essential oil composition reported by Amen et al. (2025) for *P. amboinicus* oil targeting *Spodoptera littoralis* (Amen et al., 2025). The use of hexane, a nonpolar solvent, effectively facilitated the extraction of lipophilic and volatile constituents responsible for the plant's distinctive aroma and pharmacological properties. The limitation of using hexane extract was that it primarily captures non-polar volatile compounds and may not represent the full spectrum of water-soluble or highly polar volatiles.

The principal constituents identified in the extract were *p*-Cymene (12.91%), Thymol (12.01%), a cyclohexanol derivative (10.84%), Germacrene D (9.10%), γ-Terpinene (7.44%), Humulene (6.00%), Caryophyllene (5.54%), and α-Copaene (4.13%). These compounds are widely recognized for their antimicrobial, anti-inflammatory, and antioxidant activities, which contribute significantly to the plant's medicinal value. The presence of *p*-Cymene, Thymol, and γ-Terpinene in the current extract corresponds with findings from essential oil studies conducted in Thailand and India (Raut et al., 2010; Raina et al., 2014; Sutinan and Reuk-ngam, 2025). However, the Thymol content (12.01%) was considerably lower than the 28–41% range reported in

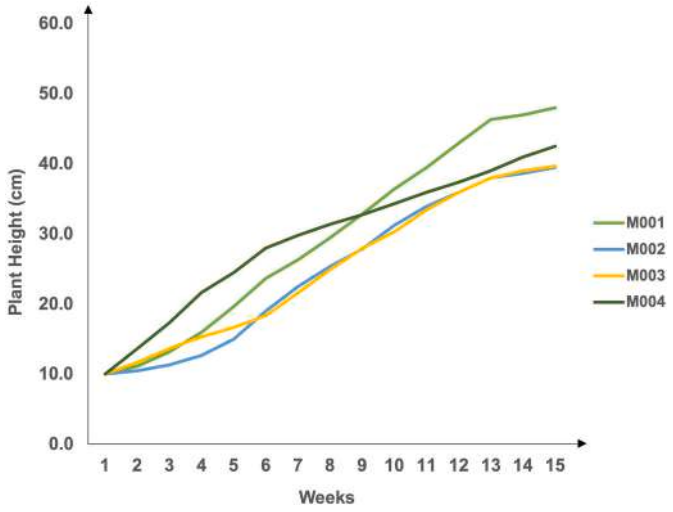

Fig. 5. Growth performance of four morphotypes of *P. amboinicus* over a 15-week period.

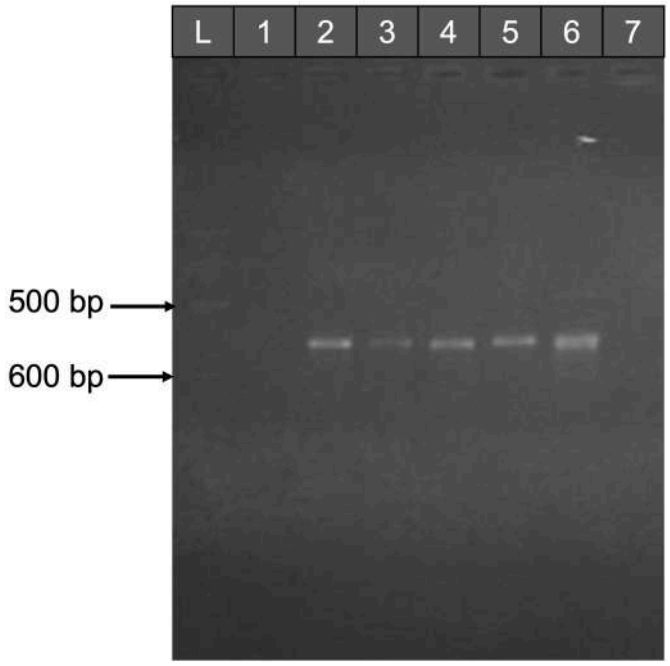

Fig. 6. Agarose gel (1.5%) electrophoresis of PCR amplicons from *Plectranthus amboinicus* samples collected from different locations showing morphological variation, amplified using the ITS4 and ITS5 primer pair. Lane 1: 100 bp DNA ladder; Lane 2: negative control; Lane 3: M001; Lane 4: M002; Lane 5: M003; Lane 6: M004; Lane 7: M005.

hydrodistilled oils by Pino et al. (2013) and Raut et al. (2010), likely due to the nonpolar nature of hexane, which extracts fewer moderately polar compounds than hydrodistillation. A particularly distinctive feature of this study is the detection of a cyclohexanol derivative (10.84%), a compound not commonly reported in previous GC–MS analyses of *P. amboinicus*, suggesting solvent-specific extraction or the presence of a novel chemotype. Moreover, the relatively high levels of Germacrene D, Humulene, and α-Copaene indicate a sesquiterpene-rich profile that deviates from the monoterpene-dominant compositions documented in studies by Vijayakumar et al. (2022) and Chagonda et al. (2000). These variations underscore the influence of extraction method, geographic origin, and plant chemotype on the resulting volatile composition. The higher concentration of sesquiterpenes observed here may contribute to

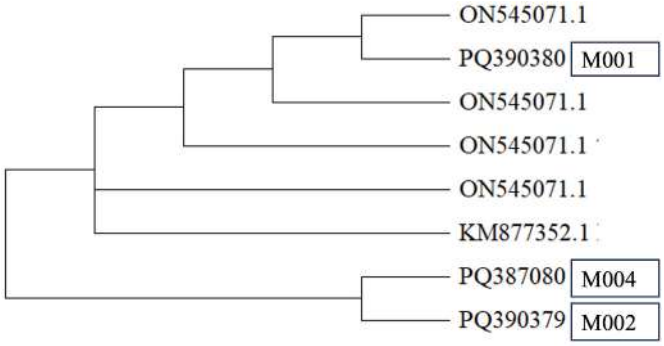

Fig. 7. Phylogenetic tree of *P. amboinicus* constructed using ITS region sequences obtained in this study (PQ387080, PQ390379, and PQ390380 from Sri Lanka) and sequences retrieved from GenBank representing other countries. The additional sequences include ON545071 (India), KM877352 (India), KM877374 (India), KM877368 (India), and OL774797 (Indonesia). The tree was generated using the Neighbor-Joining (NJ) method with 1000 bootstrap replications.

unique bioactivities not captured in earlier essential oil studies. Recent work showing NAGK-targeted bioactivity of *P. amboinicus* essential oil against *S. littoralis* (Amen et al., 2025) suggests that such chemotypic variation could have important implications for pest management as well as pharmacological applications. The chemical profile obtained in this study, particularly the enrichment of sesquiterpenes and the occurrence of a unique cyclohexanol derivative, points to a distinct chemotype of *P. amboinicus* with potential novel therapeutic applications. The relatively low Thymol content and solvent-specific compound recovery highlight the importance of extraction methodology in phytochemical investigations and open new avenues for pharmacological and chemotaxonomic exploration.

3.5. Conclusion

This study comprehensively characterized the morphological, molecular, phylogenetic, and chemical diversity of *P. amboinicus* collected from Sri Lanka. Distinct variation was observed among the morphotypes in terms of plant stature, leaf characteristics, branching pattern, and aroma intensity, indicating notable intraspecific diversity. Morphotype M004 exhibited the strongest aroma, which was associated with dense glandular trichomes and compact leaf morphology, suggesting that microstructural traits significantly influence volatile compound accumulation and emission. Molecular and phylogenetic analyses based on ITS sequences confirmed the identity of all samples as *P. amboinicus*, yet the Sri Lankan accessions formed a separate subclade distinct from Indian and Indonesian accessions, suggesting a geographically adapted or genetically diverged lineage. This has to be verified by analyzing more samples and assessing more genetic markers. This separation implies that *P. amboinicus* populations in Sri Lanka may represent a unique genetic variant or local chemotype evolved under regional environmental conditions. GC–MS profiling of the most aromatic morphotype (M004) revealed a unique volatile composition comprising 28 compounds dominated by *p*-Cymene, Thymol, Germacrene D, and a cyclohexanol derivative. The presence of the cyclohexanol derivative, rarely reported in earlier studies, along with a higher proportion of sesquiterpenes, suggests a distinct chemotype influenced by both solvent extraction and geographical factors. Considering the limitations of hexane extraction, in future other supportive methods (e.g., SPME, hydrodistillation) should also be used to capture all compounds. Collectively, these findings highlight the morphological and chemotypic richness of *P. amboinicus* in Sri Lanka and highlight its potential as a promising source of bioactive compounds for pharmaceutical, aromatic, and nutraceutical applications.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT to assist with paraphrasing and grammar checking. After using this tool the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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CRediT authorship contribution statement

Anushika Christy Mohanathas: Formal analysis, Methodology, Writing – review & editing. **Suvanthini Terensan:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Gajapathy Kanapathy:** Formal analysis, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2026.105285.

Data availability

No data was used for the research described in the article.

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