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Morphological and chemotypic diversity of a cultivated cinnamon (*Cinnamomum verum* J. Presl) collection at the mid-country research station in Sri Lanka

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ABSTRACT

Though Sri Lankan cinnamon (Cinnamomum verum J. Presl) poses a significant morphological and chemical diversity, challenging standardization, it presents opportunities for breeding tailored to niche market products. This study evaluated 71 cultivated C. verum accessions at the Mid-Country Research Station (MRS), Dalpitiya, Sri Lanka, including elite varieties Sri Gemunu and Sri Wijaya. Nine leaf traits were assessed using TURIS 2013 descriptors. Principal Component Analysis (PCA) and cluster analysis grouped accessions into six morphological clusters. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of leaf essential oils revealed 34 compounds. Eugenol ranging from 92.88-98.09% (accessions 52,64,65 and 70) and accession 41, which was unique for 94.94% benzyl benzoate and no eugenol, suggested the existence of distinct chemotypes. The study supports the idea of divergent biosynthetic pathways. These findings highlight the potential of Sri Lankan C. verum for breeding high-value varieties with tailored phytochemical profiles for use in food, pharmaceuticals, and cosmetics.

Keywords: Benzyl benzoate, *Cinnamomum verum,* cinnamon chemotypes, eugenol, germplasm characterization, leaf morphology.

INTRODUCTION

Cinnamon exports from Sri Lanka increased from 17,860 metric tons in 2018 to 19,195 metric tons in 2021, with earnings rising from 124.95 million USD to 167.07 million USD (Central Bank of Sri Lanka, 2023). Beyond the traditional use as a spice, cinnamon has gained increasing recognition in the cosmetic and pharmaceutical industries. Despite this strong global demand and recognition for quality, Sri Lankan cinnamon is still primarily exported in raw form to countries such as the USA, Mexico, Colombia, Ecuador, Peru, Spain, Guatemala, Chile, and Bolivia, often at relatively low prices. To access premium markets and enhance export value, there is a clear need for strategic value addition and cultivar development.

The genus *Cinnamomum* (family Lauraceae) comprises approximately 250 species and subspecies distributed across Asia and other parts of the world (Mabberly, 2008). There are two cinnamon varieties named, *Sri Gemunu* and *Sri Wijaya* in Sri Lanka, which were introduced by the Department of Export Agriculture. These were superior accessions selected from a germplasm collection of approximately 700 accessions and propagated vegetatively to maintain uniformity and desirable traits.

Essential oils from bark, leaves, roots, fruits, and inflorescences contain key volatile compounds such as cinnamaldehyde, eugenol, camphor, cadinene, and cinnamyl acetate (Paranagama *et al*., 2001; Kaul *et al*., 2003). These compounds have been shown to possess diverse biological activities (Ranasinghe and Galappaththy, 2016; Abeysekera *et al*., 2017; Gulcin *et al*., 2019). As such, chemical composition is not only important for quality assessment but also for functional use in various industries.

Studies have consistently shown that *C. verum* germplasm in Sri Lanka exhibits significant morphological and chemical diversity. Leaf morphology in particular has emerged as a useful trait for distinguishing among genotypes (Azad *et al*., 2016a; 2018; 2019; Geekiyanage *et al*., 2025). A significant correlation between leaf shape and oil yield was reported by Wijesinghe and Gunarathna (2001). Azad *et al* (2024) found morpho-chemo correlations of the constituents in cinnamon bark oil while Prathibhani *et al* (2024) identified two distinct cinnamon chemotypes based on leaf essential oil composition, one eugenol-dominant and the other rich in benzyl benzoate. These chemotypes were also linked to visible morphological traits such as flush colour.

Currently, cinnamon genetic resources are maintained in several major repositories across Sri Lanka. National Cinnamon Research and Training Center hosts approximately 700 cultivated accessions, while a wild cinnamon collection is conserved at the Royal Botanical Garden, Peradeniya. Mid-Country Research Station, Dalpitiya, Sri Lanka (MRS) houses a collection of 71 morphologically diverse cultivated accessions. Given this background, the identification and evaluation of morphologically and chemically diverse accessions are essential for cinnamon breeding programmes aimed at developing elite cultivars with targeted chemical profiles. Morphological characterization provides a cost-effective, early-stage screening tool to guide the selection of chemically valuable accessions, especially in resource-limited breeding programs.

Therefore, this study focuses on evaluating the leaf morphological diversity in a cultivated collection of 71 *C. verum* accessions at the MRS, including the *Sri Gemunu* and *Sri Wijaya* varieties. In parallel, we analyzed the essential oil composition of selected accessions to identify distinct chemotypes with potential applications in breeding, product differentiation and commercialization.

MATERIALS AND METHODS

This study was conducted using a cultivated cinnamon (*Cinnamomum verum*) collection of 71 accessions maintained at the MRS (GPS coordinates: 7.1333031 N, 80.590026 E).

Leaf morphological data collection and chemical profiling were carried out to assess phenotypic and biochemical diversity among these accessions. Nine leaf morphological traits {leaf arrangement (LA), leaf length (LL), leaf width (LW), petiole length (PL) leaf shape (LS), leaf margin (LM) leaf apex (LAP), leaf base (LB) and leaf venation (LV)} were assessed using standardized descriptors (Azad *et al*., 2016b). For each accession, measurements were taken from five fully expanded, mature leaves. Sampling was standardized by selecting leaves located between the 5th and 6th nodes below the apical shoot tip of a healthy branch, to minimize developmental variability.

Essential oil extraction and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were performed following the same protocols described by Prathibhani *et al* (2024). Briefly, leaf essential oils were extracted using hydro-distillation with a Clevenger-type apparatus. The extracted oils were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to determine their chemical composition.

Instrument specifications, operational parameters, and compound identification methods were consistent with those described by Prathibhani *et al* (2024), including the use of the National Institute of Standards and Technology (NIST) library for spectral matching and retention index confirmation where applicable.

Principal Component Analysis (PCA) was carried out to identify the major sources of variation among the accessions based on the morphological parameters. Subsequently Hierarchical Cluster Analysis was performed to group morphologically similar accessions. Both analyses were conducted using the FactoMine R package in R (Le *et al*., 2008).

A dendrogram was generated using Ward's method based on Euclidean distances. Five morphologically diverse accessions representing major clusters were selected for further chemical analysis.

RESULTS AND DISCUSSION

Diversity of Accessions based on leaf morphological variation

Leaf is reported to be the most variable, age and environment-independent morphological character in the genus *Cinnamomum* (Ravindran *et al*., 2004). In the present study, leaf morphological traits of 71 cultivated cinnamon (*C. verum*) accessions maintained at the MRS were characterized. Leaf length (LL) ranged from 10.54 cm (accession 26) to 17.64 cm (accession 60), leaf width (LW) from 4.3 cm (accession 26) to 7.4 cm (accession 22), and petiole length (0.9-1.94 cm). The overall means were 14.3±0.2 cm (LL), 5.8±0.1 (LW) and 1.5±0.03 cm (PL). Commercial varieties *Sri Gemunu* recorded 14.0±1.0 cm (LL), 6.7±0.5 cm (LW) and 1.6±0.1 cm (PL) while *Sri Wijaya* recorded 11.9±0.5 cm (LL), 5.9±0.3 cm (LW) and1.2 ±0.1 cm (PL).

The descriptors developed by Azad *et al* (2016b) classified four types of leaf arrangements (LA): opposite, sub-opposite, opposite to sub-opposite in different branches within the same plant and opposite to sub-opposite in the same branch. Representative leaves from the collection are presented in Figure 1. LA of all accessions in the collection at MRS was either opposite or sub-opposite in different branches, but in the same plant or opposite to sub-opposite in the same branch in the same plant, except for accession 40, which showed a purely opposite arrangement. Frequencies of qualitative variables are summarized in Table 1. Both *Sri Gemunu* and *Sri Wijaya* exhibited LA, described as opposite to sub-opposite in different branches of the same plant. According to Prathibhani *et al* (2024), among 40 accessions at the Faculty of Agriculture, University of Ruhuna, the four LA categories were recorded in a 2:1:1:9 ratio. A national survey by Azad *et al* (2019) showed that the "opposite to sub-opposite in same branch" category was the most common (125 out of 269 accessions).

Azad *et al* (2016a) also described nine types of leaf shape (LS), including elliptic, narrowly elliptic, ovate, oval, lanceolate, ovate-lanceolate, oblong-lanceolate, broadly elliptic and broadly ovate. In the Dalpitiya collection, observed LS types included elliptic, narrowly elliptic, ovate, oval and lanceolate (Table 1). Accession 53 exhibited ovate-lanceolate leaves, and accession 66 showed oblong-lanceolate leaves. The broadly ovate leaf shape was not observed in this collection, but was presented only in *Sri Gemunu*. *Sri Wijaya* displayed ovate leaf-shaped leaves. LS distribution was reported as elliptic (15/40), broadly elliptic (1/40), narrowly elliptic (12/40), ovate (2/40), broadly ovate (2/40), oval (3/40), lanceolate (4/40), ovate-lanceolate (1/40), and oblong-lanceolate (0/40) (Prathibhani *et al*., 2024). In accessions from their original locations, these proportions were: elliptic (112/269), broadly elliptic (31/269), narrowly elliptic (45/269), ovate (42/269), broadly ovate (4/269), oval (2/269), lanceolate (29/269), ovate-lanceolate (2/269), and oblong-lanceolate (2/269) (Azad *et al*., 2019). The most common LS types in Sri Lankan cinnamon are elliptic and narrowly elliptic.

The leaf apex (LAP) types observed in this study included acute, obtuse, acuminate, long acuminate, narrowly acuminate, and acuminate with broad acumen. Leaf base (LB) types included acute, subacute, cuneate, rounded, subcordate, obtuse, and contracted into the petiole, then shortly cuneate (Table 1). At the Faculty of Agriculture, University of Ruhuna, LAP types were observed in the following proportions: acute (20/40), obtuse (9/40), acuminate (7/40), long acuminate (2/40), narrowly acuminate (1/40), and acuminate with broad acumen (1/40) (Prathibhani *et al*., 2024). Azad *et al* (2019) reported acuminate as the most common LAP in the national collection. LB types in the Ruhuna collection were recorded as acute (6/40), subacute (8/40), rounded (3/40), obtuse (10/40), and obtuse, contracted into the petiole then shortly cuneate (13/40). Azad *et al* (2019) found acute to be the most common LB type. *Sri Gemunu* and *Sri Wijaya* both exhibited acute LAPs, and rounded LBs.

Leaf venation (LV) types included three-veined and three- or five-veined patterns. All accessions at Dalpitiya displayed one of these two types (Figure 1). *Sri Gemunu* showed both three-veined and five-veined venation, while *Sri Wijaya* had exclusively three-veined venation. Leaf margins (LM) were mostly undulate; only Accession 26 and *Sri Gemunu* had entire margins. These findings provide a valuable baseline for understanding leaf morphological variation in the WM2 agro-ecological zone of Sri Lanka.

Principal Component Analysis and Cluster Analysis

Principal Component Analysis (PCA) identified three components (PC1, PC2, and PC3) with eigenvalues above 1, explaining 69.6%, 18.2%, and 12.3% of the total variation, respectively. PC1, primarily influenced by petiole length (PL) and leaf width (LW), accounted for the largest variation, while PC2 contributed to differentiation mainly associated with leaf length (LL) (Figure 2).

Cluster analysis at a distance of 0.5 revealed three clusters: Cluster 1 with low PC1 values, Cluster 3 with high PC1 values, and Cluster 2 in between. The continuous nature of trait variation resulted in overlapping clusters. The PCA-biplot showed the contribution of PL and LW to PC1. There is a clear separation along PC1, reflecting PL and LW differences. Accessions on the right exhibited higher trait values, while those on the left had lower values. PC2 added minor separation, likely due to LL, with closer clusters indicating phenotypic similarity and wider separation suggesting greater diversity (Figure 2).

Variation of leaf oil composition

GC-MS analysis of leaf essential oil samples from five accessions revealed 34 chemical compounds. Accession 70 reported had the highest eugenol content (98%) followed by Accessions 64 (96%), 52 (95%), 65 (93%) and 41 (0%). Conversely, Accession 41 had the highest benzyl benzoate (BB) content (95%), while Accessions 64, 52, 65 and 70 had only 1%, 1%, 0.3%, and 0.2% respectively. Additional constituents included linalool (0.3% in Accession 41; 0.6% in Accession 65), caryophyllene oxide (0.4% in Accession 70), and α-Pinene (ranging from 0.08% to 0.25%) (Table 2).

Azad *et al* (2024) previously documented essential oil composition from bark samples of 30 cinnamon accessions. Identifying major compounds such as cinnamaldehyde (27.14-82.65%), eugenol (1.62-8.84%), cinnamyl acetate (0.70-47.57%), and BB (0.55-13.14%). They reported that cinnamaldehyde and cinnamyl acetate are negatively correlated (-0.81, $p < 0.01$), while eugenol and BB are positively correlated (0.49, $p < 0.01$). Leaf width correlated moderately with eugenol ($r = 0.37$, $p < 0.05$), and bark thickness correlated with caryophyllene ($r = 0.54$, $p < 0.01$).

Prathibhani *et al* (2024) documented eugenol levels from 0-79.47%, caryophyllene from 0.46-3.08%, linalool from 0-5.59%, and BB from 0-91.92% across different accessions. Seventeen accessions were eugenol-dominant, while two green flush accessions had BB levels of 86.8% and 91.9%, and no eugenol. They reported strong negative correlations between eugenol and linalool (-0.630) and between eugenol and BB (-0.886, $p < 0.001$). For the first time, they reported BB-rich accessions in Sri Lanka: KA11, GB17 (both >85% benzyl benzoate, 0% eugenol), and HB12 (16.5% eugenol, 22.3% benzyl benzoate). In the present study, Accession 41 from Dalpitiya is confirmed as a BB chemotype (95% benzyl benzoate, 0% of eugenol), expanding the known pool of such accessions.

Additional BB chemotypes have been reported by several researchers. Nath *et al.* (1996) identified similar accessions in India. Farias *et al* (2020) reported two chemotypes in Brazil. One accession was with 93.6% of eugenol in leaves and 89.3% of cinnamaldehyde in bark; the other was with 95.3% BB in leaves and 23.3% cinnamaldehyde in bark. Xavier *et al* (2022) also confirmed BB-rich genotypes in Brazil. These findings collectively support the mutual exclusivity of eugenol and BB biosynthesis.

Our results indicate that the chemical composition of *Cinnamomum verum* accessions at the MRS reveals substantial biochemical diversity, with clear potential for applications in food, pharmaceutical, and cosmetic industries.

This diversity holds significant value for cinnamon breeding programmes and industrial applications. Eugenol-rich accessions have strong potential in culinary and therapeutic markets, while BB-dominant chemotypes are promising for use in cosmetics, perfumery, and pharmaceuticals.

CONCLUSION

The cultivated cinnamon (*Cinnamomum verum*) germplasm collection at the MRS, Sri Lanka, demonstrated a morphological and chemical diversity. Six distinct clusters were produced based on leaf morphological trait variation of 71 accessions. GC-MS analysis of selected accessions confirmed the presence of two major chemotypes: a eugenol-rich type and a benzyl benzoate-rich type, with clear mutual exclusivity between these constituents.

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CONFLICT OF INTEREST STATEMENT

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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Table 1: Variation in qualitative leaf morphological traits of 71 cultivated cinnamon accessions at Mid-Country Research Station, Dalpitiya, Sri Lanka.

Qualitative trait	Pattern	Frequency
Leaf arrangement (LA)	Opposite	2
	Sub-opposite	0
	Opposite or sub-opposite in different branch but in same plant	11
	Opposite to sub-opposite in the same branch in same plant	58
Leaf shape (LS)	Elliptic	20
	Broadly elliptic	0
	Narrowly elliptic	23
	Ovate	7
	Broadly ovate	2
	Oval	13
	Lanceolate	5
	Ovate-lanceolate	1
	Oblong-lanceolate	0
Leaf apex (LAP)	Acute	37
	Obtuse	12
	Acuminate	6
	Long-acuminate	4
	Narrowly acuminate	2
	Acuminate with broad acumen	10
Leaf base (LB)	Acute	13
	Subacute	5
	Cuneate	3
	Rounded	15
	Subcordate	2
	Obtuse	2
	Obtuse, contracted into petiole, then shortly cuneate	31
Leaf venation (LV)	3-veined	41
	5-veined	0
	3-veined or 5-veined	30
Leaf margin (LM)	Entire	1
	Undulate	70

Table 2: Variation in major chemical constituents of five selected *Cinnamomum verum* accessions from the germplasm collection at Mid-Country Research Station compared to the *Sri Gemunu* and *Sri Wijaya* varieties

Chemical constituent	Acc. 41	Acc. 52	Acc. 64	Acc. 65	Acc. 70	*Sri Gemunu**	*Sri Wijaya**
Benzyl benzoate	94.94	0.98	1.12	0.26	0.19	-	-
Eugenol	-	95.21	96.09	92.88	98.09	82.11	90.80
Linalool	0.25	0.30	-	0.55	-	2.36	4.94
Caryophyllene	1.61	0.85	0.53	2.45	0.40	4.68	2.32

*****Prathibhani *et al*., 2021

Figure 1. Representative leaves from *Cinnamomum verum* **accessions 2, 4, 8, 18, 46, 49 and 55 in the germplasm collection at the Mid-Country Research Station, Dalpitiya, Sri Lanka. Bar indicates 1 cm.**

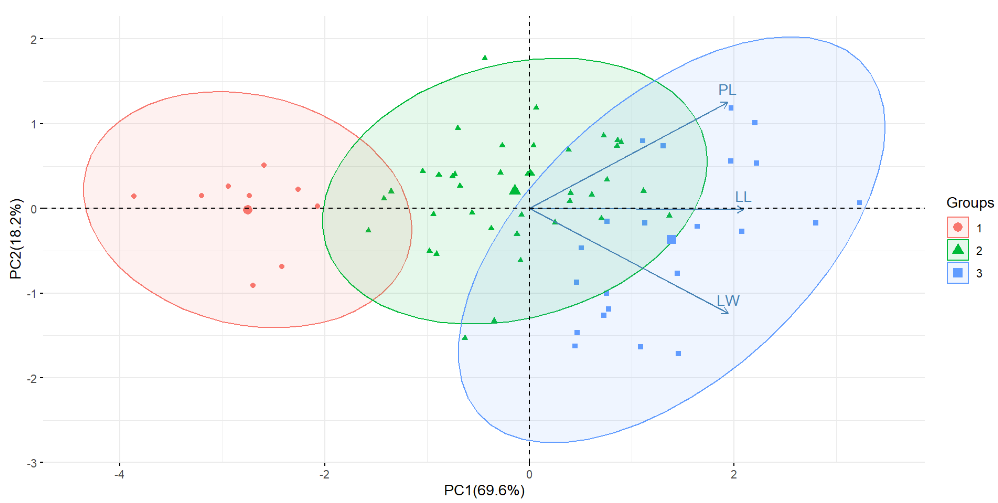

Figure 2. Principal Component Analysis (PCA)- biplot of morphological traits of cinnamon accessions.

Dots (Group 1), triangles (Group 2), and squares (Group 3) represent accessions from clusters 1, 2, and 3, respectively. The vectors PL, LL, and LW indicate the contribution of petiole length, leaf length, and leaf width to the principal components.