

RESEARCH ARTICLE

Germplasm Characterization

Variation in plant morphology and leaf essential oil composition of a representative *Cinnamomum verum* collection from Sri Lanka[†]

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Abstract: Sri Lankan cinnamon germplasm is an underexploited genetic resource of high breeding potential. A representative collection of a cultivated cinnamon germplasm with 48 accessions, is established at the University of Ruhuna, Sri Lanka. From that collection, 40 three-year-old vegetative-propagated accessions, were used in this study to study the variations in plant morphology and leaf oil composition. Flush colour was determined as four main categories of pink, red, brown, and green in all 48 accessions. Leaf length, leaf width, leaf length to width ratio and petiole length varied from 7.9 to 14.5 cm, 3.8 to 7.3 cm, 1.8 to 3.07 cm, and 1 to 2.2 cm, respectively, in the selected 40 accessions. Both the good fragrant aroma category of bark fragrance and the sweet pungent category of bark taste were recorded in 21 of the 40 accessions. Twenty (20) accessions from above collection were selected for leaf oil analysis. Gas chromatography-mass spectrometry (GC-MS) of leaf oil revealed the presence of 146 chemical compounds. Eugenol was the major compound of 17 accessions (52.2% to 79.5%). Two accessions with zero eugenol contained 86.8% and 91.9% of benzyl benzoate (BB), in contrast to that of 0%-0.65% from other accessions. One accession produced 16.6% eugenol and 22.3% BB. A green flush colour associated with a higher BB percentage suggested a potential morphological marker for BB. The PCA for 20 accessions explained 88.11% of total variance. The cinnamon accessions with high BB, KA11

and GB17, were clustered together, and HB12 was isolated on a different cluster. Positive correlations were detected between linalool and β -caryophyllene (0.649), linalool and BB (0.770) ($p < 0.01$), and negative correlations between eugenol and linalool (-0.630) and eugenol and BB (-0.886) ($p < 0.01$).

Keywords: *Cinnamomum verum* collection, GC-MS, leaf essential oil composition, leaf morphology, Sri Lanka.

INTRODUCTION

The genus *Cinnamomum* in plant Family Lauraceae, to which Sri Lankan cinnamon (*Cinnamomum verum* J. Presl) belongs, consists of 250 species and sub-species. Most of these species are distributed in Asia, some parts of South and Central America, and Australia (Mabberley, 2008). *Cinnamomum verum* exhibits three major phenological phases of leaf flushing, flowering and fruiting. According to Hansika *et al.* (2022) flushing is controlled by seasonality and rainfall after a dry period. Azad *et al.* (2018 and 2019a) reported the wide variation of leaf and flower morphology in *Cinnamomum verum* germplasm explored from major growing areas of Sri Lanka.

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There are seven wild cinnamon species in Sri Lanka, which comprise the secondary gene pool of cultivated cinnamon (Kumarathilake *et al.*, 2010). Prathibhani *et al.* (2021) have reported the protogynous dichogamy, leaf morphology, and leaf essential oil composition of selected wild cinnamon species from an *ex situ* conservation site. In 2018, the export volume of 17860 metric tons of cinnamon earned 37,315 million rupees (Central Bank of Sri Lanka, 2020). The leaf and bark essential oils of cinnamon are used primarily for spice, cosmetics, and pharmaceuticals due to their important chemical profiles (Joy *et al.*, 1998). Cinnamon bark, leaf, root, and fruit essential oils bear unique chemical profiles with cinnamaldehyde, eugenol, camphor, and cadinene, respectively, as major components (Senanayake *et al.*, 1990; Paranagama *et al.*, 2001). Cinnamon possesses several anti-diabetic, anti-inflammatory, anti-microbial, insecticidal, and antioxidant properties (Ranasinghe & Galappaththy, 2016). Many traditional Asian cultures use *Cinnamomum verum* as a medicine mainly for bloating, nausea, flatulence, colic and gastro-intestinal tract spastic conditions (Toriizuka, 1998).

Azad *et al.* (2019a) reported an eco-geographical survey in major cinnamon growing areas of the Matara, Galle, Ratnapura, Kalutara, Kurunegala, and Hambantota districts of Sri Lanka to develop a core collection of Sri Lankan cinnamon germplasm. Intensity of leaf spot and rough bark diseases in major cinnamon growing areas was reported by Azad *et al.* (2019b). A vegetative propagated cinnamon collection was established at the Faculty of Agriculture, University of Ruhuna for *ex situ* conservation (Azad *et al.*, 2019a). Azad *et al.* (unpublished data) have reported that there is a variation in essential oil composition of cinnamon bark, collected from plantations more than 30 years old, in major cinnamon growing areas of Sri Lanka. Sri Lankan cinnamon germplasm exhibits a wide variation in morphology that may be associated with distinct chemical profiles (Azad *et al.*, 2016; Azad *et al.*, 2019a).

Determination of morphological markers for the identification of the variation recorded in the chemical composition is important in breeding new varieties. Wijesinghe and Gunarathna (2001) reported a relationship between leaf shape and size with the yield in seven genotypes of true cinnamon, as trees with large round inwardly curved leaves had produced a higher cinnamaldehyde content in bark oil, while high quality leaf oil was obtained from the small round leaves. Flush colour of cinnamon was recorded to be a highly variable qualitative leaf character (Azad *et al.*, 2016; Azad *et al.*,

2019a). The anthocyanins cyanidin glucoside, cyanidin xyloside, and cyanidin galactoside were reported to be the pigments responsible for cinnamon flush colour (Joy *et al.*, 1998). The present study is an attempt to determine the variations of leaf essential oil chemical profiles and plant morphology in Sri Lankan cinnamon germplasm at one location, irrespective of differential environmental effects. Further, the information on characterization of chemical constituents may provide an insight to the biochemical pathways of these chemical constituents. The reports on cinnamon chemical composition by Wijesekera *et al.* (1974), Senanayake and Wijesekera (1990), and Paranagama *et al.* (2001) are based on a limited number of cinnamon genotypes. Morphological characterizations of whole cinnamon plant by Azad *et al.* (2016; 2019a), and flower by Azad *et al.* (2018) were based on *in situ* data from different locations. Azad *et al.* (2015) carried out the *ex situ* evaluation of age and the environmentally independent morphological characters of leaf shape, leaf apex, and leaf base of two *Cinnamomum verum* progenies and mother plants at the same location. Both progenies were different from their mother plants and produced new phenotypes for leaf shape and leaf base. Cross pollination due to protogynous dichogamy in genus *Cinnamomum* including the seven wild cinnamon species in Sri Lanka (Kumarathilake *et al.*, 2010), had led to the wide variation among accessions. Prathibhani *et al.* (2021) have reported the protogynous dichogamy in selected wild cinnamon species from an *ex situ* conservation site and suggested its effect on variation of leaf morphology and leaf essential oil composition. There are no reports on chemical and morphological characterization of a representative cinnamon germplasm collection established at one location in Sri Lanka, which may be independent of environmental effects on morphology and chemical composition. Therefore, this study was conducted to determine the variations of leaf oil composition and plant morphology in a collection of three-year-old vegetative-propagated accessions, established at University of Ruhuna, which were originally collected from major cinnamon growing areas (Azad *et al.*, 2019a).

MATERIALS AND METHODS

Location

The cinnamon collection, established at the Faculty of Agriculture, University of Ruhuna (GPS 6°03'27.7"N 80°34'02.9"E) was used as a representative collection for Sri Lankan cinnamon germplasm (Azad *et al.*, 2019a).

The Faculty of Agriculture, University of Ruhuna is located at Mapalana, Kamburupitiya, in Sri Lanka in the agro-ecological region WL2 (low country wet zone with >1900 mm of annual rainfall). Mean monthly temperatures of the location during the research period of August to October, 2018 were 28.8 °C, 30.4 °C, and 28.4 °C, respectively. Mean monthly rainfalls were 2.14 mm, 4.91 mm, and 9.79 mm, respectively.

Morphological characterization

Material: The representative cinnamon collection (Azad *et al.*, 2019a) was comprised of 48 vegetative-propagated, three-year old accessions, which were used for flush colour determination. For data collection of other characters, only 40 accessions were selected based on leaf availability and plant growth. All morphological characters except the flush colour of the accessions were based on the Descriptors for Cinnamon (Team of TURIS 2013 Project, 2016).

Leaf characters

The above mentioned 40 cinnamon accessions in the collection were characterized based on both quantitative and qualitative leaf morphological characters, *viz.*, leaf length (LL), leaf width (LW), Petiole length (PL), leaf arrangement (LA), leaf shape (LS), leaf apex (LAP), leaf base (LB), leaf texture (LT), upper surface leaf texture (UST), lower surface leaf texture (LST), leaf venation (LV), and leaf margin (LM), during August and September, 2018. For flush colour (FC) determination, a young active bud within two days of emerging was selected from each accession. Observations were taken on five consecutive days. At the fourth to fifth day of the fully emerged flush, colour was recorded.

Bark characters

Bark characters *viz.*, bark fragrance (BF), bark thickness (BTH), bark taste (BT), bark colour (BC), peel quality (PQ) and bark surface (BS) were recorded.

Tree characters

Tree characters, *viz.*, tree height (TH), tree vigor (TV), tree shape (TS), trunk circumference (TC), trunk surface (TSU), crotch angle of main branches (CA), inter-nodal length of twigs (INLT), distribution of branches (DIBR), twig diameter (TWD), and branching pattern (BP) were recorded. The plants were observed for the presence or absence of flower buds, flowers and fruits at the time

of data recording. The occurrence of leaf spot disease was recorded in order to determine the level of resistance to leaf spot disease among cinnamon accessions. The variations of selected qualitative characters are graphically presented, while quantitative character variations are given in the text.

Chemical characterization of leaf oil

Leaf oil extraction

Material: Twenty (20) accessions were selected from the collection, considering the diversity of leaf morphology and high leaf availability. For oil extraction, healthy, mature, fresh leaves were collected from selected accessions in October 2018.

Methods: Collected leaves were shade dried at room temperature for two weeks until the colour changed from green to metallic brown. Dried cinnamon leaves were cut into small pieces of ~1-1.5 cm before hydro-distillation in a light oil clever-type apparatus to extract cinnamon leaf essential oil at the National Cinnamon Research and Training Center (NCRTC), Pallolpitiya, Thihagoda, Sri Lanka. The round bottom flask with 50 g of dried leaf sample and 250 mL of tap water was settled on the heater. The settled Clever arms were filled with 2 mL of hexane ether solvent (2 : 1 ratio of hexane to ether) and 10 mL of distilled water. Hydro-distillation was performed for 4 h for one sample. Cinnamon leaf essential oil was separated from the mixture of hexane ether, water and leaf essential oil after an overnight settling period.

GC-MS analysis

Material: Twenty (20) cinnamon leaf essential oil samples were prepared for the GC-MS analysis.

Method: Each essential oil sample of 50 µL was dissolved in 950 µL of absolute methanol. All samples were filtered using Agilent 0.22 µm PTFE syringe filters. An Agilent 7890A gas chromatograph system (Agilent, USA) coupled with a 5975C inert XL EI/CI triple axis mass selective detector was used to perform GC-MS analysis at the Instrumental Center, University of Sri Jayawardenapura, Sri Lanka. A 19091s-433HP-5MS 5% phenyl methylpolysiloxane capillary column (of 30 m length and 250 µm inner diameter, 0.25 µm film thickness) was used for separation. One microliter of the sample was injected using a split injector with a split ratio of 1:100. The initial oven temperature was set at 40 °C and increased up to 230 °C at a rate of 5 °C/min.

Total run time was 38 min. The injector temperature was maintained at 250 °C. The ion source temperature was 230 °C. The carrier gas was Helium (He) with a flow rate of 1 mL/min. Leaf essential oil constituents were identified based on their mass spectra with authentic standard samples or with those recorded in the National Institute of Standards and Technology database. The relative abundance of identified chemical compounds of each accession were recorded. Morphological and chemical variables were subjected to PCA followed by cluster analysis through FactoMine R (Le *et al.*, 2008).

RESULTS AND DISCUSSION

Morphological characterization

Leaf characters: Leaf is one of the most useful morphological characters in genus *Cinnamomum* as it varies greatly among species (Ravindran *et al.*, 2004). Mean values of LL, LW, length to width ratio and PL were 11.40 cm (± 0.24), 5.2 cm (± 0.14), 2.2 (± 0.04) and 1.5 cm (± 0.04) respectively. In Table 1, the variation of quantitative leaf characters such as LL, LW, leaf length to width ratio, and PL of the accessions in the collection established at the Faculty of Agriculture, University of Ruhuna, Sri Lanka was compared and contrasted with the reported ranges of above characters at the original locations by Azad *et al.* (2019). In the same table, the means of above characters of selected wild cinnamon species (Prathibhani *et al.*, 2021), are compared. Leaf arrangement patterns of opposite, sub-opposite, opposite or sub-opposite in different branch but in same plant, and opposite to sub-opposite in the same branch in the same plant, as indicated, are illustrated in Figure 1(a), following the Descriptors for Cinnamon by Team of TURIS 2013 Project (2016). The above leaf arrangement patterns were recorded in 7/40, 3/40, 4/40, and 26/40 accessions, respectively (Figure 1A). There were eight types of leaf shapes as described in the Descriptors from elliptic to oblong-lanceolate (Figure 1(b), in different frequencies. Elliptic, broadly elliptic, narrowly elliptic, ovate, broadly ovate, oval, lanceolate and ovate-lanceolate categories were recorded in 15/40, 1/40, 12/40, 2/40, 2/40, 3/40, 4/40, and 1/40 accessions, respectively (Figure 1B). Six types of leaf apices, *viz.*, acute, acuminate, long acuminate, narrowly acuminate, acuminate with broad acumens and obtuse (Figure 1(c)) were recorded in 20/40, 9/40, 7/40, 2/40, 1/40, and 1/40 accessions, respectively (Figure 1C). Five categories of leaf base, acute, sub-acute, rounded, obtuse, and obtuse

contracted into petiole then shortly cuneate (Figure 1(d)) were recorded in 6/40, 8/40, 3/40, 10/40, and 13/40 accessions, respectively from the collection (Figure 1(D)). Three types of leaf venation patterns, *viz.*, three-veined, five-veined and three- and five-veined on the same plant were observed in 26/40, 1/40, and 13/40 accessions, respectively in the *Cinnamomum verum* collection. Two types of leaf margins, entire and undulate, were recorded at frequencies of 6/40 and 34/40 accessions, respectively. In *Cinnamomum verum*, observations were made on the flushes emerging directly from the main stem, from branches, from twigs, or from the cut base of the stem. The colour patterns varied in flushes of different origin on the same plant. A gradual colour change was observed in flush with time from emergence to maturation. The variation of flush colour of a plant in terms of spatial and temporal variations may be due to genetic response to the environmental cues. The frequency of pink, red, brown, and green colour categories of the same aged flushes is depicted in Figure 2. Photographs of different flushes under each colour category are presented: The pink and red are in Figure 3, while brown and green are in Figure 4. In Figure 3, the GAM2 (A), KE5 (B) and GK 17 (C) accessions represent the light pink leaf blade with a light green base. A pink leaf blade with a light green base is represented by accessions GB13-2 (D), KA11 (E) and KA14 (F). Accessions KB 14 (G), GB13-1(H) and GE7 (I) represent the red leaf blade with a green leaf base. In Figure 4, the MaDS5 (J), 2MDS5-2 (K) and Palol-1(L) accessions represent the light brown throughout the leaf. A light brown leaf blade with a green leaf base was represented by accession MKG14 (M). Brown colour throughout the leaf is represented by 2 MDS4 (N), while a brown leaf blade with a light green leaf base was represented by 2-MDS5-1(O) and MKG 13 (P). A light green colour throughout the leaf was represented in HB12 (Q) while a whitish green colour throughout the leaf blade with light pink leaf apex was represented by GB17 (R).

Bark characters: The frequencies of different bark fragrance types, bark tastes, bark peeling qualities, and bark surface types of the cinnamon accessions in the collection were reported (Figure 5). The good fragrant aroma category of bark fragrance and the sweet pungent category of bark taste were recorded at the frequency of 21/40 accessions. Weak bark peeling qualities were recorded at the frequency of 16/40, while the good peeling quality was at 10/40 accessions. Slightly rough bark surface was at the frequency of 22/40 accessions.

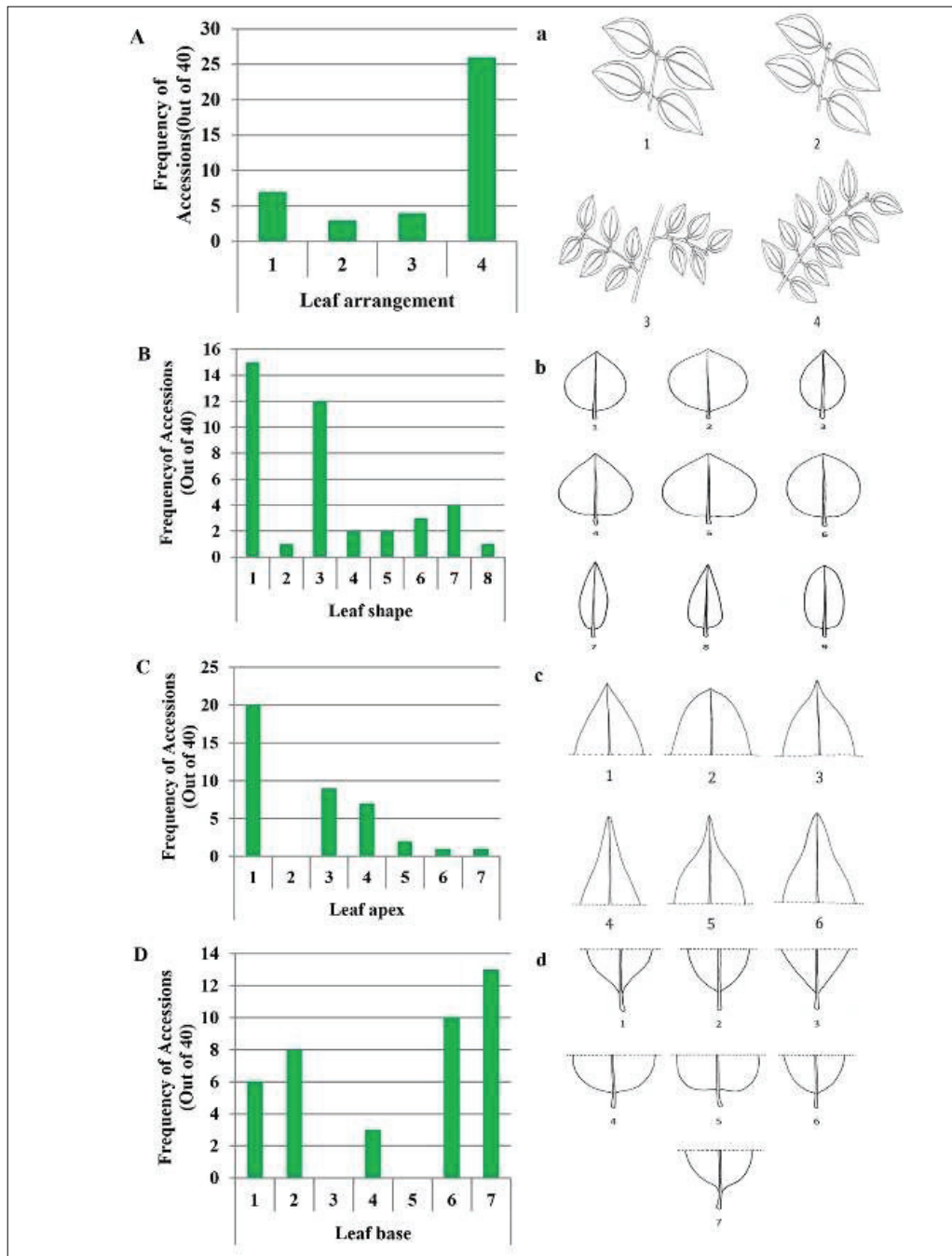


Figure 1: Variation of leaf morphology in the cinnamon collection at University of Ruhuna based on the Descriptors for Cinnamon (*Cinnamomum verum*) (Team of TURIS 2013 Project, 2016); (A) Frequency of leaf arrangement types (a) Descriptors for leaf arrangement patterns of *Cinnamomum verum*; 1. opposite 2. sub-opposite 3. opposite to sub-opposite in a different branch in same plant 4. opposite to sub-opposite in the same branch in the same plant (B) Frequency of different leaf shapes (b) Descriptors for leaf shapes of *Cinnamomum verum*; 1. elliptic 2. broadly elliptic 3. narrowly elliptic 4. ovate 5. broadly ovate 6. oval 7. lanceolate 8. ovate-lanceolate 9. oblong-lanceolate (C) Frequency of leaf apex types (c) Descriptors for leaf apex types of *Cinnamomum verum*; 1. acute 2. obtuse 3. acuminate 4. long acuminate 5. narrowly acuminate 6. acuminate with broad acumen 7. other (D) Frequency of leaf base types (d) Descriptors for leaf base types of *Cinnamomum verum*; 1. acute 2. subacute 3. cuneate 4. rounded 5. subcordate 6. obtuse 7. obtuse, contracted into petiole, then shortly cuneate.

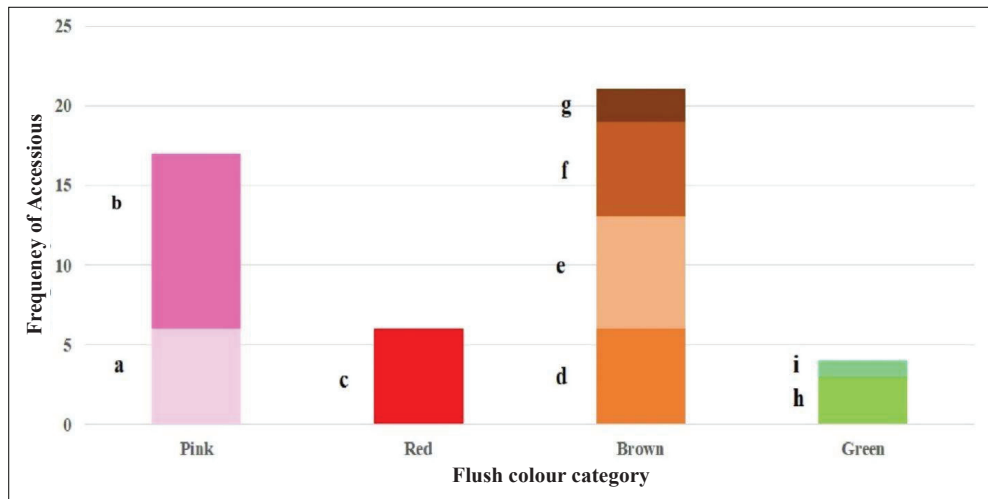


Figure 2: Variation of flush colour of the cinnamon collection at University of Ruhuna Pink; (a) light pink colour leaf blade with light green colour base (6/48) (b) pink colour leaf blade with light green leaf base (11/48) Red; (c) red colour leaf blade with green leaf base (6/48) Brown; (d) light brown colour throughout the leaf (6/48) (e) light brown colour leaf blade with green leaf base (7/48) (f) brown colour throughout the leaf (6/48) (g) brown colour leaf blade with light green leaf base (2/48) Green; (h) light green colour throughout the leaf (3/48) (i) whitish green colour throughout the leaf blade with light pink leaf apex (1/48)

Table 1: Variation of leaf quantitative characters of cultivated and wild cinnamon species

Leaf character	Range						
	1	2	3	4	5	6	7
Leaf length (cm)	7.9 to 14.5	7.43 to 20.26	14.02 ± 2.13	11.88 ± 1.22	13.98 ± 1.44	12.52 ± 1.53	7.44 ± 0.44
Leaf width (cm)	3.8 to 7.3	3.4 to 10.08	6.68 ± 1.13	5.92 ± 0.64	4.20 ± 0.67	3.78 ± 0.05	2.66 ± 0.23
Leaf length to width ratio	1.08 to 3.07	1.58 to 3.1	2.10	2.01	3.33	3.31	2.81
Petiole length (cm)	1.0 to 2.2	0.9 to 2.7	1.60 ± 0.26	1.16 ± 0.21	1.00 ± 0.24	0.66 ± 0.21	0.88 ± 0.21

1 Accessions of the collection at Faculty of Agriculture, University of Ruhuna, during the current study, 2 Accessions at the original locations (Azad et al., 2019), 3 Cultivated cinnamon variety *Sri Gemunu* at Mid-Country Research Station, 4 Cultivated cinnamon variety *Sri Wijaya* at Mid-Country Research Station 5 *Cinnamomum capparu-coronde* Blume at Mid-Country Research Station, 6 *Cinnamomum litsaeifolium* Thwaites at Mid-Country Research Station (Prathibhani et al., 2021)

Tree characters: TH of accessions ranged from 0.53 m to 2.51 m. TV of accessions was reported as weak (8/40), intermediate (20/40) and strong (12/40). Pyramidal (7/40), circular (3/40), and irregular (30/40) shaped TS were reported. The frequencies of TSU of accessions were reported as smooth (10/40), rough (25/40) and very rough (5/40). CA of accessions was reported as 90° (14/40) and other (26/40). The average INLT of accessions

was reported from 1.2 to 8 cm. The frequencies of DIBR was reported as ascendant (26/40), irregular (3/40), axial (1/40), and horizontal (10/40). The presence of leaf spot disease was reported in all accessions. The average TWD of accessions ranged from 0.9 to 2.3 cm. Nine accessions were reported with the extensive BP and 31 were reported with the intensive BP. Six accessions were at flowering during the period of data collection.



Figure 3: Representative accessions for different flush colour categories of pink and red in the cinnamon core collection: Light pink colour leaf blade with light green colour base (A) GAM2 (B) KE5 (C) GK17, Pink colour leaf blade with light green leaf base (D) GB13-2 (E) KA11 (F) KA14 and Red colour leaf blade with green leaf base (G) KB14 (H) GB13-1 (I) GE7.

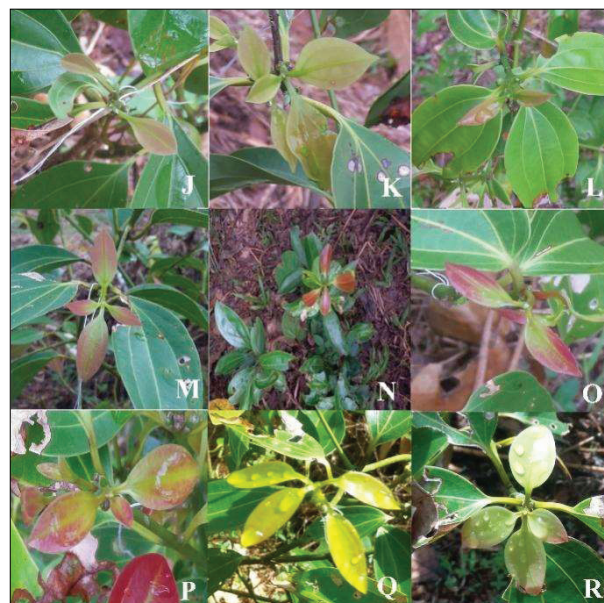


Figure 4: Representative accessions for different flush colour categories of brown and green in the cinnamon core collection: Light brown colour throughout the leaf (J) MaDS5 (K) 2MDS5-2 (L) Palol-1, Light brown colour leaf blade with green leaf base (M) MKG14, Brown colour throughout the leaf (N) 2MDS4, Brown colour leaf blade with light green leaf base (O) 2MDS5-1 (P) MKG13, Light green colour throughout the leaf (Q) HB12 and Whitish green colour throughout the leaf blade with light pink leaf apex (R) GB17.

Variation in quantitative leaf characters LL, LW, length to width ratio, PL, and in qualitative leaf characters LS, LAP, LB, LA, and LM of cinnamon accessions at the collection at the Faculty of Agriculture, in contrast to their original locations, reflects the intra-specific genetic diversity of *Cinnamomum verum*. Plants acquire morphological plasticity with confounding environmental conditions. In most plant species, leaf morphological plasticity is incompletely understood. In *Quercus acutissima*, stable leaf morphological characters at species level are the main characters that adapt to environmental changes (Zhang *et al.*, 2018). During data collection, the accessions KA 12, KA14, KD5-1, GAK9, RL16-1 and RL16-2 were at the first flowering. Environment dependent leaf morphological characters could be identified based on the variability of the characters, which was valid for wild cinnamon as well (Table 1).

GC-MS analysis: One hundred and forty-six chemical compounds were reported during the GC-MS analysis of 20 leaf oil samples. Eugenol was the major compound of

seventeen accessions with the content ranging from 52.2-79.5%, while BB was the major compound of other three accessions ranging from 22.3-91.1%. Zero percentage of eugenol with 86.8 and 91.9% of BB were recorded in two accessions. The accession which produced 22.3% of BB, recorded 16.5% of eugenol (Table 3). The variation of eugenol, linalool, β -caryophyllene and BB in twenty cinnamon accessions collected from different cinnamon growing districts and established at the Faculty of Agriculture is illustrated in Figure 6. The range of selected fatty acids from 20 cinnamon leaf oil samples detected through GC-MS is listed (Table 3).

Principal component analysis (PCA): Fifty-five morphological and chemical variables (including 31 morphological variables and 24 chemical variables) were subjected to PCA. Eight PCs explained 83.11% total variance. In Table 4, percentage of variance of

each PC is presented. Methyl palmitate (MetP), methyl stearate (MS), methyl myristate (MeM), methyl laurate (MeL), toluene (To), prococene-2 (HB), ethyl palmitate (EthP), methyl linolelaidate (MeLIn), methyl elaidate (Octa), methyl iso-eugenol (MeIE), 2-oxazolidone

(Oxa), ethyl oleate (EO), α -caryophyllene (AlCa), benzyl benzoate (BB), (1~{Z},4~{Z},7~{Z})-1,5,9,9-tetramethylcycloundeca-1,4,7-triene (Cyc) and linalool (Li) were among major components of total variance ($p < 0.05$).

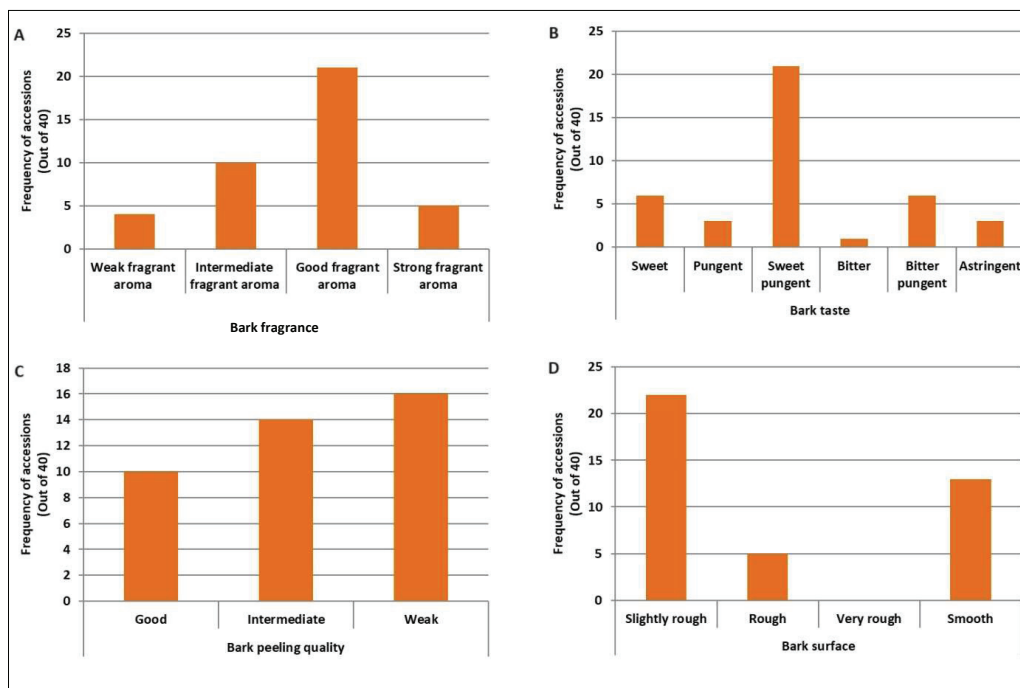


Figure 5: (A) Frequency of bark fragrance types in the cinnamon collection at University of Ruhuna based on Descriptors for Cinnamon (Team of TURIS2013 Project, 2016): (B) Frequency of bark tastes in the cinnamon core collection: (C) Frequency of bark peeling qualities in the cinnamon core collection: (D) Frequency of bark surface types in the cinnamon core collection

Correlation matrixes of major chemical compounds:

Pearson correlation coefficients were generated among eight major chemical variables, viz., toluene, eucalyptol, linalool, eugenol, α -caryophyllene, β -caryophyllene, caryophyllene oxide, and BB, through IBM SPSS version 24.0 statistical software (Table 5). Strong positive correlations were found between linalool and β -caryophyllene (+0.649), and linalool and BB (+0.770), at the 0.01 level of significance. Strong negative correlations were found between linalool and eugenol (-0.630), and eugenol and BB (-0.886), at the 0.01 level of significance. At the 0.05 level of significance, positive correlations between eucalyptol and α -caryophyllene

(0.472), and β -caryophyllene and BB (0.498), and negative correlations between toluene and linalool (0.483), eucalyptol and caryophyllene oxide (0.457), and eugenol and β -caryophyllene (0.497) were significant.

Cluster analysis: Cluster analysis, followed by PCA using the average linkage method, classified the selected twenty cinnamon accessions into five clusters at an average distance of 2 (Figure 7). Cluster analysis assigned the two cinnamon accessions with highest BB percentages, KA11 and GB17 (86.8% and 91.9% respectively) into one cluster and HB12 of (22.3% of BB) into a different cluster.

Table 2: A comparison of eugenol, β -caryophyllene, linalool, benzyl benzoate, methyl eugenol, eucalyptol, and alpha-terpineol in leaf oil of cultivated and selected wild *Cinnamomum* species from Sri Lanka

Source	Relative abundance (%)						
	Eugenol	β -Caryophyllene	Linalool	Benzyl benzoate	Methyl eugenol	Eucalyptol	Alpha-terpineol
Wijesekara <i>et al.</i> , 1974	87	1.85	1.5	2.67	-	-	0.15
Paranagama <i>et al.</i> , 2001	76.74	3.47	2.77	4.01	-	-	0.28
Senanayake <i>et al.</i> , 1978	68.5	3.33	2.4	4.06	0.01	-	0.4
Schmidt <i>et al.</i> , 2006	74.9	4.1	2.5	3.0	-	-	0.3
Liyanage <i>et al.</i> , 2017	85.66	1.08	0.97	-	-	-	-
Accessions of collection at the Faculty of Agriculture, Univ. of Ruhuna during the current study	0-79.47	0.46-3.08	0-5.59	0-91.92	-	0.06-0.43	0.11-0.42
<i>Cinnamomum</i> <i>Capparu-coronde</i> Blume (Prathibhani <i>et al.</i> , 2021)	33.11	1.81	11.76	-	-	-	0.37
<i>Cinnamomum</i> <i>dubium</i> Nees (Prathibhani <i>et al.</i> , 2021)	-	-	3.41	-	-	51.19	13.18
<i>Cinnamomum</i> <i>litsaeifolium</i> Thwaites (1) (Prathibhani <i>et al.</i> , 2021)	24.99	-	7.16	-	59.27	-	0.63
<i>Cinnamomum</i> <i>litsaeifolium</i> Thwaites (2) (Prathibhani <i>et al.</i> , 2021)	-	3.04	30.93	-	-	0.12	10.01

According to previous records on leaf chemical composition of *Cinnamomum verum* from Sri Lanka, eugenol accounts for the highest percentage in leaf essential oil mainly with low percentages of linalool, β -caryophyllene, cinnamaldehyde, and BB (Wijesekera *et al.*, 1974; Liyanage *et al.*, 2017; Ravindran *et al.*, 2004; Schmidt *et al.*, 2006; Paranagama *et al.*, 2001). In agreement with those reports, seventeen accessions in the core collection were detected with eugenol ranging from 52.2-79.5%. However, *Cinnamomum dubium* is reported to possess less or zero eugenol in leaves (Kumarathilake, 2009; Prathibhani *et al.*, 2021). Deviating from other accessions, three accessions from Kalutara (KA11), Hambantota (HB12), and Galle (GB17), produced chemical profiles, mainly with BB (Figure 7). Both

HB12 and GB17 were associated with green flush colour, which needs to be further investigated as a potential morphological marker. Determination of flavonoid composition and variation of Sri Lankan cinnamon germplasm would be important in the production of therapeutic products. This is a recent record of the presence of BB as the major constituent in cinnamon leaf essential oil and the first record from Sri Lanka. GC and GC/MS analysis of leaf and bark essential oils from *Cinnamomum verum* grown in the Brahmaputra valley, India, recorded BB as the main constituent at 65.42% and 84.69%, respectively (Nath *et al.*, 1996). GC-MS analysis reported cinnamaldehyde, cinnamyl acetate, eugenol, BB, β -caryophyllene, and linalool as the major chemical compounds in bark essential oil profiles from the

accessions at their original locations. There were positive correlations between eugenol and BB (0.49), and linalool and β -caryophyllene (0.52), and negative correlations between cinnamaldehyde and cinnamyl acetate (0.81), cinnamaldehyde and BB (0.37), and cinnamaldehyde and

β -caryophyllene (0.37) (Azad *et al.*, unpublished data). There was a positive correlation between eugenol and BB in bark essential oil profiles, which had cinnamaldehyde as the major constituent. In contrast, there is a negative correlation between eugenol and BB in leaf essential oil

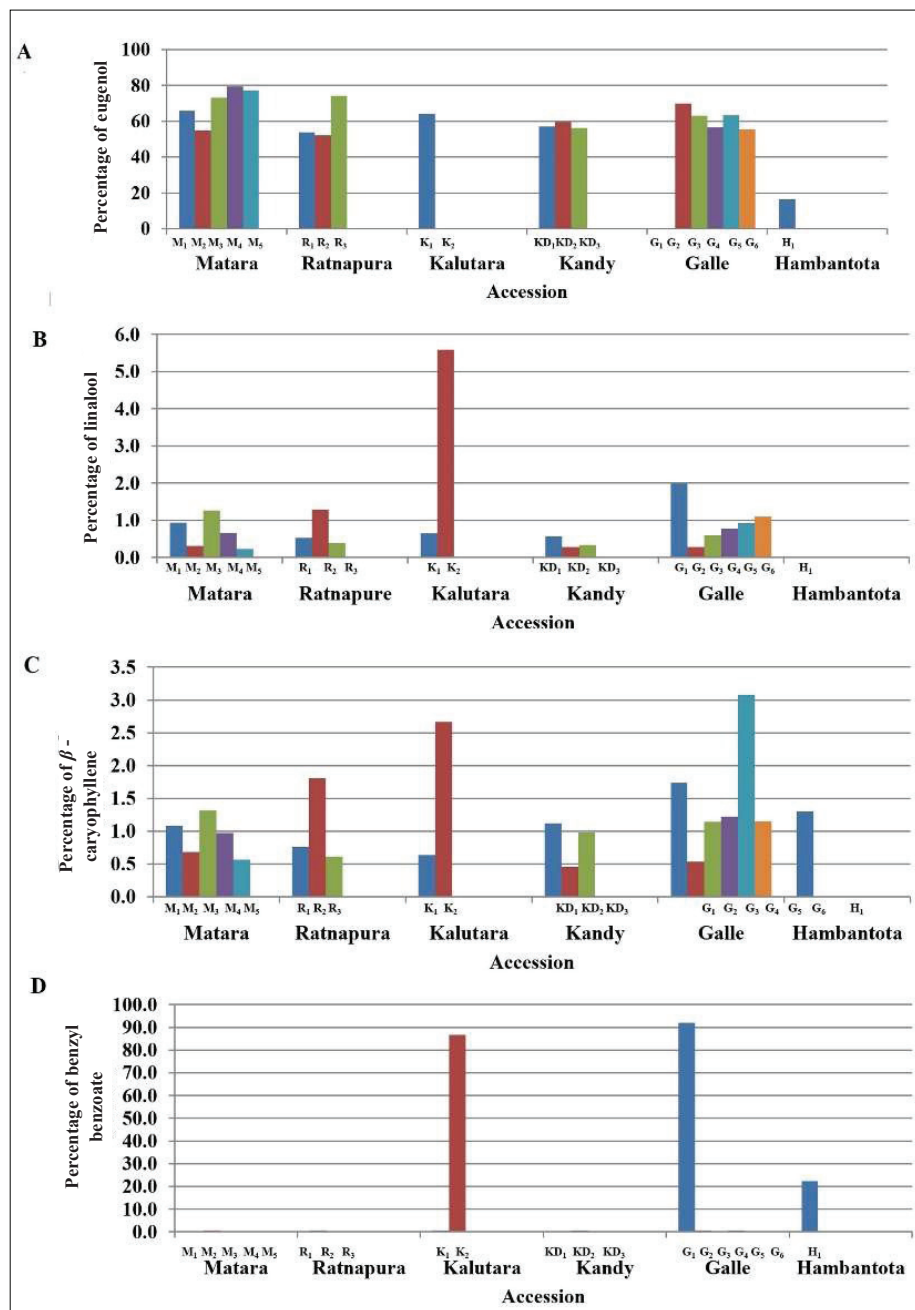


Figure 6: Variation of percentages of eugenol (A), β -Caryophyllene (B), Linalool (C) and Benzyl benzoate (D) in selected accessions of core collection originally from different districts and grown at Faculty of Agriculture, University of Ruhuna. M1, M2, M3, M4, M5, R1, R2, R3, K1, K2, KD1, KD2, KD3, G1, G2, G3, G4, G5, G6 and H1 represent accessions MKG14, MKG13, MaDS5, 2MDS5-1, 2MDS4-1, RL16-2, RL16-1, RL15, KA14, KA11, KD5-2, KD5-1, KD2-1, GB17, GK17, GE11, GAM2, GAK9, GAK3-1 and HB12 respectively.

profiles in the current study. The fatty acid methyl esters methyl laurate, and methyl myristate are used as flavoring agents and may be responsible for the characteristic

odour and taste of cinnamon. Most of these fatty acids recorded from cinnamon leaf essential oil are identified as plant metabolites. Fatty acid analysis of *Cinnamomum verum* bark oil using gas liquid chromatography has shown the presence of undecanoic acid (2.43%), lauric acid (0.5%), palmitic acid (15.58%), linoleic acid (26.08%), stearic acid (47.68%), and oleic acid (7.73%) (Jamil *et al.*, 2012). Schmidt *et al.* (2006) reported that the presence of considerably higher concentrations of the phenolic compound, eugenol, was responsible for the antioxidant capacity of cinnamon leaf essential oil. Cinnamon essential oil could be an alternative to replace the synthetic carcinogenic antioxidants of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), with a higher antioxidant activity and the lowest IC₅₀ value among them. Cinnamon oil was effective over BHT in inhibition of secondary product formation

Table 3: Range of fatty acids in cinnamon leaf essential oil from 20 cinnamon accessions

Fatty acid	Range of relative abundance%
Methyl laurate	0-3.74
Methyl myristate	0-1.89
Methyl palmitate	0-8.96
Ethyl palmitate	0-0.20
Methyl stearate	0-1.37
Ethyl oleate	0-0.21
Methyl linolelaidate	0-2.95

Table 4: Variance (%) of each principal component (PC) of principal component analysis (PCA)

PC	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	10 th
Variance	9.250	5.057	3.346	3.209	2.107	1.840	1.350	1.155	0.986	0.737
% Variance	29.838	16.314	10.794	10.351	6.798	5.936	4.356	3.726	3.182	2.378
Cumulative % of variance	29.838	46.153	56.947	67.297	74.095	80.031	84.387	88.113	91.295	93.673

Table 5: The correlation matrix of eight identified chemical compounds in cinnamon leaf essential oil collected from 20 accessions of the core collection.

Variable	To	Euc	Li	Eu	Alca	Ca	CaOx	BB
To	1.000	0.191	-0.483*	0.145	0.437	0.004	0.205	-0.417
		0.420	0.031	0.543	0.054	0.987	0.386	0.067
Euc		1.000	-0.185	0.298	0.472*	0.285	-0.457*	-0.345
			0.434	0.203	0.036	0.223	0.043	0.136
Li			1.000	-0.630**	-0.238	0.649**	0.007	0.770**
				0.003	0.311	0.002	0.977	0.000
Eu				1.000	0.035	-0.497*	0.171	-0.886**
					0.884	0.026	0.472	0.000
Alca					1.000	0.369	-0.056	-0.205
						0.110	0.813	0.386
Ca						1.000	0.017	0.498*
							0.942	0.026
CaOx							1.000	0.011
								0.962
BB								1.000

To: toluene; Eu: eucalyptol; Li: linalool; Eu: eugenol; Alca: α -caryophyllene; Ca: β -caryophyllene; CaOx: caryophyllene oxide and BB: benzyl benzoate

by lipid peroxidation. However, all twenty accessions have distinct chemical profiles differentiating from each other even in the same environmental conditions. The different chemical cultivars in the cinnamon plantations had been recognized as sweet, honey, camphoraceous, mucilaginous, wild, and bloom by planters based on sensory evaluation. Only the sweet and honey varieties had been selected for cultivation (Ravindran *et al.*, 2004). Accessions with more than 70% of eugenol and zero percentage of BB were recorded as having a sweet pungent taste (RL15, MaDS5, 2MDS5-1 and 2MDS4-1). KA11 and GB17 with more than 85% of BB and zero percentage of eugenol recorded sweet pungent and sweet tastes, respectively. HB12 with 22.3% of BB and 16.6% of eugenol produced a bitter pungent taste. According to the above results, there is a possibility that bulk oil yields of cinnamon bark and leaf from commercial plantations contain variable chemical compositions suggesting a great potential in utilization for diverse industries of food, pharmaceuticals, and cosmetics. The results of the study showed that there is an immediate requirement in standard grading systems and quality estimating for chemical profiles in export bulks of cinnamon products

from Sri Lanka. Continuous cross pollination over generations may have created a wide chemical diversity and leaf diversity including a variety of flush colours among accessions of Sri Lankan cinnamon germplasm. Accessions GB 17 and HB 12 recorded zero or low eugenol and exceptionally higher benzyl benzoate, in contrast to other tested accessions contained flushes of green colour variations. Our results suggest the utilization of germplasm to optimize economic potential: The identified accessions with distinct chemical profiles could be released to the farmers after evaluation of their agronomic feasibility by the Department of Cinnamon Development. Simultaneously, the results provide an insight into the importance of the cinnamon germplasm: It would be a breeding resource for future cultivars of novel chemical compositions leading to desired aroma and flavour. On the other hand, this germplasm will provide information on elucidating the relationship between the pigment composition of flush colour and the essential oil biosynthesis pathway, to reveal the potential morphological markers for distinct chemical cultivars.

CONCLUSION

There is a variation in leaf morphological characters among accessions in the collection. There are four main categories of flush colour – pink, red, brown, and green. Leaf length, leaf width, leaf length to width ratio, and petiole length vary from 7.9 to 14.5 cm, 3.8 to 7.3 cm, 1.8 to 3.07 cm, and 1 to 2.2 cm, respectively. The collection comprised different bark fragrance types. According to the analysis of gas chromatography-mass spectrometry (GC-MS) of leaf oil, we report that there are eugenol (52.2% to 79.5%) rich accessions and, for the first time in Sri Lanka, zero to 16.6% eugenol containing, benzyl benzoate (BB) rich (23.3%, 86.8% and 91.9%) accessions. The green flush colour is associated with a higher BB percentage, which needs to be further investigated as a visual marker.

The dendrogram followed by the PCA, which is derived through variations of plant morphological characters and leaf oil constituents, explained 88.11% of total variance. The cinnamon accessions with exceptionally high BB, were clustered as distinct.

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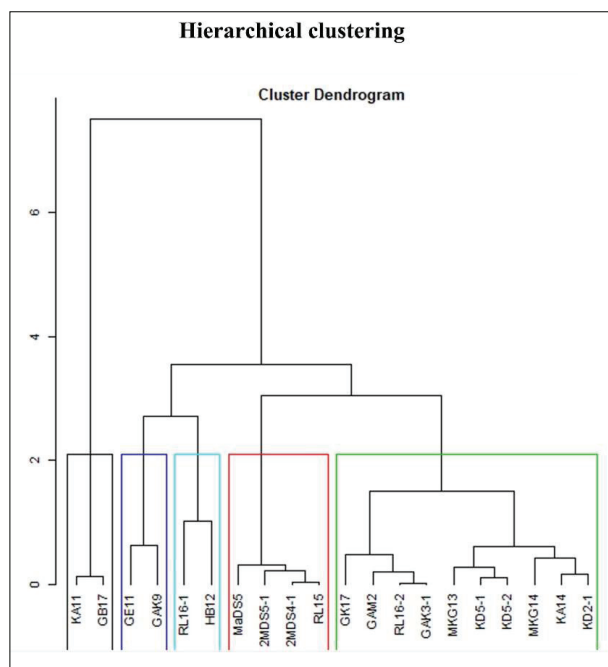


Figure 7: Dendrogram classified the twenty accessions of the core collection at the Faculty of Agriculture, University of Ruhuna, based on both morphological and chemical variables.

(2018) to SG. Authors wish to acknowledge the Staff of the Division of Post-Harvest Technology, National Cinnamon Research and Training Center, Pallolpitiya, Sri Lanka and the Instrument Center, Faculty of Applied Sciences, University of Sri Jayawardhanapura, Sri Lanka for technical support of this study.

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