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RESEARCH ARTICLE

PROTOGYNOUS DICHOGAMY, LEAF MORPHOLOGY AND LEAF ESSENTIAL OIL COMPOSITION OF SELECTED *Cinnamomum* SPECIES IN SRI LANKA

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ABSTRACT

Wild relatives of cultivated cinnamon (*Cinnamomum verum* J. Presl) are underutilized and endangered in Sri Lanka. There is a lack of knowledge on wild relatives of cinnamon, hampering their utilization in breeding and industry. Morphology, floral behaviour and leaf essential oil composition of selected wild relatives were determined under *ex-situ* conservation in Dalpitiya, Sri Lanka. Floral cycles were determined in *Cinnamomum dubium* Nees (*Cd*) and *Cinnamomum litsaeifolium* Thwaites (*Cl*) along with *Cinnamomum verum* (*Cv*) variety *Sri Gemunu* (*SG*). *Cl* and *SG* belonged to type A, while *Cd* was type B of protogynous dichogamy. Partial overlapping of male and female phases in types A and B may lead to self-pollination. Leaf morphological characters varied among species. Gas Chromatography Mass Spectrometry revealed 34, 34, 12, 48, 8 and 18 chemical compounds from *Cinnamomum capparu-coronde* Blume (Cc), *Cd*, *Cl* (1), *Cl* (2), *SG* and variety *Sri Wijaya* (SW) respectively. The highest abundant chemical compound in leaf oil varied as Eugenol in *Cc*, *SG* and *SW* (33.11%, 82.11% and 90.80% respectively), Methyl eugenol in *Cl* (1) (59.27%), Eucaliptol in *Cd* (51.19%) and Linalool in *Cl* (2) (30.93%). The above variation of wild cinnamon provides insights on future cinnamon breeding and industry.

Keywords: Chemical Composition, *Cinnamomum capparu-coronde* Blume, *Cinnamomum dubium* Nees, *Cinnamomum litsaeifolium* Thwaites, Floral behaviour

INTRODUCTION

Genus *Cinnamomum* of Family Lauraceae consists of about 250 species and sub-species grown in South and Central America, Asia and Australia (Mabberley 2008). The true cinnamon produced from the cultivated species, *Cinnamomum verum* J. Presl (*Cv*) is one of the most important spices in the world. Sri Lanka is the world's largest true cinnamon producer. *Cinnamomum dubium* Nees (*Cd*) (*Sewel Kurundu* in *Sinhala*), *Cinnamomum ovalifolium* Wight (*Wal Kurundu* or *Bola Kurundu* in *Sinhala*), *Cinnamomum litsaeifolium* Thwaites (*Cl*) (*Kudu Kurundu* in *Sinhala*), *Cinnamomum rivulorum* Kosterm (*Wal Kurundu* in *Sinhala*), *Cinnamomum sinharajaense* Kosterm (*Sinharaja Kurundu* in *Sinhala*), *Cinnamomum capparu-coronde* Blume (*Cc*) (*Kapuru Kurundu* in *Sinhala*) and *Cinnamomum citriodorum* Thwaites (*Pangiri Kurundu* in *Sinhala*) are the crop wild relatives of Sri Lankan cultivated cinnamon (Kumarathilake *et al*. 2010). Except for *Cl*, *Cd* and *Cinnamomum ovalifolium* Wight, other species are considered to be endemic to Sri Lanka. *Cl* and *Cinnamomum ovalifolium* Wight are native to India and Sri Lanka and *Cd* is considered to be native to Myanmar and Sri Lanka (Plants of the World Online 2021). Kumarathilake *et al*. (2010) had carried out an eco-geographic survey determining the

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extinction risk level of wild cinnamon species in Sri Lanka. According to Kumarathilake (2009), *Cinnamomum citriodorum*, *Cinnamomum rivulorum*, *Cinnamomum sinharajaense*, *Cc* and *Cl* are critically endangered at the global level. *Cinnamomum citriodorum* and *Cc* are highly threatened at the national level. *Cc* is distributed in low country rain forests. *Cl* is a threatened species at the national level and is only found in the Knuckles forest of Sri Lanka. *Cd* with medicinal and timber value is not a threatened species and is grown in tropical rain forests and secondary forests in Sri Lanka. Habitat destruction for urbanization and intensive agricultural practices had been identified as the major threats to wild cinnamon species in Sri Lanka by Kumarathilake (2009), who had proposed conservation strategies such as the declaration of restricted areas, *in-situ* management in forest reservations and development of new policies. The *ex-situ* conservation sites of wild cinnamon species established by Kumarathilake (2009) in Sri Lanka, are at Kanneliya forest reserve, Royal Botanical Garden in Peradeniya, Horticultural Research and Development Institute in Gannoruwa, Faculty of Agriculture, University of Ruhuna in Kamburupitiya and Mid Country Research Station, Department of Export Agriculture in Dalpitiya, Bandaranayake Memorial Ayurveda Research Institute in Nawinna, Forest Department Research Station in Badulla and Barbarian Beach Resort in Weligama. However, these *ex-situ* conservation sites consist of heterogenous plant collections as seedlings from exploration sites had been used for the establishment. During his study, *Cc*, *Cinnamomum citriodorum* and *Cd* were identified as potential candidates for *Cv* breeding. Among them, *Cc* was identified as the most suited species for domestication due to its desirable characters of the high amount of eugenol in leaf essential oil, the erect stem and medicinal and timber value. However, Kumarathilake (2009) reported that the above species were not successful in vegetative propagation. Prathibhani *et al*. (2020) reported the variation of shoot regeneration capacity of stem cuttings from several *Cv* genotypes. Geethakumary *et al*. (2007; 2012) reported the distribution of *Cd* and *Cl* in the southern part of Western Ghats, India while Ananthakrishnan *et al*. (2018) reported the variation of the chemical composition of several *Cinnamomum* species found in the Western Ghats, India including *Cl* and *Cd*. There are no reports on genetic relatedness among Sri Lankan and Indian *Cl* and *Cd* genotypes. Yang *et al*. (2019) proposed an identification method for morphologically similar *Cinnamomum* species using leaf images and deep convolutional neural networks classifiers with 96.7% test accuracy. Ho and Hung (2011) proposed inter-simple sequence repeats (ISSRs) and ribosomal DNA internal transcribed spacer (ITS) molecular markers to identify the cladistic relationship of 12 endemic *Cinnamomum* species in Taiwan. Ho *et al*. (2015) reported the use of the same ITS for authentication of *Cinnamomum osmopholoeum* and related species in Taiwan. Azad *et al*. (2016; 2019a; 2019b) carried out a detailed study on *Cv* germplasm in Sri Lanka with the assistance of the Department of Export Agriculture, Sri Lanka. Morphologically different 269 cinnamon accessions were selected for the study from more than 3000 cinnamon plants in 51 farmer fields located in major cinnamon growing areas in Sri Lanka. The same team (Team of TURIS 2013 project) developed a set of descriptors for cinnamon based on the observed morphological variation of Sri Lankan *Cv* germplasm during the study. Variation of leaf and flower morphology and bark chemical composition of *Cv* germplasm under the *in-situ* conditions in Sri Lanka was reported by Azad *et al*. (2016; 2018; 2019a and Unpublished data). Azad *et al*. (2019a) established a vegetatively propagated collection of above 269 cinnamon accessions at the Faculty of Agriculture, University of Ruhuna. Variations of leaf morphology and leaf essential oil composition of the above collection at the Faculty of Agriculture revealed the genetic diversity of *Cv* germplasm in Sri Lanka (Prathibhani *et al*. 2019).

The leaf is one of the most useful morphological characters in genus *Cinnamomum* as it is highly varied among species (Ravindran *et al*. 2004).

Kumarathilake (2009) had classified *Cinnamomum* species using a key based on the *in-situ* variation of leaf and bark morphological characters.

In Family Lauraceae, protogynous dichogamy is observed: Flowers open in two phases, where the female phase is prior to the male phase. The first opening of flowers occurs during the morning in the type A plants, while it occurs during noon in type B plants. At the first phase only, the stigma appears as receptive. The first and third stamen whorls appear fused during this stage. First opening lasts for about five hours. The second phase begins after 24 hours of first opening. The third whorl of the stamens adheres to the pistil. The anthers become dehiscent after 1/2-1 hour of second opening. The stigma is shriveled and become non-receptive. Again, the flower is kept open for about five hours (Joseph 1981; Kubitzski and Kurz 1984; Sedgley and Griffin 1989). According to Azad *et al*. (2018), there is a variation in inflorescence as in panicle length, panicle type, flower colour, flower length, flower width, tepal length, tepal width, tepal number and tepal pubescence within *Cv* germplasm. Information on inflorescence type, size and flower colour of fifteen accessions from a field survey conducted in 15 locations of cultivated lands and wild habitats in Matara district, Sri Lanka has been reported by Azad *et a*l. (2018). The same authors suggested the possible linkage between large flower size and type A flower behaviour.

There may be inter-species variation in the time of occurrence and active duration of the female and male phases among *Cinnamomum* species. Protogynous dichogamy, which leads to cross-pollination, contributes to allele richness in *Cv* germplasm (Azad *et al*. 2015). Bark, leaf, root and fruit essential oils of *Cv* bear each of unique chemical profiles has majored with cinnamaldehyde, eugenol, camphor and cadinene respectively (Senanayake *et al*. 1989; Paranagama *et al*. 2001). *Cv* possesses antidiabetic, anticholinergic, antilipidemic, anti-inflammatory, antioxidant, anticarcinogenic, antimicrobial and insecticidal properties (Ranasinghe *et al*. 2012; Ranasinghe and Galappaththy 2016; Abeysekera *et al*. 2017; Gulcin *et al*. 2019; Unlu *et al*. 2010; Kim *et al*. 2015). Many traditional Asian cultures use cinnamon in bloating, nausea, flatulence, colic and gastro-intestinal tract spastic conditions (Toriizuka 1998). There is unpublished information on the use of wild cinnamon species for medicinal purposes in Sri Lanka. Kumarathilake (2009) reported the *in-situ* chemical compositional variation of *Cc* and *Cd* through Gas Liquid Chromatography (GLC) analysis.

The genetic diversity of wild cinnamon germplasm in Sri Lanka would be depicted through environment-independent leaf morphological variation. The wide chemical profiles of wild cinnamon species in Sri Lanka would be potential sources in food, pharmaceuticals and cosmetics. Revealing the extent of protogynous dichogamy among wild cinnamon would be useful in their utilization in breeding programmes.

One plant of *Cc*, one plant *Cd* and two plants of *Cl* through the exploration of Kumarathilake (2009) were maintained in the *ex-situ* conservation site at Mid Country Research Station. Seed setting had been observed at the above *ex-situ* conservation site in 2018 by the second author. As there were single plants of *Cc*, *Cd* and two plants of *Cl* (named as *Cl*-1 and *Cl*-2) at the site, we speculated that selfing within the single plant or cross-pollination with another *Cinnamomum* spp. had taken place.

Therefore, this study was conducted in the following year (2019) to determine the morphological and chemical characters and flower type of each spp. as such information would be useful in identifying potential hybrids in the future. Further, the information on essential oil composition would be useful in industrial applications.

MATERIALS AND METHOD

The present study was based on the *ex-situ* wild cinnamon conservation site at Mid-Country Research Station, Department of Export Agriculture, Dalpitiya (WM2) (GPS:

7.1333031 N, 80.590026 E) established by Kumarathilake (2009). Observations on flowering of above *Cinnamomum* spp. were made. According to the availability of flowers during the study period of February to March 2019, the floral behaviour of two wild cinnamon species of *Cd* and *Cl* along with *SG* was determined. Floral cycles were determined through visual observation of flowers for two consecutive days from 8 am to 4 pm. Leaf morphology and leaf essential oil composition of wild cinnamon species of *Cc*, *Cd* and *Cl* along with *SG* and *SW* were determined. Mature leaves of one *Cd*, one *Cc*, two *Cl* (1), (2), *SG* and *SW* were collected randomly at 5th to 6th leaf from the tip of the branch for both morphological characterization and leaf essential oil analysis. Length, width and petiole length were measured as quantitative leaf morphological characters. Qualitative leaf morphological characters of shape, apex, base, texture, venation and margin were characterized using the Descriptors for Cinnamon (Team of TURIS 2013 Project 2016). The leaf essential oil was extracted using the hydro-distillation method and analyzed using Gas Chromatography Mass Spectrometry (GC-MS).

RESULTS AND DISCUSSION

There was a variation in leaf morphological characters of leaf length, leaf width, leaf shape, leaf apex, leaf base, leaf texture, leaf venation, petiole length and leaf margin among *Cinnamomum* species (Table 1, Figure 1). *Cc* and *Cd* were lanceolate leaf-shaped while *Cl* (1) and *Cl* (2) were elliptic leaf-shaped. *SG* and *SW* were broadly ovate and ovate leaf-shaped respectively. *Cl* (1), (2), *SG* and *SW* had acute leaf apexes. *Cc* and *Cd* leaf apexes were acuminate with broad acumen and long acuminate respectively. Leaf base of *Cd*, *SG* and *SW* was round. *Cc*, *Cd* and *Cl* (1) leaf textures were thin to stiffly coriaceous. *SG* and *SW* had the same chartaceous leaf

Table 1: Leaf morphological characters of *Cc*, *Cd*, *Cl* (1), *Cl* (2), *SG* and *SW*

Species	**Leaf character***								
	LL (cm)	LW (cm)	LS	LA	LB	LT	LV	PL (cm)	LM
Cc	13.98±1.44	4.20±0.67	7	6	2	4	3	1.00±0.24	1
Cd	12.52±1.53	3.78±0.50	7	4	4	4	3	0.66±0.21	1
***Cl* (1)**	7.88±1.24	2.88±0.40	1	1	6	4	1	1.10±0.17	1
***Cl* (2)**	7.44±0.44	2.66±0.23	1	1	7	5	1	0.88±0.21	1
SG	14.02±2.13	6.68±1.13	5	1	4	6	3	1.60±0.26	1
SW	11.88±1.22	5.92±0.64	4	1	4	6	1	1.16±0.21	2

**According to the Descriptors for Cinnamon (Team of TURIS, 2013 project) LL: Leaf length; LW: Leaf width; LS: Leaf shape 1-Elliptic, 4-Ovate, 5-Broadly ovate, 7-Lanceolate; LA: Leaf apex 1-Acute, 4-Long acuminate, 6-Acuminate with broad acumen; LB: Leaf base 2-Subacute, 4-Rounded, 6-Obtuse, 7-Obtuse, contracted into petiole, then shortly cuneate; LT: Leaf texture 4-Thinly to stiffly coriaceous, 5-Chartaceous to rigidly chartaceous, 6-Chartaceous; LV: Leaf venation 1-Three veined, 3-Three veined or Five veined; PL: Petiole length; LM: Leaf margin*

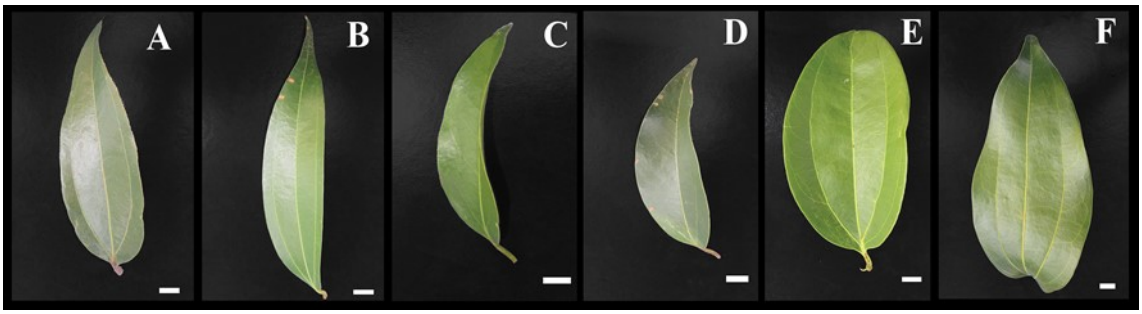

Figure 1: Leaves of wild and cultivated cinnamon
Cc: A; *Cd*: B; *Cl* (1): C; *Cl* (2); D; *SG*: E; *SW*: F (Scale: 1 cm)

texture, while *Cl* (2) was of chartaceous to rigidly chartaceous leaf texture. *Cl* (1), (2), *SG* and *SW* had three-veined leaves while *Cc* and *Cd* had three-veined or five veined leaves. All *Cc, Cd*, *Cl* (1), (2) and *SG* had the entire leaf margin. *SW* was with the undulate leaf margin.

We observed wild cinnamon flowering from February to March 2019 in Dalpitiya. Sri Lanka. The flowering season of *Cv* begins in November and continues until early March. *Cv* fruits ripen from May to June (Joseph 1981; Kubitzki and Kurz 1984; Mohankumar *et al*. 1985). According to our observations in Southern Sri Lanka, *Cv* flowering starts from August to September and extends up to April in the following year. There are evidences that the floral behaviour of cinnamon might be affected by the environmental factors of day length, photon flux density and temperature (Sedgley 1985). According to Schaffer and Anderson (2018), the floral induction of avocado was not directly influenced by day length, while day length determined the floral behaviour of protogynous dichogamy. The floral cycle of the avocado was completely disrupted under continuous light conditions as the male phase and female phase flowers were open throughout the day and the shorter day lengths shortened its floral cycle (Sedgley 1985).

All *Cl*, *SG* and *Cd* produced axillary panicles (Figure 2). *Cl* and *Cd* produced equally dense panicles at the axil and the apex. *SG* panicles got long peduncles and were dense at the apex of the branchlets. According to the visual observation of inflorescences and individual flowers of three *Cinnamomum* spp, the inflorescences carried flowers and buds of different physiological maturity (Figure 2).

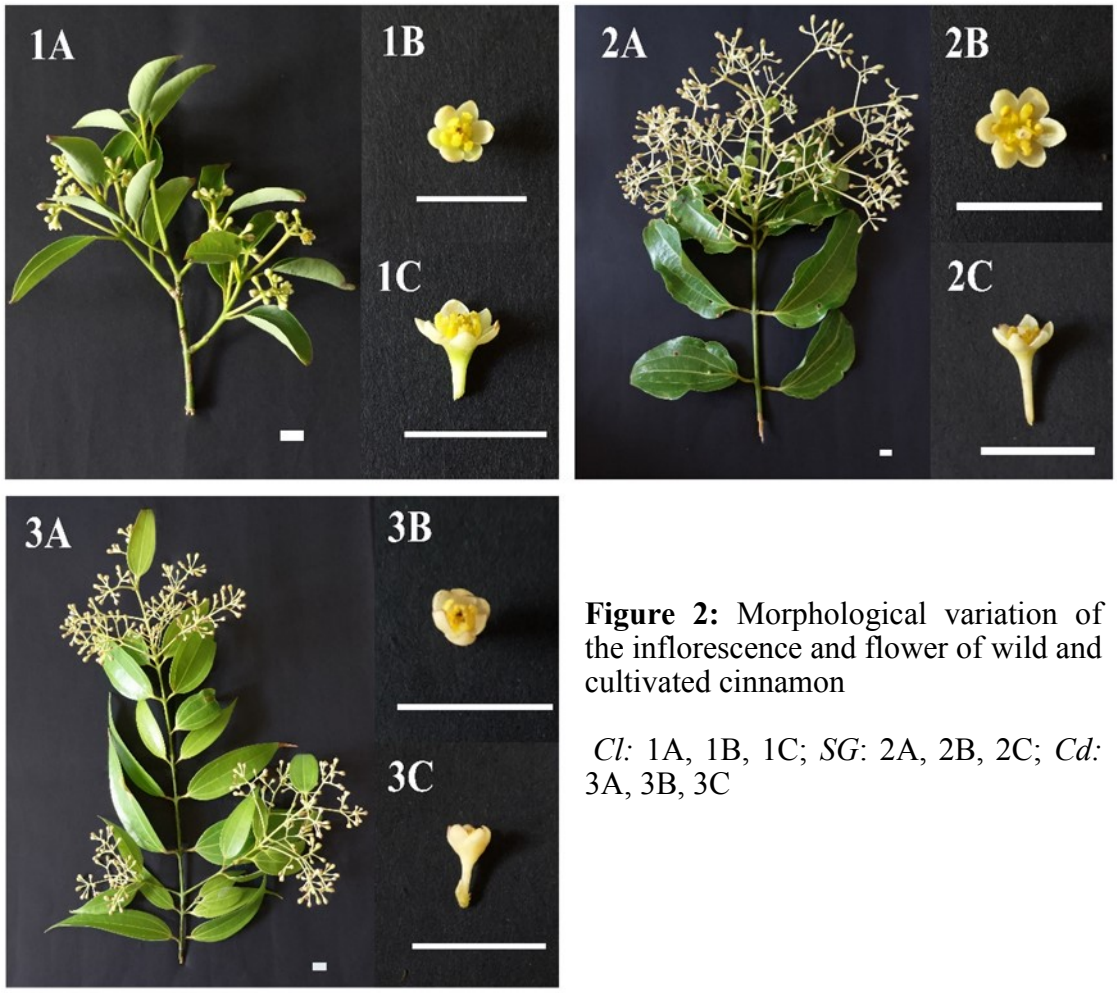

Figure 2: Morphological variation of the inflorescence and flower of wild and cultivated cinnamon

Cl: 1A, 1B, 1C; *SG*: 2A, 2B, 2C; *Cd:* 3A, 3B, 3C

The inflorescence of the variety *SG* was large and produced a comparatively large number of flowers while *Cd* and *Cl* inflorescences were small in size with a small number of flowers (Figure 2). Immature and mature unopened flowers of *Cd* were pinkish-yellow while *Cl* and *SG* were yellowish-green.

The morphology of flowers of all three species in Figure 2, was in accordance with the previous records on typical *Cv* flowers (Azad *et al*. 2018; Heslop-Harrison and Shivanna 1977) and on typical Lauraceae flowers by Zeng *et al* (2017). The standard floral characters comprise of six tepals in two whorls and fifteen stamens in three whorls; both whorl I and whorl III contain six stamens, while whorl II contains only three stamens. A style arises from the ovary. Three staminal glands are present in between stamen whorl II and the stigma (Azad *et al*. 2018).

In *SG* and *Cl*, the inflorescence produced open flowers of two physiological stages during the morning. Flowers of one of the stages opened from 8.00 to 9.00 am with white colour stigma. They remained open around five hours till 1.00 pm and closed. They opened again on the next day around 11.00 am with brown colour stigma and tepals. After about five hours they permanently closed completing the floral cycle. According to the classification of flowers on dichogamy, variety *SG* and the studied *Cl* plant are included under type-A.

The studied *Cd* plant belonged to the type-B category. The flowers started opening in the afternoon around 1.00 pm with fresh white colour stigma and tepals. The stamen whorls appeared as fused. They remained opening about 3½ hours and closed around 4.00 pm. They opened again on the next day around 8.00 am. There were anthers dehiscent with pollens around 9.00-10.00 am. They were with brown colour stigma and brown colour tepals at this opening and remained open about 7 hours. According to the above observations, partial overlapping of functional male and functional female stages may lead to self-pollination in all three species.

Joseph (1981) reported that the female and male phases of cinnamon flowers separated by almost one day. According to our observations in Dalpitiya, Sri Lanka, the above observation was confirmed for the type A plants. The floral cycle of type B plants consisted of the female and male phases separated by twelve hours. In accordance with the report of Mohankumar *et al*. (1985), we also observed the maximum flower breath at the second phase of the floral cycle.

There are reports on the presence of type A and type B plants in *Cv* and *C. camphora* populations. Within a *Cv* population, 3/5 of the plants are type A and 2/5 of the plants are type B (Joseph 1981). In a natural population, A and B plant types are mixed for the availability of functional male and female flowers at any given time (Joseph 1981; Kubitzski and Kurz 1984). The majority of cultivars belong to type B among the studied ten cultivars of *Cv* for floral behaviour at the National Cinnamon Research and Training Center, Sri Lanka (Kumari *et al*. 2008). There is some unpublished information on floral behavior of two commercial varieties of *Cv* in Sri Lanka as *SG* is type A and *SW* is type B. Future work should be focused on the molecular basis of type A and type B plants of cinnamon, floral morphological markers for identifying the two types of plants and the environmental effects on floral behaviour of cinnamon.

There were 122 chemical compounds in *Cc*, *Cd*, *Cl* (1), (2), *SG* and *SW* (Table 2). A total of 34, 34, 12, 48, 8 and 18 chemical compounds were detected from *Cc*, *Cd*, *Cl* (1), (2), *SG* and *SW* respectively. Eugenol was the major chemical compound of leaf essential oils from *Cc* (33.11%), *SG* and *SW* (82.11% and 90.80% respectively). Other than eugenol, 11.76% of linalool, 15.93% of methyl palmitate and 20.04% of methyl elaidate were reported in *Cc*. There were 2.36% of linalool, 4.68% of caryophyllene and 9.55% of eugenyl acetate in *SG*. There were 4.94% of linalool and 2.32% of caryophyllene in *SW*. Eucalyptol was the major chemical compound of *Cd* (51.19%). With eucalyptol, *Cd* got 13.18% of alpha-

Table 2: Chemical composition of leaf essential oil of wild and cultivated cinnamon

	Chemical compound	*Cc*	*Cd*	*Cl* (1)	*Cl* (2)	*SG*	*SW*
1	Eucalyptol	-	51.19	-	0.12	-	0.097
2	Linalool	11.761	3.406	7.163	30.93	2.362	4.939
3	1-H-indazole, 4,5,6,7-tetrahydro-7-methyl	-	-	-	-	-	-
4	Isoborneol	0.075	-	-	-	-	-
5	4-Terpinenyl acetate	-	-	-	0.208	-	-
6	alpha-Terpineol	0.367	13.18+0.72	0.63	10.013	-	0.27
7	delta-Cadinene	-	0.078	0.392	-	-	-
8	alpha-Amorphene	-	-	0.136	0.714	-	-
9	Pentane, 3-methylene	0.064	-	-	-	-	-
10	delta-Elemene	0.078	-	-	-	-	-
11	Eugenol	33.105	-	24.993	-	82.107	90.80
12	Benzene, 1,2,4-triethyl	2.206	-	-	-	-	-
13	Cyclohexene, 1-butyl	0.304	-	-	-	-	-
14	Caryophyllene	1.818	-	-	3.044	4.682	2.316
15	1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z	0.264	-	0.159	-	-	0.264
16	1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4methylene-,[1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]	0.216+0.212	-	-	-	-	0.05
17	trans-isolongifolene	0.062	-	-	-	-	-
18	Copaene	0.074	-	2.243	0.555+0.931	-	-
19	beta-Gurjunene	0.136	-	-	-	-	-
20	5-Muuroladiene	0.098	-	-	-	-	-
21	Bicyclogermacrene	0.331	-	-	-	-	-
22	alpha-Muurolene	0.188	-	-	0.597	-	-
23	delta-Cadinene	1.116	-	-	1.181+3.583	-	0.052
24	a-Triazolo[4,3-a]pyridine, 3,5,7-trimethyl-	0.054	-	-	-	-	-
25	3-Undecen-1-yne, (E)	0.083	-	-	-	-	-
26	Cyclopentanecarboxamide, N-(2-fluorophenyl)-	1.71	-	-	-	-	-
27	Espatulenol	0.787	-	-	-	-	-
28	beta-Selinene	0.732	-	-	-	-	-
29	Bicyclo[5,2,0]nonane, 4,8,8-trimethyl-2-methylene	0.14	-	-	-	-	-
30	3-Hexyne	0.333	-	-	-	-	-
31	Cadinadiene-1,4	0.528	-	-	-	-	-
32	gamma-Cadinene	0.856+0.228	-	-	-	-	-
33	Cyclohexene,1-methyl-3-vinyloxy-	0.525	-	-	-	-	-
34	Methyl myristylate	0.356	-	-	-	-	-
35	Phthalic acid, isobutyl undecyl ester	0.217	-	-	-	-	-

Table 2 continued…..

	Chemical compound	***Cc***	***Cd***	***Cl* (1)**	***Cl* (2)**	***SG***	***SW***
36	Methyl palmitate	15.928	-	-	0.233	-	-
37	Methyl linoleate	3.519	-	-	-	-	-
38	Methyl elaidate	20.035	-	-	-	-	-
39	Methyl stearate	1.494	-	-	-	-	-
40	1R-alpha-Pinene	-	1.361	-	-	-	-
41	(-)- 4 – Terpineol	-	5.421	-	-	-	-
42	alpha-Santalene	-	4.583	-	-	-	-
43	2-Norpinene	-	2.64	-	-	-	-
44	Norbonane	-	2.955+ 3.610	-	0.163	-	-
45	beta-Bisabolene	-	0.204	-	-	-	-
46	gamma.-Elemene	-	-	0.167	-	-	-
47	Eugenyl acetate	-	-	-	-	9.549	-
48	1,7,7-Trimethyl-2-vinylbicyclo [2.2.1]hept-2-ene	-	-	-	9.057	-	-
49	alpha-Thujene	-	0.222	-	-	-	-
50	Camphene	-	0.051	-	-	-	-
51	beta-Thujene	-	2.815	-	-	-	-
52	beta-Pinene	-	1.552	-	-	-	-
53	beta-Myrcene	-	0.077	-	-	-	-
54	alpha-Terpinene	-	0.282	-		-	-
55	m-Cymene	-	0.633	-	-	-	-
56	gamma-Terpinene	-	0.711+ 0.192	-	-	-	-
57	beta-Terpineol	-	0.079	-	-	-	-
58	2-Cyclohexen-1-ol, 1-methyl-4 -(1-methylethyl)-.trans	-	0.222	-	-	-	-
59	3,4-Dimethyl-1H-pyrrole-2-carboxylic acid	-	0.097	-	-	-	-
60	Phenol.4-amino	-	0.208	-	-	-	-
61	Succinic acid, di(3,5-dimethylcyclohexyl) ester	-	1.133	-	-	-	-
62	trans-alpha.-Bergamotene	-	0.182	-	-	-	-
63	beta-Farnesene	-	0.914	-	-	-	-
64	Phenol,2,6-bis(1,1-dimethylethyl)-	-	0.162	-	-	-	-
65	cis-alpha,-Bisabolene	-	0.09	-	-	-	-
66	Cyclohexane,ethylidene-	-	0.077	-	-	-	-
67	(S)-cis-Verbenol	-	0.343	-	-	-	-
68	Silane,[[4-[1,2-bis [(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)bis [trimethyl-	-	0.226+ 0.070	-	-	-	0.093
69	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy) tetrasiloxane	-	0.079	-	-	-	0.043

Table 2 continued…..

	Chemical compound	*Cc*	*Cd*	*Cl* (1)	*Cl* (2)	*SG*	*SW*
70	Cycloheptasiloxane,tetradecamethyl-	-	0.062	-	-	-	-
71	Oxacyclotetradecan-2-one, 1,3-methyl-	-	0.106	-	-	-	-
72	Heptasiloxane, hexadecamethyl	-	0.067	-	-	-	0.092
73	Isobenzofuran-1(3H)-one,3-(3-furyl)-3a,4,5,6-tetrahydro-3a,7-dimethyl-	-	-	-	-	0.094	-
74	Bicyclo[4.2.0]octa-2,4-diene-7-carbonitrile	-	-	-	-	0.113	-
75	alpha-caryophyllene	-	-	-	1.119	0.674	-
76	Spiro[2,4]heptane,1,5-dimethyl-6-methylene	-	-	-	-	0.419	-
77	2-Pentyn-1-ol	-	-	-	0.117	-	-
78	(S)-Camphor	-	-	-	0.36	-	-
79	L-Borneol	-	-	-	0.354	-	-
80	Acetic acid, Borneol ester	-	-	-	0.186	-	-
81	delta-Elemene	-	-	-	3.363	-	-
82	1S-alpha-Pinene	-	-	-	1.486	-	-
83	4-Amino-6-hydroxypyrimidine	-	-	-	3.565	-	-
84	alpha-Cubebene	-	-	-	0.922	-	-
85	beta-Cubebene	-	-	-	2.471	-	-
86	gamma-Cadinene	-	-	-	0.2	-	-
87	alpha-farnesene	-	-	-	0.305	-	-
88	alpha-Cadinene	-	-	-	0.114	-	-
89	Germacrene D	-	-	2.279	8.338	-	-
90	Bicyclo[6.4.0]dodeca-9,11-diene	-	-	-	0.222	-	-
91	Longifolene	-	-	-	1.164	-	-
92	1R,3Z,9S-2,6,10,10-Tetramethylbicyclo[7,2,0]undeca-2,6-diene	-	-	-	0.619	-	-
93	p-Cyanophenyl p-(2-butoxyethoxy) benzoate	-	-	-	0.189	-	-
94	1H-Indene,2,3,3a,4-tetrahydro-3,3a,6-trimethyl-1-(1-methylethyl)-	-	-	-	0.145	-	-
95	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1R-(1.alpha.,4a.alpha.,8a.alpha.)]-	-	-	-	0.146	-	-
96	Carbonic acid, ethyl 2,2,2-trichloroethyl ester	-	-	-	0.233	-	-
97	2,8-Decadiyne	-	-	-	0.283	-	-
98	Tricyclo[6.3.0.0(2,4)]undec-8-ene,3,3,7,11-tetramethyl	-	-	-	1.743	-	-
99	Isocaryophillene	-	-	-	1.119	-	-
100	Undec-10-ynoic acid	-	-	-	0.32	-	-

Table 2 continued…..

	Chemical compound	*Cc*	*Cd*	*Cl* (1)	*Cl* (2)	*SG*	*SW*
101	4-Ethoxy-2-(methylamino)tropone	-	-	-	0.641	-	-
102	gamma-Cadinene	-	-	-	0.826+ 2.189	-	-
103	alpha-Cadinol	-	-	-	3.094	-	-
104	[4,4-Bipyrimidine]-2,2,6 (1H,1H,3H)-trione,5-methyl	-	-	-	0.191	-	-
105	Hydrazine,2-[fluorobis(1-methylpropyl)silyl]-1,1-dimethyl-	-	-	-	0.075	-	-
106	Benzene,1-(5,5-dimethyl-1-cyclopenten-1-yl)-2-methoxy-	-	-	-	0.33	-	-
107	Benzaldehyde,2-hydroxy-3-(2-propenyl)-	-	-	-	0.166	-	-
108	Ethanol,1-(2-benzimidazolyl)-	-	-	-	0.222	-	-
109	2,4-Dihydroxy-1,5-naphthyridine	-	-	-	0.184	-	-
110	7-Hexadecyn-1-ol	-	-	-	0.095	-	-
111	1,6-Octadecanoic acid, methyl ester	-	-	-	0.554	-	-
112	Bicyclo[3.3.0]octan-3-one,7-ethylidene	-	-	-	-	-	0.107
113	1,2,4-Triazolo[4,3-a]pyridin-3(2H)-one, 5-methyl-	-	-	-	-	-	0.198
114	Benzene, (2-methylpropoxy)-	-	-	-	-	-	0.057
115	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)	-	-	-	-	-	0.078
116	3-Ethylidenecycloheptane	-	-	-	-	-	0.428
117	2-Cyclohexen-1-one,4,4,6-trimethyl	-	-	-	-	-	0.067
118	Cyclononasiloxane,octadecamethyl	-	-	-	-	-	0.057
119	(+)-4-Carene	-	-	0.169	-	-	-
120	beta-Cubebene	-	-	0.663	-	-	-
121	Methyleugenol	-	-	59.268	-	-	-
122	Bicyclo[7,2,0]undec-4-ene,4,11,11-trimethyl-8-methylene	-	-	1.738	-	-	-

terpineol, 4.58% of alpha-santalene, 5.42% of 4-terpineol and 3.41% of linalool. The highest chemical constituent in two *Cl* (1) and (2) plants varied as methyl eugenol (59.27%) in one plant and linalool (30.93%) in the other. There were 7.16% of linalool, 24.99% of eugenol, 2.24% of copaene and 2.28% of germacrene D in *Cl* (1). *Cl* (2) contained 10.01% of alpha-terpineol, 3.04% of caryophyllene and 8.34% of germacrene D. *Cl* plants at the same location exhibited a variation in their leaf essential oil composition. According to Kumarathilake (2009), the essential oil extracted from *Cc* leaves from Walakanda forest possessed 90.25% of eugenol and 5.98% of linalool. Kumarathilake (2009) reported the *in-situ* chemical compositional variation of four *Cd* leaf essential oil samples: two samples from Gongala forest reserve (G1 and G2), one from Rakwana and one from Hay's Group Estate. All four samples contained citral – b as the major chemical compound of *Cd* leaf essential oil. There was 27.43% of cinnamyl alcohol with 29.51% citral-b in G1. G2 reported the presence of 21.21% of citral-b, 16.65% of *β*-caryophyllene and 13.77% of eucalyptol. According to Kumarathilake (2009), *Cd* plants from the same location exhibited a variation of leaf essential oil composition. *Cd* leaf oil from Rakwana contained 18.57% of citral-b, 16.08% of cinnamyl alcohol and 16.52% of myrcene, while 35.8% of citral-b, 28.18% of cinnamyl alcohol and 10.91% of linalool were present in *Cd* leaf oil sample from Hay's group. Leaf essential oil of *Cl* and *Cd* from the Western Ghats, India contained 32.1% of alpha-phellandrene and 64.6% of

caryophyllene oxide respectively (Ananthakrishnan *et al*. 2018).

CONCLUSION

There was a variation in leaf morphological characters in *Cc*, *Cd*, *Cl*, *SG* and *SW*. The observed *Cl* plant was of type A and *Cd* was of type B. Partial overlapping of functional male and female phases should be further investigated to determine the possibility of self-pollination. Eugenol was the major chemical compound of leaf essential oils from *Cc* (33.11%). Eucalyptol was the major chemical compound of *Cd* (51.19%). The highest chemical constituent in two *Cl* plants varied as methyl eugenol (59.27%) in one plant and linalool (30.93%) in the other. This information signifies the necessity of understanding the variation within a spp. and identification of chemotypes.

AUTHOR CONTRIBUTION

MRP, RAAKR and SG conceptualized and designed the study. MRP performed the experiments and analyzed the data. MRP, SAR and SG interpreted the data. MRP and SG drafted the manuscript. RAAKR and SG critically revised the manuscript.

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