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ESSENTIAL OIL COMPONENTS IN SELECTED SPECIES OF ALPINIEAE (ZINGIBERACEAE) FROM SARAWAK AND ITS TAXONOMIC CORRELATION

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Alpinieae is recognised as the most complex tribe in the Zingiberaceae family, in terms of defining the morphological characters that delimit the genera. In the present study, essential oils from the rhizomes of selected Alpinieae species from Sarawak such as *Alpinia galanga, A. ligulata, Conamomum cylindrostachys, C. xanthophlebium, Etlingera coccinea, E. nasuta, Hornstedtia leonurus, Plagiostachys strobilifera* var. *strobilifera, P. strobilifera* var. *conica, Sundamomum corrugatum* and *S. laxesquamosum* were analysed using GC-MS and hierarchical clustering. Phytochemical analysis revealed the major and specific components that grouped and characterised the studied taxa in their respective cluster. Close relationship between the studied *Conamomum, Sundamomum* and *A. ligulata* with *P. strobilifera* var. *conica* were also shown at 70%, 94% and 60% similarity index, respectively. The clustering based on chemometric data corresponded to the morphology of the inflorescence, anther crest, lateral staminodes and fruit. The present findings had provided supplementary evidence to a better understanding especially on the ambiguity status of some problematic genera in the tribe including *Alpinia, Plagiostachys* and *Hornstedtia.*

Keywords: *Alpinia*, Alpinioideae, Borneo, chemotaxonomy, *Hornstedtia, Plagiostachys,* systematics

INTRODUCTION

Zingiberaceae is the largest family in the order of Zingiberales. They were generally found in the tropics and subtropics, and the Asian tropics hold the highest diversity and number of taxa (Lamb et al. 2013, WCVP 2020). Various species of Zingiberaceae throughout the world are utilised locally and commercially for numerous purposes such as for medicines, foods, food additives, beverages, ornamental plants, fragrances, cosmetics or in rituals and ceremonies (Larsen et al. 1999, Ibrahim 2006).

Most Bornean species belonged to the subfamily Alpinioideae of which was diagnosed by the plane of distichous leaves perpendicular to the rhizome and the absence or reduction of the two lateral staminodes. Alpinioideae was subdivided into two tribes, i.e. Riedelieae and Alpinieae. They were mainly characterised by having indehiscent fruits and traditionally lacking extrafloral nectarines (Smith 1985, Smith 1986, Kress et al. 2002). Presently, following recent molecular phylogenetic studies and including two newly described taxa such as *S. corrugatum* and *P. strobilifera* var. *conica* by the authors, the total number of Alpinieae species established in Borneo were at least 145 taxa from 13 genera (Salasiah & Meekiong, 2020, Salasiah et al. 2020, POWO 2020).

As molecular phylogenetic studies of the family progressed, taxonomic status of the tribe Alpinieae was proven to require further clarification especially of the non-monophyletic genera, i.e. *Alpinia* that consisted of different clades, *Plagiostachys* which was nested within the *Alpinia Zerumbet* clade, and *Hornstedtia* in which certain species were nested within the *Amomum* and *Etlingera* clades (Pedersen 2004, Kress et al. 2005, Julius et al. 2008, De Boer et al. 2018). The previously-known paraphyletic *Amomum* was reclassified into several genera which included *Conamomum* and *Sundamomum* based on the comprehensive molecular phylogenetic and morphological analyses, and the anther crest and fruit morphologies were shown to be significant in delimiting the allied *Amomum* s.l. (De Boer et al. 2018)

Biochemical systematics or chemotaxonomy enabled plants classification by relying on the

differences and similarities of their chemical structures. The chemical constituents of the secondary metabolites derived from primary metabolites in plants were commonly restricted between different taxa (Harborne 1973, Waterman 2007). The application of chemotaxonomy was considered reliable because it provides useful phytochemical details related to plant systematics (Waterman 2007). The method would certainly help on certain taxa in which morphological characters for identification especially floral parts were uncertain or difficult to retrieve throughout the year.

Chemotaxonomic study based on the secondary constituents such as volatile oil was continuously applied on several types of plants, such as *Ferula* of family Apiaceae (Kanani et al. 2011), *Ocimum* of family Lamiaceae (Pirmoradi et al. 2013), *Lippia* of family Verbenacaeae (Sandasi et al. 2013), *Juniperus* of family Cupressaceae (Rajcevic et al. 2013), *Ribes* of family Grossulariaceae (Dordevic et al. 2014), as well as *Helichrysum* and *Pulicaria* of family Asteraceae (Maggio et al. 2015, Kladar et al. 2015).

Terpenoids were among the richest compounds found in the essential oils of the family Zingiberaceae, which provided the plants with a specific aroma and flavour. In the manipulation of essential oils as a chemical marker related to family Zingiberaceae, Dan et al. (2007) discovered the interspecific correlations in four South Indian taxa of *Hedychium* based on the distribution and percentages of mono- and sesquiterpenes in their rhizomes essential oils, which also corresponded to their morphological characteristics. Another study on three Bornean *Etlingera* species reported several chemotypes of the genus, in particular six, four, and eight markers in the essential oils of the leaves, leafy shoots, and rhizomes (Nur-Anwariah et al. 2011). Moreover, essential oil components were used to classify several species of *Amomum* s.l. (Setyawan, 2002), *Alpinia* (Padalia et al. 2010), as well as *Zingiber* (Theanphong et al. 2016). Phytochemical components of essential oil extracted from the rhizome of *Wurfbainia uliginosa* from Peninsular Malaysia was studied and added to the species taxonomic knowledge (Mailina et al. 2007).

The current state of the taxonomic status of several genera in the tribe were not sufficiently understood, therefore the approach that integrated evidence from other disciplines such as chemotaxonomy would provide descriptive explanations to better understand the generic boundaries, especially on the problematic ones including *Alpinia, Plagiostachys* and *Hornstedtia*.

MATERIALS AND METHODS

Specimens collection and identification

The taxa for the study were chosen based on their ambiguity status, species with close morphological resemblance, as well as the availability of the plant materials in the field. Plants specimens were collected and documented with preference to fertile material from various localities throughout Sarawak, Malaysia as shown in Table 1. Morphological characters such as floral and reproductive parts of each collected plant were measured and recorded to provide a primary basis for species identification.

Specimens were described and verified with type materials through cross examination from several herbaria (SAR, HUMS), digital images of types (K, E), protologues and taxonomic data from online databases (BHL 2020, IPNI 2020, Newman 2020, POWO 2020, WVCP 2020) and published reference materials on related species. Herbarium specimens were then deposited at the Herbarium of Forest Department Sarawak (SAR) while the duplicates were kept at the herbarium of Universiti Malaysia Sarawak (HUMS) and other herbaria. Figure 1 showed photographs of the studied species in the habitat localities.

Essential oils extraction

The rhizomes from mature plants mainly during anthesis of selected species were cleaned by rinsing under running water to remove soil and dirt and then air-dred. The samples were then chopped into smaller pieces of about 1 cm^3 and ground into coarse form. The plant materials in various weight were placed in a round bottom flask of a Clevenger type apparatus and filled with distilled water at ratio 1:10 or to a level to immerse the entire plant material. Essential oils were isolated by hydrodistillation for 6–7 hours and then stored at 4 °C before further analysis.

Table 1 Details of plant species

Plant samples	Voucher No.	Collection date	Localities	Forest types	GPS
Conamomum cylindrostachys (K.Schum.) Skornick. & A.D.Poulsen	0024	13 Oct 2018	Lambir Hills National Park, Miri	Primary lowland forest	4° 11' N 114° 02' E
Etlingera coccinea (Blume) S.Sakai & Nagam.	0020	12 May 2018			
Hornstedtia leonurus (J.Koenig) Retz.	0026	13 Oct 2018			
Plagiostachys strobilifera var. *strobilifera* (Baker) Ridl.	0021	13 May 2018			
Conamomum xanthophlebium (Baker) Skornick. & A.D. Poulsen	0004	3 Mar 2018	Similajau National Park, Bintulu	Coastal, heath and primary lowland forests	3° 20' N 113° 09' E
Plagiostachys strobilifera var. *conica* Salasiah & Meekiong	0003	24 Feb 2018			
Alpinia ligulata K.Schum.	0028	20 Oct 2018	Niah National Park, Miri	Forest over limestone, seasonal swamp forest	3° 48' N 113° 47' E
Alpinia galanga (L.) Willd.	0050	14 Apr 2019	Tanjung Kidurong, Bintulu	Disturbed forest	3° 12' N 113° 06' E
Etlingera nasuta (K.Schum.) R.M.Sm.	0007	18 Mar 2018			
Sundamomum corrugatum Salasiah & Meekiong	0040	9 Nov 2019	Tubau, Bintulu	Old secondary forest	3° 08'N 113° 41'E
Sundamomum laxesquamosum (K.Schum.) A.D.Poulsen & M.F.Newman	0063	7 Mar 2020	Gunung Podam, Bau	Forest over limestone	1° 20'N 110° 03'E

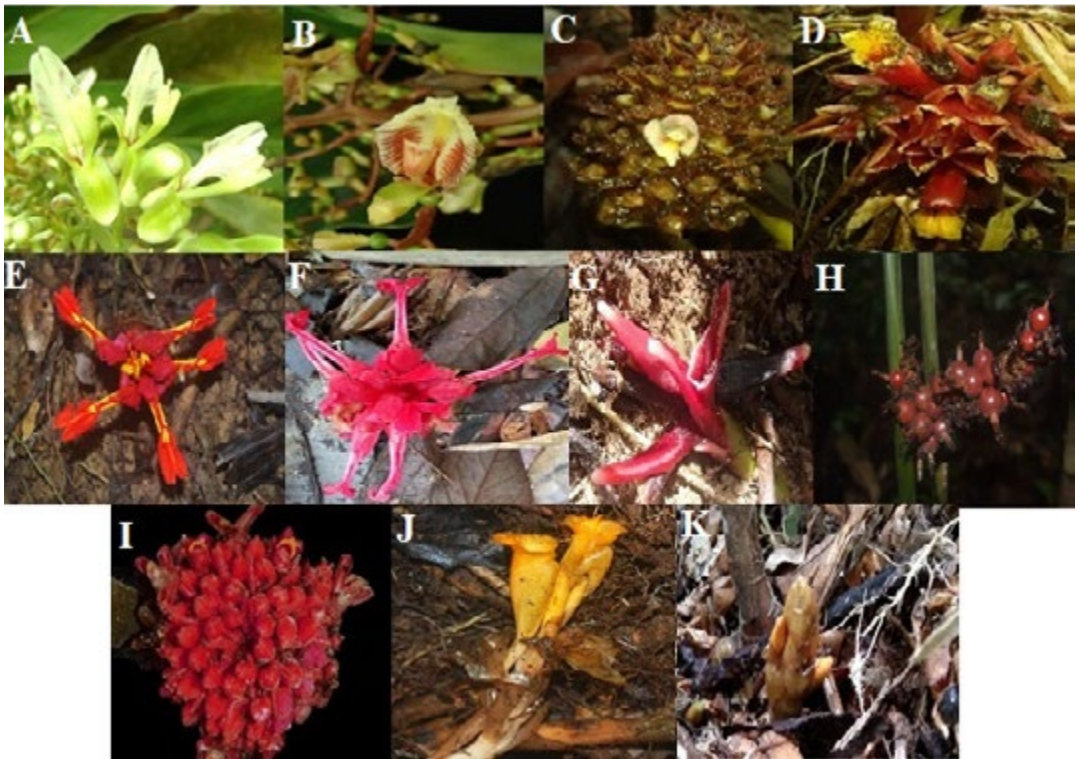

Figure 1 Inflorescences and infructescences of studied taxa in habitat localities

A = *A. galanga,* B = *A. ligulata,* C = *C. cylindrostachys,* D = *C. xanthophlebium,* E = *E. coccinea,* F = *E. nasuta,* G = *H. leonurus,* H = *P. strobilifera* var. *strobilifera,* I = *P. strobilifera* var. *conica,* J = *S. corrugatum,* K = *S. laxesquamosum* (Salasiah Mohamad)

Gas Chromatography-Mass Spectrometry (GC-MS)

Chemical constituents of the essential oils were analysed by gas chromatography equipped with mass spectrometry. A BPX-5 capillary column with 30 m × 0.25 m I.D × 0.25 µm film thickness was used in the analysis. The injector port was heated to 280 °C and injection was performed in splitless mode. Helium was used as carrier at a flow rate of 1.0 mL min^{-1}. The oven temperature was set at 50 °C for 1 minute, then raised to 260 °C at 6.5 °C min^{-1} and maintained for 10 minutes with a total run of 43 minutes. Mass spectra were obtained from the range m/z 45–450. The analyses of samples were conducted in duplicates. Immediately after each GC analysis of essential oils, a mixture containing a homologous series of n-alkanes ranging from C8 to C27 was injected into the column under identical operating conditions. The hydrocarbons were used as standards in the calculation of retention indices (RI). Identification of compounds was based on computer matching of mass spectra against a database from the commercial library of the National Institute of Standards and Technology (NIST17). Compounds were further confirmed by comparing the experimentally calculated retention indices or arithmetic index (Van den Dool & Kratz 1963) values with that of published standards by Adams (2017) from the formula:

$$AI(x) = 100\,P_z + 100\,[(RT(x) - RT(P_z)) / RT(P_{z+1}) - RT(P_z))]$$

where, AI = Arithmetic Index
RT = retention time
x = sample compound
P_z = number of carbons atom in the smaller alkane
P_{z+1} = number of carbon atoms in the larger alkane

Hierarchical clustering

All compounds with contents > 0.05% were scored with either absent (0) or present (1) for hierarchical clustering. Phenetic similarities of different characters were mainly enumerated through clustering using PAST3 (PAlentological STatistics) Software Version 3.22. A dendrogram was constructed using the algorithm of unweighted pair-group method using arithmetic average or UPGMA (Sokal 1986) based on the Bray-Curtis similarity index. The clustering process was estimated by the cophenetic correlation coefficient. The Bray-Curtis index was based on shared similarities divided by total similarities, which was calculated using the formula:

$$BC_{ij} = \Sigma\,|(n_i - n_j)| / \Sigma\,(n_i + n_j)$$

where, BC_{ij} = Bray-Curtis Dissimilarity of two species i and j
n_i = number of characters present in i
n_j = number of characters present in j
BC_{ij} Similarity Index = $(1 - BC_{ij}) \times 100$

RESULTS AND DISCUSSION

Phytochemical compounds of the studied species

The essential oils from the ginger rhizomes revealed a combination of camphoraceous, spicy, floral, fruity, sweet, citrusy to lemony smells. *H. leonurus* recorded relatively higher yield of essential oils at approximately 3.7% as compared to other taxa. The rhizomes of *E. nasuta* and *P. strobilifera* var. *conica* produced a yield about 2.9%, in contrast to *A. galanga* and *A. ligulata* that produced the least amount of essential oils at only 0.2%. Table 2 elucidated the essential oil components of all studied species including the essential oils yield.

In the present study, compounds that showed peak area of more than 0.05% were considered as major constituents. Overall, *Conamomum* and *Sundamomum* species recorded relatively higher major compounds, in particularly at 37 and 34 for *C. xanthophlebium* and *C. cylindrostachys*, and at 36 and 35 for *S. laxesquamosum* and *S. corrugatum,* respectively. The following were *P. strobilifera* var. *strobilifera* at 31, *E. nasuta* at 30, *A. ligulata, E. coccinea* and *P. strobilifera* var. *conica* at 28 each, *E. coccinea* at 27, *H. leonurus* at 26, and *A. galanga* at 24. All ten species showed the presence of n-hexadecanoic acid (1.09–10.56%) and linalool (0.08–25.02%), whereas β-elemene (1.05–10.62%) and *α*-terpineol (0.8–13.11%) were present in ten species except for *A. galanga* and *E. coccinea*.

Oxygenated monoterpenes were relatively high in the rhizomes oils of all species (15.27–53.85%) especially in *Etlingera*,

Table 2 Major compounds from the rhizomes essential oils of the studied Alpinieae species

	Taxa / Compounds	AIa	AIb	AL	AG	CC	CX	EC	EN	HL	PSC	PSS	SC	SL
							Monoterpene hydrocarbons							
1.	(Z)-β-ocimene	1027	1032	-	-	0.13	0.05	-	-	-	-	-	-	-
2.	4-carene	929	-	-	2.35	-	1.94	1.08	-	-	-	-	-	-
3.	camphene	948	946	-	1.25	0.26	-	1.69	-	-	-	15.29	12.01	13.92
4.	limonene	1030	1024	-	4.51	-	1.09	5.53	-	1.12	-	3.85	-	-
5.	p-cymene	1030	1026	-	-	3.6	1.37	-	-	-	-	-	-	-
6.	p-mentha-1,5,8-triene	1018	-	-	-	-	0.05	1.06	-	-	-	-	-	-
7.	sabinene	967	969	-	-	17.74	16.4	-	-	-	-	-	-	-
8.	α-terpinene	1020	1014	-	-	4.55	5.07	-	-	-	-	-	-	-
9.	α-thujene	922	924	-	-	2.02	0.94	1.13	7.18	2.1	-	-	-	-
10.	α-pinene	940	932	-	6.95	4.14	2.25	3.59	7.09	6.5	-	-	-	-
11.	β-pinene	980	974	-	8.94	4.16	-	9.69	-	3.3	-	-	-	-
12.	δ-terpinene	1050	1054	-	1.06	5.59	6.55	1.29	-	1.05	-	-	-	-
	Total			0.00	26.06	42.19	35.71	25.06	14.27	14.07	0.00	19.14	12.01	13.92
							Oxygenated monoterpenes							
13.	(2E)-decenal	1268	1260	-	-	-	-	2.87	1.51	-	-	-	-	-
14.	(E)-isocitral	1206	-	-	-	-	-	0.92	-	-	-	0.45	-	-
15.	2-cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, trans-	1346	-	-	-	-	-	-	-	-	-	-	0.59	0.65
16.	borneol	1168	1169	-	2.79	-	-	-	1.1	-	-	-	1.85	2.22
17.	bornyl acetate	1255	1254	0.5	2.15	0.21	0.17	-	-	-	-	0.63	0.8	1
18.	camphenol, 6-	1120	1111	-	-	-	-	-	-	-	-	-	2.67	3.9
19.	cis-carveol	1233	1226	-	-	-	-	1.52	0.38	-	-	-	-	-
20.	cis-piperitol	1175	-	-	-	0.94	-	-	-	-	-	0.52	-	-
21.	cis-p-mentha-1(7),8-dien-2-ol	1230	1227	-	-	-	-	2.5	0.4	-	-	-	-	-
22.	cis-sabinol	1085	-	-	-	-	-	-	1.63	-	-	-	1.11	1.77
23.	eucalyptol	1029	1026	-	-	-	-	-	7.38	20.78	-	-	4.32	4.43
24.	eugenol	1350	1356	45.31	-	1.42	-	-	-	-	-	-	-	-
25.	geranial	1277	1267	-	-	0.67	0.35	-	-	-	-	7.74	25.4	25.29
26.	geraniol	1250	1249	-	-	-	-	-	-	-	2.7	-	1.6	1.14
27	geranyl acetate	1374	1379	2.27	-	0.11	0.23	-	-	-	1.31	1.8	1.8	1.57

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Table 2 Continued

28.	isoascaridole	1011	-	-	-	-	-	-	-	-	-	-	4.21	5.75
29.	linalool	1100	1095	1.59	1.23	0.08	3.15	0.5	1.21	2.15	4.63	25.02	0.64	0.74
30.	myrtenol	1200	1194	-	-	-	-	6.42	5.43	-	-	-	-	-
31.	neral	1248	1235	-	-	0.27	0.12	-	-	1.15	3.09	3.23	4.15	4.22
32.	nerol	1228	1227	-	-	0.14	0.15	-	-	0.15	3.12	0.05	-	-
33.	p-cymen-7-ol	1284	1290	-	-	0.5	-	-	-	-	-	2.27	-	-
34.	pinocarveol	1136	1135	-	-	-	-	4.33	1.77	0.51	-	-	-	-
35.	pinocarvone	1168	1160	-	-	-	-	2.8	1.05	-	-	-	-	-
36.	sabinene hydrate	1061	1065	-	-	3.11	2.02	-	-	-	-	-	-	-
37.	terpinen-4-ol	1310	1299	2.95	2.53	-	13.7	-	-	0.3	5.53	-	-	0.57
38.	terpinen-4-ol acetate	1300	1299	-	-	0.28	0.08	-	-	-	-	-	-	-
39.	trans-pinocarveol	1140	1135	-	-	-	-	4.3	2.72	-	-	-	-	-
40.	trans-p-menth-2-en-1-ol	1109	-	-	-	2.33	0.99	-	-	-	-	-	-	-
41.	α-campholenal	1126	1122	-	-	-	-	2.5	0.4	-	-	-	-	-
42.	α-terpineol	1190	1186	1.23	6.57	13.11	2.26	-	11.97	0.8	1.5	5.16	1.06	1.18
43.	α-terpinyl acetate	1349	1346	-	-	1.96	1.55	0.62	-	-	-	-	-	-
44.	carvacrol	1300	1298	-	-	-	0.07	-	3.87	1.5	-	0.46	-	-
	Total			53.85	15.27	25.13	24.84	29.28	40.82	27.34	21.88	47.33	38.86	40.47
							Sesquiterpene hydrocarbons							
45.	(E)-β-farnesene	1450	1454	-	6.18	1.17	2.11	8.83	-	1.8	1	-	-	-
46.	(Z)- -bisabolene	1507	1506	-	-	-	-	-	-	-	-	-	0.85	1.63
47.	4-ethenyl-1,4-dimethyl-3-(2-methylprop-1-enyl) cycloheptene	-	-	-	-	-	-	-	-	-	0.34	1.3	-	-
48.	aromadendrene	1440	1439	0.46	-	-	-	-	2.08	3.54	-	-	-	-
49.	cis-calamene	1527	1528	0.37	-	-	-	-	1.76	1.51	-	-	-	-
50.	E-caryophyllene	1422	1417	1.33	30.84	-	-	4.13	3.03	2.7	4.2	-	-	-
51.	germacrene D	1488	1480	0.55	-	-	-	-	1.37	2.77	-	-	-	-
52.	sesquithujene	1394	1405	-	-	-	1.05	-	-	-	0.69	-	-	-
53.	zingiberene	1491	1493	0.7	-	3.86	2.61	-	1.75	-	-	1.48	0.75	1.55
54.	α-bergamotene	1430	1432	0.65	0.56	2.19	2.33	-	-	-	0.15	1.37	-	-

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Table 2 Continued

55.	α-copaene	1370	1374	1.25	4.91	-	2.09	-	-	-	2.04	-	-	-
56.	α-cubebene	1350	1345	-	-	-	-	-	-	2.59	1.48	-	-	-
57.	α-curcumene	1489	1479	2.95	-	-	2.9	-	-	-	6.8	3.79	4.01	-
58.	α-farnesene	1500	1505	3.28	0.48	-	-	-	1.13	-	2.45	1.38	1.5	-
59.	α-humulene	1459	1452	0.98	1.14	-	2.1	-	-	-	2.3	1.11	-	-
60.	α-santalene	1420	1416	0.51						2.18				
61.	β-bisabolene	1510	1505	-	0.53	2.46	-	4.42	2.5	-	-	2.14	2.04	3.19
62.	β-elemene	1390	1389	6.33	-	2.12	2.49	4.78	1.05	6.45	10.62	1.29	1.3	1.5
63.	β-selinene	1489	1489	0.87	1.35	-	-	-	4.09	6.34	-	-	-	-
64.	β-sesquiphellandrene	1528	1521	2.35	3.54	8.41	-	-	1.3	-	4.36	3.51	-	3.94
65.	γ-elemene	1428	1434	-	-	5.56	2.34	-	-	-	-	-	-	-
	Total			22.58	49.53	25.77	20.02	22.16	20.06	29.88	36.43	17.37	10.45	11.81
							Oxygenated sesquiterpenes							
66.	caryophyllene oxide	1586	1582	-	5.5	-	-	7.17	8.75	-	0.47	-	-	-
67.	elemol	1522	-	-	-	-	-	-	-	-	-	3.64	-	2.16
68.	neointermedeol	1663	1658	6.83	-	-	-	-	-	-	7.98	-	-	-
69.	nerolidyl acetate	1754	-	-	-	-	8.71	-	-	-	19.23	-	-	-
70.	spathulenol	1578	1577	-	-	-	-	-	-	-	1.08	3.4	-	-
71.	zingiberenol	1591	-	-	-	4.13	-	-	-	-	-	2.68	2.57	.56
72.	α-cadinol	1650	1652	3.35	-	-	-	-	-	10.18	-	2.16	-	-
73.	β-eudesmol	1643	1649	-	-	-	-	-	-	-	-	2.91	2.51	3.53
	Total			10.18	5.5	4.13	8.71	7.17	8.75	10.18	28.76	14.00	5.08	8.25
							Diterpene hydrocarbons							
74.	trachylobane	2061	-	-	-	-	-	-	-	-	-	-	3.82	3.63
	Total			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.82	3.63
							Oxygenated diterpenes							
75.	ent-kauran-16-β-ol	2311	-	-	-	-	-	-	-	-	-	-	8.82	6.85
76.	ent-kaurenal	2184	-	-	-	-	-	-	-	-	-	-	2.01	1.23

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Table 2 Continued

77.	phytol	1950	1942	0.87	1.08	-	2.37	-	-	1.32	-	-	1.00	1.38
78.	thunbergol	2227	-	-	-	-	-	-	-	-	-	-	1.20	0.84
	Total			0.87	1.08	0.00	2.37	0.00	0.00	1.32	0.00	0.00	13.03	10.3
							Non-terpenoids							
79.	3-o-acetyl-6-methoxy-cycloartenol	2450	-	-	-	-	-	-	-	-	-	-	6.92	5.22
80.	4,4-dimethyl-cyclohex-2-en-1-ol	1252	-	-	-	-	-	-	-	-	-	-	0.47	0.64
81.	1-aminononadecane, N-trifluoroacetyl-	1435	-	-	-	-	-	-	-	-	-	-	0.71	0.62
82.	apiole	1685	1677	1.4	-	-	-	-	-	-	-	-	0.49	0.49
83.	2-undecanone	1291	1293	-	-	-	-	-	-	5.12	1.42	-	-	-
84.	2-undecanol	1305	1301	2.21	-	-	-	-	-	-	2.38	-	-	-
85.	2-acetoxy-1,8-cineole	1386	-	-	-	0.07	-	1.3	-	-	-	-	-	-
86.	(2E)-dodecenal	1470	1464	-	-	-	-	6.82	4.81	-	-	-	-	-
87.	cryptone	1190	1183	-	-	-	2.05	-	-	-	-	-	0.45	0.67
88.	bis(2-ethylhexyl) phthalate	2704	-	-	-	-	-	-	-	-	-	0.29	2.15	0.52
89.	guaiacol, 4-butyl-	1502	-	-	-	0.13	2.05	-	-	-	-	-	-	-
90.	methyl eugenol	1408	1403	-	-	-	-	2.56	3.78	-	-	-	-	-
91.	tricyclo[4.2.2.0(2,5)]dec-7-ene, 7-(5-hexynyl)-	1447	-	2.72	1.63	-	-	-	-	-	1.23	0.46	2.2	0.92
92.	tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	869	-	-	-	-	-	-	-	-	2.03	0.47	-	-
93.	n-hexadecanoic acid	1968	1959	2.5	1.15	1.28	4.07	4.41	6.6	10.56	3.99	1.09	2.47	1.31
94.	n-heptadecane	1710	1700	2.11	-	1.27	-	-	-	-	-	-	-	-
	Total			10.94	2.78	2.75	8.17	15.09	15.19	15.68	11.05	2.31	15.86	10.39
	Yield (% v/w)			0.2	0.2	0.7	1.3	3.3	2.9	3.7	0.8	2.9	0.3	1.5

AIa = Arithmetic Indices calculated based on GC-MS analysis, AIb = Arithmetic Indices based on Adams (2017), AL = *A. ligulata*, AG = *A. galanga*, CC = *C. cylindrostachys*, CX = *C. xanthophlebium*, EC = *E. coccinea*, EN = *E. nasuta*, HL = *H. leonurus*, PSC = *P. strobilifera* var. *conica*, PSS = *P. strobilifera* var. *strobilifera*, SC = *S. corrugatum*, SL = *S. laxesquamosum*

Sundamomum, A. ligulata and *P. strobilifera* var. *strobilifera*. Similarly, sesquiterpene hydrocarbons (10.45–49.53%), oxygenated sesquiterpenes (4.13–28.76%) and non-terpenoid compounds (2.31–15.86%) were found in all species. Moreover, sesquiterpene hydrocarbons were the major compounds detected in *A. galanga* (49.53%), *H. leonurus* (29.88%) and *P. strobilifera* var. *conica* (36.43%). Monoterpene hydrocarbons were not recorded in *A. galanga* and *P. strobilifera* var. *conica*, but dominated the rhizomes oils of *C. cylindrostachys* at 42.19% and *C. xanthophlebium* at 35.71%. Diterpene hydrocarbon was only found in *Sundamomum* species (3.63–3.82%), and oxygenated diterpenes were also mainly detected in the genus and in certain taxa.

Geranial, an oxygenated monoterpene, produced the highest percentage area in *S. corrugatum* (25.40%) and *S. laxesquamosum* (25.29%). Similarly, sabinene, an oxygenated monoterpene was the highest in *C. cylindrostachys* (17.74%) and *C. xanthophlebium* (16.42%). For *Alpinia* species, the maximum peak area was detected for (E)-caryophyllene (a sesquiterpene hydrocarbon) with 30.84% in *A. galanga* and eugenol (an oxygenated monoterpene) with 45.31% in *A. ligulata*. Meanwhile, β-pinene (a monoterpene hydrocarbon) and α-terpineol (an oxygenated monoterpene) showed the highest peak area of 9.69% in *E. coccinea* and 11.97% in *E. nasuta*, respectively. Eucalyptol (an oxygenated monoterpene) was the main peak in *H. leonurus* with 20.78%. Two varieties of *P. strobilifera* revealed different highest peak percentage; linalool (an oxygenated monoterpene) at 25.02% in var. *stobilifera,* and nerolidyl acetate (an oxygenated sesquiterpene) at 19.23%) in var. *conica*.

Essential oils yield and compositions are highly dependent on endogenous factors including plant age or maturity and part of the plant used, while exogenous factors such as climate, weather, light, precipitation, soil composition, pH, habitat elevation or recent attack by herbivores (Waterman & Mole 1989, Waterman 2007, Padalia et al. 2010). Due to the location and nature of the secretory structures vary interspecifically, each studied taxon demonstrated different qualitative and quantitative phytochemical profiles. These might also explain the difference of the oil percentage in some species that were not exactly similar with the findings of the other research works. For example, the rhizomes of *A. galanga* produced 0.8% of essential oils as reported by Jantan et al. (2003) as compared to only 0.2% in the present study. Nevertheless, the major components in *A. galanga* including β-farnesene, β-bisabolene, β-pinene, β-selinene and α-bergamotene as reported by Padalia et al. (2010) were also present in the current study. Padalia et al. (2010) also reported the presence of a single marker compound, endo-fenchyl acetate and other major constituent, 1,8-cineole or eucalyptol in their research but absent in the current study and was probably due to the endogenous and exogenous factors too.

Similarly, the reported highest peak percentage in the *Etlingera* was not comparable with the data by Nur-Anwariah et al. (2011) who used GC/FID analysis. For instance, germacrene D (a sesquiterpene hydrocarbon) at 17.91% was reported as the highest peak area in *E. nasuta*, as opposed to only 1.37% in the current study. For *E. coccinea,* ethylfuranone at 26.18% was reported as the highest percentage area, instead of β-bisabolene at 4.42% as recorded in the current study. Nonetheless, several major constituents were still recorded in either two species, including camphene, carene, zingiberene, caryophyllene, β-bisabolene, β-elemene, β-farnesene, β-selinene, β-sesquiphellandrene, caryophyllene oxide, and methyl eugenol (Nur-Anwariah et al. 2011). It was difficult to compare the rhizome oil components of the remaining species as only few previous related or detailed studies were carried out.

Chemotaxonomic significance

Clustering analysis of all compounds had preliminarily divided the studied species into four main clusters as shown in Figure 2. In addition to the chemical profile similarity index, each cluster was further defined by several significant constituents that were only shared by species of the same group and could potentially be specific chemical markers. The potential chemotaxonomic markers of each genus were eluted at approximately similar retention time per minute.

The first cluster consisted of *A. galanga*, *A. ligulata* and the two *P. strobilifera* varieties, which shared no less than 40% similarity index with at least 12 similar major compounds. Between the two *Plagiostachys* species, 16 shared compounds with 50% similarity were recorded and three of them only occurred in the genus. The specific components for var. *strobilifera* and var. *conica*

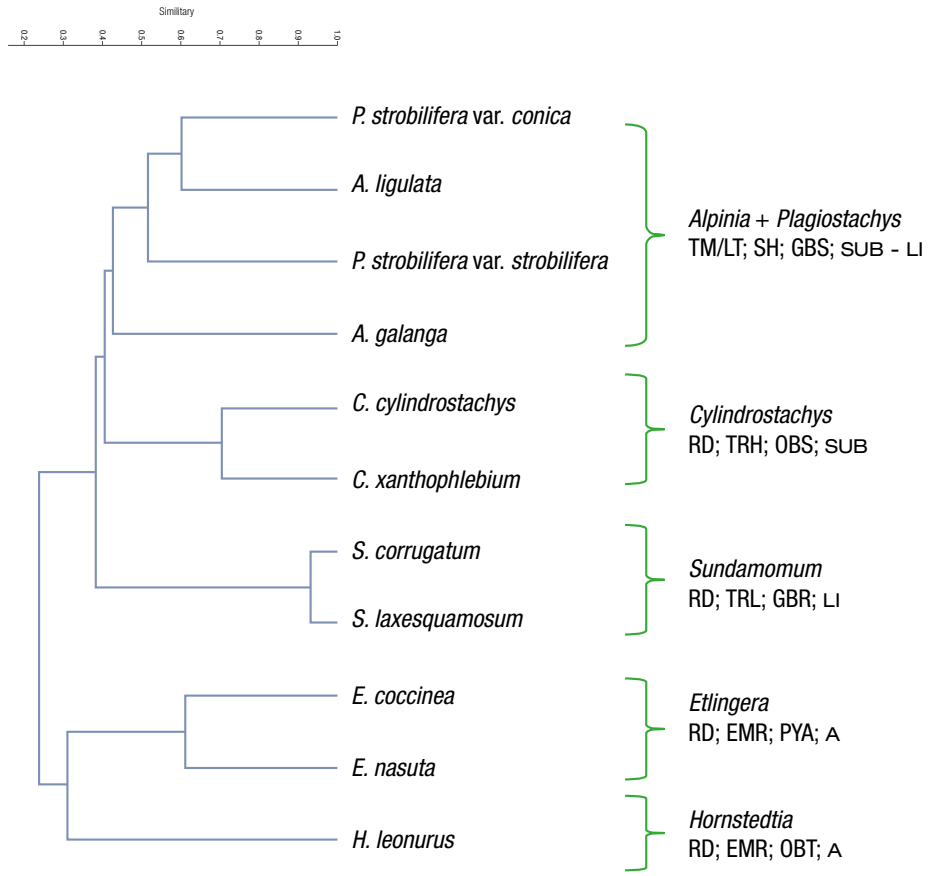

Figure 2 Dendrogram from hierarchical clustering implies phenetic relationship of 11 Alpinieae species based on the rhizome oils compounds using Bray-Curtis similarity (r = 0.864) and UPGMA algorithm

Morphology of the inflorescence, anther crest, fruit and lateral staminodes were also plotted in the dendrogram.
Inflorescence type: LT = Lateral, RD = Radical, TM = Terminal;
Anther crest shape: A = Absent, EMR = Emarginated-ridge, SH = Short, TRH = Trilobed-horned, TRL = Trilobed-lobules;
Fruit type: GB = Globose, OBS = Obovoid-smooth, OBT – Obovoid, thin-walled, OBR = Obovoid-ridged, PYA = Pyriform-angular;
Lateral staminodes shape: A - Absent; LI - Linear; SUB – Subulate.

comprising tricyclo [3.1.0.0 (2,4)] hexane, 3,6-diethyl-3, 6-dimethyl-, trans- (RT 22.394 vs 22.362), spathulenol (RT 22.500 vs 22.460), as well as 4-ethenyl-1,4-dimethyl-3-(2-methylprop-1-enyl) cycloheptene (RT 22.852 vs 22.816). Moreover, the rhizomes oils of *A. ligulata* and *P. strobilifera* var. *conica* indicated close relationship with 16 shared components and 60% similarity index. The rhizomes oils of two species of *Alpinia* recorded 14 similar components, however no specific compounds were recorded. The lack of specific compounds between these two *Alpinia* supported the disjunction of the species into separate clades i.e. *Zerumbet* and *Galanga* clades, as proposed by Kress et al. (2005). In fact, molecular findings had placed *Plagiostachys* along with several *Alpinia* species including *A. ligulata* and *A. nieuwenhuizii* (Kress et al. 2005, Julius et al. 2008).

For species belonging to the monophyletic genera, i.e. *Conamomum* and *Sundamomum,* their chemical components were considerably corresponding to their generic boundaries. For *Conamomum* cluster with 70% similarity index, it was dominated by monoterpene hydrocarbons and further characterised by 25 shared compounds, including nine specific compounds. Sabinene was considered as the highest specific compound that occurred only in this genus with 17.74% (RT at 8.567 min) in *C. cylindrostachys* and 16.42% (RT at 8.359 min) in *C. xanthophlebium.* Another eight distinct compounds in *C. cylindrostachys* and C. *xanthophlebium* were α-terpinene (RT 9.509

vs 9.418), p-cymene (RT 9.754 vs 9.659), (Z)-β-ocimene (RT 10.102 vs 10.060), sabinene hydrate (RT 10.910 vs 10.851), trans-p-menth-2-en-1-ol (RT 12.290 vs 12.253), terpinen-4-ol acetate (RT 16.215 vs 16.190), γ-elemene (19.168 vs 19.159), and guaiacol, 4-butyl- (RT 20.042 vs 20.020).

The *Sundamomum* cluster was characterised by high percentages of oxygenated monoterpenes and 33 shared compounds of a mixture of terpenoids and non-terpenoids, together with 11 specific components. The highest percentage of specific component recorded was ent-kauran-16-β-ol with values of 8.82% (RT at 33.773 min) in *S. corrugatum* and 6.85% (RT at 33.770 min) in *S. laxesquamosum*. The remaining special constituents in *S. corrugatum* and *S. laxesquamosum* in terms of RT (min) comprised of 4,4-dimethyl-cyclohex-2-en-1-ol (14.740 vs 14.744), camphenol, 6- (16.282 vs 16.262), isoascaridole (17.091 vs 17.101), 2-cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methethyl)-, trans- (19.520 vs 19.521), 1-aminononadecane, N-trifluoroacetyl- (20.830 vs 20.829), (Z)-α-bisabolene (29.218 vs 29.218), trachylobane (29.937 vs 29.938), ent-kaurenal (31.825 vs 31.824), thunbergol (32.483 vs 32.478) and 3-o-acetyl-6-methoxy-cycloartenol (35.962 vs 35.960). Chemicals profile in these two *Sundamomum* species signified highest similarity index of 94% in the dendrogram.

Subsequently, *Etlingera* cluster which formed 60% similarity index was described through 18 similar compounds including nine specific ones. The cluster was mainly dominated by oxygenated monoterpenes and myrtenol was the highest specific compound with the values of 6.42% (RT at 14.212 min) in *E. coccinea* and 5.43% (RT at 14.428 min) in *E. nasuta*. Other specific components in *E. coccinea* and *E. nasuta* in terms of RT (min) were α-campholenal (12.233 vs 12.161), trans-pinocarveol (12.830 vs 12.594), pinocarvone (13.287 vs 13.125), cis-carveol (14.630 vs 14.428), cis-*p*-mentha-1(7),8-dien-2-ol (14.884 vs 14.688), (2E)-decenal (15.258 vs 15.312), methyl eugenol (18.579 vs 18.430), and (2E)-dodecenal (20.079 vs 19.789).

In the context of close association between *H. leonurus* and *Etlingera* clade through molecular-based study (De Boer et al. 2018), phytochemical analysis had revealed several identical compounds between these two clades in particular 14 and 11 similar compounds with *E. nasuta* and *E. coccinea*, respectively. The chemical profile of *H. leonurus* showed at least 32% similarity with *E. nasuta*.

Morphological Relationship

The hierarchical clustering of the studied species based on chemometric data was in agreement to their morphological characters especially the inflorescence position on a leafy shoot, anther crest shape, lateral staminodes presence and shape, labellum shape and fruit type. For the first cluster, *Plagiostachys* showed close correlation with *Alpinia* through the morphology of terminal inflorescence, although borne laterally from the leafy shoot. The short anther crest, globose-smooth fruit and constant presence of lateral staminodes in various shapes were other shared morphological similarity in both genus members. Additionally, the strongly paniculate inflorescence in *A. ligulata* that more or less resembled a branching pattern in *Plagiostachys* might explain they were phylogenetically united in one cluster. However, it was noted that the mucilaginous nature of the inflorescence in several *Plagiostachys* species had never occur in *Alpinia*, and the lateral position of the inflorescence was consistent for *Plagiostachys* members.

The remaining clusters were mainly consisted of species with the radical inflorescences. *Conamomum* cluster was characterised by the obovate-trilobed labellum, subulate lateral staminodes, prominent horned anther connective where the lateral lobes pointed outwards as well as elipsoid-smooth capsule. Likewise in the third cluster, both species of *Sundamomum* shared high similarity through the obovate orange labellum, linear lateral staminodes and trilobed anther crest where the mid-lobe extended and larger than the lateral lobes. As for *Etlingera* cluster, the studied species were described through the trilobed labellum with long mid-lobe, emarginated anther crest as well as pyriform, flat-topped fruit. *H. leonurus* was linked to *Etlingera* through the resemblance of the trilobed labellum, and occurrence of the tubular bracteoles. Additionally, Holttum (1950) had pointed out the disjunction of *H. leonurus* from other species in the genus which laid somewhere in between *Etlingera,* and recent phylogenetic analysis by De Boer et al. (2018) approved the placement of the species together with *Etlingera*. The comparison of important morphological characteristics of the taxa studied was shown in Table 3. The morphology of the inflorescence, anther crest, lateral staminodes and fruit were also represented in the dendrogram in Figure 2.

Table 3 Comparison of key morphological characteristics of the studied taxa

Taxa	Ligule	Inflorescence	Bracteole	Labellum	Lateral staminodes	Anther crest	Stigma	Fruit
A. galanga	*ca.* 6 mm long, apex entire	*ca.* 25 cm long, terminal, paniculate, 2–3 flowers per cincinnus	*ca.* 2 cm long, open to the base	*ca.* 22 mm long, spathulate, apex bilobed, white with red markings	*ca.* 7 mm long, subulate	Absent	*ca.* 1 mm wide, infundibuliform-tubular, gradually widening, white; ostiole apical, round	*ca.* 1.6 × 1.5 cm, globose to ellipsoid, smooth
A. ligulata	*ca.* 51 mm long, apex entire	*ca.* 59 cm long, terminal, strongly paniculate, single flower per cincinnus	*ca.* 1 cm long, open to the base	*ca.* 11 mm long, obovate, yellowish with red markings	*ca.* 1 mm, subulate	Short, *ca.* 1 mm long, erose	*ca.* 1 mm wide, infundibuliform, gradually widening, yellow; ostiole dorsal, transverse	*ca.* 2.2 × 2.8 cm, globose-oblate, smooth
C. cylindrostachys	*ca.* 7 mm long, apex entire	*ca.* 22 cm long, radical, compact, mucilaginous, flowers borne singly	*ca.* 2 cm long, open to the base	*ca.* 10 mm long, obovate, trilobed, yellowish with red markings	*ca.* 2 mm long, subulate	Horned, two lobed	*ca.* 0.9 mm wide, infundibuliform, white; ostiole dorsal, transverse	*ca.* 1.3 × 1 cm, ellipsoid, smooth
C. xanthophlebium	*ca.* 7 mm long, apex entire	*ca.* 16 cm long, radical, compact, flowers borne singly	*ca.* 3.6 cm long, open to the base	*ca.* 41 mm long, obovate, broadly trilobed, yellowish-orange with red markings	*ca.* 4.6 mm long, subulate	Horned, trilobed	*ca.* 1.25 mm wide, infundibuliform, pale yellow; ostiole dorsal, transverse	*ca.* 3 × 1.5 cm, ellipsoid, smooth
E. coccinea	*ca.* 12 mm long, apex entire	*ca.* 19 cm, radical, compact, subterranean, flowers borne singly	*ca.* 3.45 cm long, tubular	*ca.* 58 mm long, trilobed, red with yellow centre	Absent	Emarginate, ± ridged	*ca.* 1.5 mm wide, obcordate-triangular, pale pink; ostiole dorsal, transverse	*ca.* 4 × 3.5 cm, pyriform, radiating ridged, flat-topped
E. nasuta	*ca.* 14 mm long, apex entire	*ca.* 11 cm long, radical, compact, subterranean, flowers borne singly	*ca.* 3 cm long, tubular	*ca.* 43 mm long, trilobed, red with white centre	Absent	Emarginate, ± ridged	*ca.* 2.1 mm wide, rounded-triangular, red; ostiole dorsal, transverse	*ca.* 2.5 × 3 cm, pyriform, flat-topped and lateral ridges
H. leonurus	*ca.* 12 mm long, apex entire	*ca.* 14 cm long, radical, compact, rigid, 2 flowers per cincinnus	*ca.* 5.8 cm long, tubular*	*ca.* 30 mm long, trilobed, dark red with white margins	Absent	Emarginate-ridged	*ca.* 1 mm wide, infundibuliform, dark red; ostiole dorsal, transverse	*ca.* 1.9 cm long, obovoid, smooth
P. strobilifera var. *strobilifera*	*ca.* 3 mm long, apex entire to bilobed	Lateral, *ca.* 10 cm long, compact, non-mucilaginous, flowers borne singly	*ca.* 5 mm long, tubular at base, apex decayed	*ca.* 10 mm long, ovate, apex bilobed, pale yellow with red margin	*ca.* 3 mm long, linear	Short, truncate	*ca.* 1 mm wide, infundibuliform, red; ostiole dorsal, transverse	*ca.* 1.9 × 1.7 cm, globose, smooth

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Table 3 Contineud

P. strobilifera var. *conica*	*ca.* 6 mm long, apex bilobed	Lateral, *ca.* 16 cm long, compact, non-mucilaginous, flowers borne singly	*ca.* 2.4 cm long, tubular at base, persistent	*ca.* 8 mm long, ovate, apex bilobed, scarlet with red markings	*ca.* 3.5 mm long, oblong	Short, entire, *ca.* 2 mm long	*ca.* 1.3 mm wide, infundibuliform, red; ostiole dorsal, transverse	Not seen
S. corrugatum	*ca.* 4 mm long, apex bilobed	Radical, *ca.* 13.4 cm long, compact, flowers borne singly	*ca.* 4.1 cm long, open to the base	*ca.* 48 mm long, obovate, apex trilobed, yellowish with dark markings	*ca.* 8 mm long, linear	*ca.* 9 mm long, trilobed, sidelobes narrow	*ca.* 1.4 mm wide, infundibuliform, pale pink with red dots; ostiole dorsal, transverse	Not seen
S. laxesquamosum	*ca.* 10 mm long, bilobed	Radical, *ca.* 13 cm long, compact, flowers borne singly	*ca.* 1.5 cm long, tubular	*ca.* 28 mm long, obovate, apex trilobed, orange with burgundy margin	*ca.* 4 mm long, linear	*ca.* 7 mm long, trilobed, sidelobes ± larger	*ca.* 1 mm wide, infundibuliform, white with red dots; ostiole dorsal, transverse	*ca.* 2.3 × 1.8 cm, ribbed, ± globose, smooth

* Occasionally opened to the base for species from Lambir Hills National Park

CONCLUSION

The availability of phytochemical data involving established protocols for assessing phylogenetic relationships is vital in providing valuable counterpoint to DNA fingerprinting. The use of GC–MS coupled with statistical analysis enabled the identification of rhizome oil constituents which characterised and grouped the current studied taxa. Based on the present findings, it seemed premature to classify the recorded specific compounds in a particular genus as chemotaxonomic markers. Appropriate future studies to evaluate further species across the range of the genus and tribe, under controlled conditions from different climate, habitats, harvesting periods, plant age as well as focusing on the biosynthetic pathways especially on the specific constituents should be conducted. The results from this study should be used as a compilation of current knowledge in understanding the diversity and classification of the many genera of the tribe Alpinieae on the floristically rich island of Borneo.

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