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## Rapid-communication

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# Chemical composition of the essential oils of four *Polyalthia* species from Malaysia

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**Abstract:** *Polyalthia* is one of the largest genera in the Annonaceae family, and has been widely used in folk medicine for the treatment of rheumatic fever, gastrointestinal ulcer, and generalized body pain. The present investigation reports on the extraction by hydrodistillation and the composition of the essential oils of four *Polyalthia* species (*P. sumatrana*, *P. stenopetalla*, *P. cauliflora*, and *P. rumphii*) growing in Malaysia. The chemical composition of these essential oils was determined by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The multivariate analysis was determined using principal component analysis (PCA) and hierarchical clustering analysis (HCA) methods. The results revealed that the studied essential oils are made up principally of bicyclogermacrene (18.8%), *cis*-calamenene (14.6%) and  $\beta$ -elemene (11.9%) for *P. sumatrana*;  $\alpha$ -cadinol (13.0%) and  $\delta$ -cadinene (10.2%) for *P. stenopetalla*;  $\delta$ -elemene (38.1%) and  $\beta$ -cubebene (33.1%) for *P. cauliflora*; and finally germacrene D (33.3%) and bicyclogermacrene for *P. rumphii*. PCA score and HCA plots revealed that the essential oils were classified into three separated clusters of *P. cauliflora* (Cluster I), *P. sumatrana* (Cluster II), and *P. stenopetalla*, and *P. rumphii* (Cluster III) based on their characteristic

chemical compositions. Our findings demonstrate that the essential oil could be useful for the characterization, pharmaceutical, and therapeutic applications of *Polyalthia* essential oil.

**Keywords:** bicyclogermacrene; essential oil; germacrene D; hydrodistillation; multivariate analysis; *Polyalthia*.

## 1 Introduction

The Annonaceae is a large family comprising aromatic trees, shrubs, or climbers represented by 200 genera and more than 2500 species. This family is economically important as a source of spices or edible fruits, and several species are used in folk medicine for various purposes [1]. *Polyalthia* is one of the largest and notable genera in the Annonaceae family with approximately 346 species of shrubs and trees, reported in The International Plant Names Index [2]. In Peninsular Malaysia, about 30 species are known to occur [3]. The genus *Polyalthia* is widely distributed in the palaeotropical area, especially in Southeast Asia, extending from Southern India and Sri Lanka, through continental Southeast Asia to Northern Australia and Melanesia, even with some occurrences in the lowland humid regions of East Africa and Madagascar [4]. There are several general characteristics of *Polyalthia* such as the flowers, which are usually one to many-flowered and it has six petals. The number of sepals is ranging from three to six and the number of ovules per carpel can be one or up to three. The sexuality of *Polyalthia* flowers is usually bisexual and the number of ovules is usually one to three per carpel [5]. The *Polyalthia* genus is considered to be of medicinal importance because of the presence of clerodane diterpenoids and alkaloids in various parts of the plant [6, 7]. *Polyalthia longifolia* is the one most commonly used in traditional medicine to treat skin disease, urinary tract infection, fever, headache, bone fracture, postpartum depression, and reduce blood pressure [8]. There are several reports on the composition of the essential oils of *Polyalthia* species. As a continuation of our

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systematic studies of the volatile oils of Malaysian plants [9–12] we describe in this paper the chemical composition of the essential oils of *P. stenopetalla*, *P. sumatrana*, *P. cauliflora*, and *P. rumphii*.

*P. stenopetalla* (local name: *jambul cicit*) is used in some places in Peninsular Malaysia to treat rheumatic fever and diarrhea. *P. sumatrana* (local name: *karai puteh*) is widely used in Southeast Asia for treating cough and anemia. *P. cauliflora* (local name: *semukau*) is used by the ‘Kalabit’ community in Borneo for birth control, whereas *P. rumphii* (local name: *merpadi*) locally used in Indonesia to treat skin disease [13, 14]. To the best of our knowledge, there is no report on the essential oil composition of these species, except for *P. stenopetalla* [15]. Additionally, the chemical components common in all species were submitted to principal components analysis (PCA) and hierarchical clustering analysis (HCA) to evaluate the chemical similarity in species.

## 2 Material and methods

### 2.1 Plant materials

The fresh samples of *P. sumatrana* (SK30/19), *P. stenopetalla* (SK31/19), *P. cauliflora* (SK122/18), and *P. rumphii* (SK410/16) were collected from Gambang, Pahang in September 2019, and identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimens were deposited at UKMB herbarium Universiti Kebangsaan Malaysia.

### 2.2 Isolation of essential oils

The fresh leaves of each sample (300 g) were chopped into small pieces and then subjected to hydrodistillation process in Clevenger-type apparatus for 4 h. The essential oils obtained were dried over anhydrous magnesium sulfate and stored at 4–6 °C. The oil yield (%) was calculated based on the fresh weight (w/w).

### 2.3 Analysis of essential oils

Gas chromatography (GC-FID) was performed on Shimadzu GC-2010 Plus gas chromatograph equipped with HP-5MS column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. The injector and detector (FID) temperatures were set at 250 and 280 °C, respectively. The oven temperature was kept at 50 °C, then gradually raised to 280 °C at 5 °C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times and the peak area percent were reported as means ± SD of triplicates.

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using Agilent GC-MS 7890A/5975C Series MSD (70 eV direct inlet) equipped with HP-5MS column (30 m × 0.25 mm

internal diameter × 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was 250 °C. The oven temperature was programmed from 50 °C (5 min hold) to 250 °C at 10 °C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50–400 amu.

### 2.4 Identification of chemical components

For identification of the essential oil components, co-injection with the standards (major components:  $\delta$ -elemene,  $\alpha$ -cubebene,  $\beta$ -cubebene,  $\beta$ -elemene, germacrene D, bicyclogermacrene,  $\delta$ -cadinene, *cis*-calamenene,  $\alpha$ -cadinol) were used, together with correspondence of retention indices (relative to the retention times of *n*-alkanes from C<sub>6</sub> to C<sub>30</sub>) and mass spectra with respect to those reported in Adams, NIST08, and FFNSC2 libraries [16]. Semi-quantification of the essential oil components was undertaken by peak area normalization (GC-FID) considering the same response factor for all volatile components. Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds. Percentage values were the mean of three chromatographic analyses.

### 2.5 Multivariate statistical analysis

The retention index and percentage content of every component for all essential oils were entered into an Excel spreadsheet and the data were analyzed using Unscrambler X version 10.0.1 programme (CAMO Software Inc.). PCA plots of scores, loadings, residuals, and influence were analyzed and sample outliers were removed prior to generating the HCA using squared Euclidean distance of Ward’s method.

## 3 Results and discussion

The hydrodistillation from the leaves of *P. sumatrana*, *P. stenopetalla*, *P. cauliflora*, and *P. rumphii* gave pale yellow oils with a pungent smell in mean yields of 0.028, 0.028, 0.013, and 0.034% (w/w), respectively. A total of 52 components were identified in the essential oils, accounting for 72.8–94.9% of the total oil composition. The lists of components identified in the oils are shown in order of elution on the HP5 column in Table 1.

The essential oils of *P. sumatrana* revealed the presence of 30 components with a percentage of 91.8% of the total oil. The essential oil was characterized by the presence of a high concentration of sesquiterpene hydrocarbons (84.8%), followed by oxygenated sesquiterpenes (7.0%). The oil was characterized by the abundance of bicyclogermacrene (18.8%), *cis*-calamenene (14.6%),  $\beta$ -elemene (11.9%), and  $\alpha$ -cubebene (10.7%). There were also a significant amount of compounds that displayed more than 2% which were germacrene D (3.9%),

**Table 1:** Chemical components identified from the essential oils of four *Polyalthia* species.

Components	KI <sup>a</sup>	KI <sup>b</sup>	Percentage (%) <sup>c</sup>				Methods <sup>d</sup>
			PSUO	PSTO	PRUO	PCAO	
Bicycloclemene	1330	1330	0.5 ± 0.1				RI, MS
δ-Elementene	1336	1335		2.6 ± 0.1	3.2 ± 0.1	38.1 ± 0.2	RI, MS, Std
α-Cubebene	1345	1345	10.7 ± 0.3				RI, MS, Std
α-Ylangene	1370	1373		0.4 ± 0.2			RI, MS
Isodene	1374	1374			1.8 ± 0.2	0.7 ± 0.1	RI, MS
α-Copaene	1375	1374	1.1 ± 0.1	0.2 ± 0.2	4.6 ± 0.2		RI, MS
β-Patchoulene	1380	1379		2.6 ± 0.1			RI, MS
β-Bourbonene	1384	1387	1.3 ± 0.1				RI, MS
β-Cubebene	1386	1387	1.4 ± 0.1			33.1 ± 0.2	RI, MS, Std
β-Elementene	1390	1389	11.9 ± 0.2	2.1 ± 0.2	0.9 ± 0.2	1.1 ± 0.2	RI, MS, Std
α-Gurjunene	1402	1409	1.0 ± 0.1	1.9 ± 0.2			RI, MS
α-Bergamotene	1405	1411		0.5 ± 0.1			RI, MS
β-Caryophyllene	1412	1417	3.6 ± 0.2	1.5 ± 0.2	3.5 ± 0.2	1.4 ± 0.2	RI, MS
β-Gurjunene	1430	1431			0.7 ± 0.1		RI, MS
γ-Elementene	1435	1434	-	5.4 ± 0.1		0.6 ± 0.1	RI, MS
α-Guaiene	1438	1437	1.4 ± 0.1				RI, MS
Aromadendrene	1440	1439		2.3 ± 0.2			RI, MS
Seychellene	1445	1444	0.2 ± 0.1				RI, MS
α-Caryophyllene	1445	1444	0.6 ± 0.1				RI, MS
α-Humulene	1450	1452		0.7 ± 0.1	0.9 ± 0.2	0.7 ± 0.2	RI, MS
3,7-Guaiadiene	1460	1461	1.1 ± 0.2	1.1 ± 0.2	0.8 ± 0.2		RI, MS
γ-Murolene	1480	1478	0.6 ± 0.1		1.3 ± 0.2		RI, MS
Epi-bicyclosesqui-phellandrene	1482	1482	0.4 ± 0.1				RI, MS
α-Amorphene	1482	1483	2.1 ± 0.2	4.3 ± 0.2		0.4 ± 0.1	RI, MS
Germacrene D	1484	1484	3.9 ± 0.3	5.4 ± 0.2	33.3 ± 0.2		RI, MS, Std
γ-Selinene	1485	1484	0.5 ± 0.2				RI, MS
Eudesma-3,7(11)-diene	1488	1489		0.6 ± 0.1			RI, MS
β-Selinene	1490	1489	2.2 ± 0.2				RI, MS
Ledene	1490	1490	0.3 ± 0.1				RI, MS
β-Guaiene	1492	1492		0.5 ± 0.1			RI, MS
δ-Selinene	1495	1492		1.3 ± 0.1		0.6 ± 0.1	RI, MS
Cadina-1,4-diene	1496	1495		1.4 ± 0.1			RI, MS
Bicyclgermacrene	1500	1500	18.8 ± 0.2		13.0 ± 0.1		RI, MS, Std
α-Murolene	1501	1500	0.8 ± 0.2		1.5 ± 0.1		RI, MS
Epizonarene	1502	1501		0.8 ± 0.2			RI, MS
(E,Z)-α-Farnesene	1505	1505		2.5 ± 0.2			RI, MS
Guaia-1(10),11-diene	1510	1509	2.7 ± 0.1				RI, MS
γ-Cadinene	1515	1513	0.5 ± 0.1		1.1 ± 0.1		RI, MS
δ-Cadinene	1520	1522	2.0 ± 0.2	10.2 ± 0.2	5.9 ± 0.2	0.6 ± 0.1	RI, MS, Std
cis-Calamenene	1525	1528	14.6 ± 0.2				RI, MS, Std
α-Cadinene	1538	1537		0.9 ± 0.2			RI, MS
Germacrene B	1560	1559	0.6 ± 0.1	2.9 ± 0.3	4.6 ± 0.2	2.7 ± 0.2	RI, MS
Spathulenol	1575	1577	3.3 ± 0.2	0.5 ± 0.1			RI, MS
Globulol	1590	1590		1.0 ± 0.2	2.7 ± 0.2		RI, MS
Viridiflorol	1592	1592	1.7 ± 0.2				RI, MS
Fonenol	1595	1596			0.7 ± 0.1		RI, MS
Guaiol	1601	1600		0.5 ± 0.1			RI, MS
t-Murolol	1645	1644	1.6 ± 0.2	4.5 ± 0.2	5.6 ± 0.2		RI, MS
β-Eudesmol	1650	1649	0.4 ± 0.1				RI, MS
α-Cadinol	1652	1652		13.0 ± 0.2	6.4 ± 0.1		RI, MS, Std
α-Bisabolol	1685	1685		0.4 ± 0.1			RI, MS
Eudesma-4(14),11-diene	1744	1745		0.8 ± 0.1			RI, MS
Sesquiterpene hydrocarbons			84.8	52.9	77.1	80.0	
Oxygenated sesquiterpenes			7.0	19.9	17.8		
Total identified (%)			91.8	72.8	94.9	80.0	

PSUO – *P. sumatrana* oil; PSTO – *P. stenopetalla* oil; PRUO – *P. rumphii* oil; PCAO – *P. cauliflora* oil.

<sup>a</sup>Linear retention index, experimentally determined using homologous series of C<sub>6</sub>-C<sub>30</sub> alkanes.

<sup>b</sup>Linear retention index taken from Adams or NIST08 and literature.

<sup>c</sup>Relative percentage values are means of three determinations ±SD.

<sup>d</sup>Identification methods: Std, based on comparison with authentic compounds; MS, based on comparison with Wiley, Adams, FFNSC2 and NIST08 MS databases; RI, based on comparison of calculated RI with those reported in Adams, FFNSC2 and NIST08.

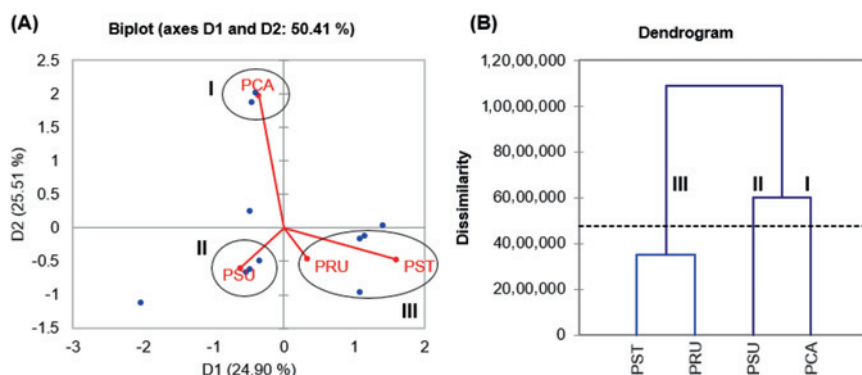
$\beta$ -caryophyllene (3.6%), spathulenol (3.3%), guaia-1(10),11-diene (2.7%),  $\beta$ -selinene (2.2%), and  $\alpha$ -amorphene (2.1%). In *P. stenopetalla* oil, a total of 30 components were detected with the constitution of 72.8% of the total oil. The oil was made up predominantly of sesquiterpene hydrocarbons and oxygenated sesquiterpenes, constituting 52.9 and 19.9%, respectively. The most abundant components were  $\alpha$ -cadinol (13.0%),  $\delta$ -cadinene (10.2%),  $\gamma$ -elemene (5.4%) and germacrene D (5.4%). The quantitatively significant leaf oil compounds which show percentage more than 2% consisted of t-murolol (4.5%),  $\alpha$ -amorphene (4.3%), germacrene B (2.9%),  $\delta$ -elemene (2.6%),  $\beta$ -patchoulene (2.6%), (E,Z)- $\alpha$ -farnesene (2.5%), aromadendrene (2.3%), and  $\beta$ -elemene (2.1%). 20 components were identified from the essential oil of *P. rumphii*, representing 94.9% of the total oil. The content of sesquiterpene hydrocarbons was 77.1% along with oxygenated sesquiterpenes, 17.8%. The most abundant components were germacrene D (33.3%), bicyclogermacrene (13.0%),  $\alpha$ -cadinol (6.4%),  $\delta$ -cadinene (5.9%) and t-murolol (5.6%). There were also significant amounts of germacrene B (4.6%),  $\alpha$ -copaene (4.6%),  $\beta$ -caryophyllene (3.5%), and  $\delta$ -elemene (3.2%). In the case of *P. cauliflora*, the oil consisted of only 11 components, representing 80.0% of the total oil. The high contents of sesquiterpene hydrocarbons were present in the oil, comprised mainly of  $\delta$ -elemene (38.1%) and  $\beta$ -cubebene (33.1%).

Based on the studies of the essential oils composition of *Polyalthia* species, all of them consisted mainly of sesquiterpene hydrocarbons (52.9–84.8%) and oxygenated sesquiterpenes (7.0–19.9%). However, oxygenated sesquiterpene was absent in the oil of *P. cauliflora*. In another study, Kamaruddin et al. [15] have reported that the essential oil of *P. stenopetalla*, collected from Selangor contains curzerene (37.56%), viridiflorol (11.59%), germacrene B (3.77%) and aromadendrene (4.01%) as the major components. However, curzerene and viridiflorol were not detected in the current oil. It is well known that medicinal

plant materials derived from the same species can show significant differences in quality when collected at different sites, owing to the influence of soil, climate, and other factors [17]. Meanwhile, in this study, no monoterpenes were found in all *Polyalthia* essential oils. The chemical differences among *Polyalthia* species could be due to the extraction procedures, stages of development, and distinct habitat in which the plant was collected. Besides, the chemical and biological diversity of aromatic and medicinal plants depends on such factors as climatic conditions, vegetation phase, and genetic modifications. These factors influence the plant's biosynthetic pathways and consequently, the relative proportion of the main characteristic compounds [18].

A review of the existing literature on essential oils of the genus revealed the presence of several studies. Similar to our results, germacrene D as the major component in *P. rumphii*, and its richness has been reported in *P. jucunda* (Vietnam: leaf oil 12.0%; stem oil 20.1%) [19], *P. thorelii* (Vietnam: leaf oil 10.5%; stem oil 6.9%) [19], and *P. suaveolens* (Cameroon: stem bark oil 8.5%) [20]. In addition, bicyclogermacrene was found predominantly in *P. sumatrana* and *P. rumphii* oils, and was also identified from *P. harmandii* (Vietnam: leaf oil 20.9%; stem oil 27.9%) [19], *P. thorelii* (Vietnam: leaf oil 6.3%) [19], and *P. michaelii* (Australia: leaf oil 4.0%) [21]. Meanwhile, monoterpenes sabinene,  $\alpha$ -copaene,  $\alpha$ -pinene, and myrcene have been reported as the most abundant components in the essential oils of *P. jucunda* [19], *P. longifolia* [22], *P. sessiliflora* [19], and *P. suaveolens* [23], respectively.

In this study, a total of 52 data set were used in the analysis that generated a PCA score plot having a total sample variance of 50.41% for the first two principal components (D1: 24.9% and D2: 25.5%). The plot showed distinctive separation of the essential oils into three clusters with *P. cauliflora* grouped in Cluster I, *P. sumatrana* in Cluster II, whereas *P. stenopetalla* and *P. rumphii* grouped in Cluster III. Comparison of the PCA score and loading plots allows identification of relationships between the oil



**Figure 1:** Principal component analysis (PCA) plot (A) and hierarchical cluster analysis (HCA) (B) dendrogram of four *Polyalthia* essential oils.



samples and the chemical composition variables. The loading plot (Figure 1A) indicated that *P. cauliflora* oil could be differentiated based on the presence of  $\delta$ -elemene and  $\beta$ -cubebene. On the other hand, *P. sumatrana* oil could be characterized by  $\alpha$ -cubebene,  $\beta$ -elemene, bicyclogermacrene, and cis-calamenene. Additionally,  $\gamma$ -elemene, germacrene D,  $\delta$ -cadinene,  $\alpha$ -cadinol, bicyclogermacrene, and t-murolol could be used to distinguish *P. stenopetalla* and *P. rumphii* oils. Thus, these chemical components could be utilized to characterize and differentiate the *P. sumatrana*, *P. stenopetalla*, *P. cauliflora*, and *P. rumphii* essential oils. To evaluate the accuracy of this classification, the cluster obtained was confirmed by HCA (Figure 1B). Similarly, the species of *Polyalthia* were also divided into three groups. The approach of PCA and HCA analysis is practical and efficient to identify essential oils obtained from different species or varieties; to authenticate oils from adulteration; and to determine quality of the oil based on different cultivation sites, harvesting time, processing method and biological activity [24].

## 4 Conclusion

The GC-FID and GC-MS analysis of the essential oils allowed us to identify bicyclogermacrene,  $\alpha$ -cadinol,  $\delta$ -elemene, and germacrene D as the major components from *P. sumatrana*, *P. stenopetalla*, *P. cauliflora*, and *P. rumphii*, respectively. Although the significant correlation between the multivariate analysis and essential oil components allows the identification of chemical similarity among *Polyalthia* species, there is a remarkable variability in oil composition that needs to be minimized with further genetic studies. The next step will be to evaluate the biological activities of the essential oils in order to valorize this species with a special ecological character. This study also provides valuable and useful information and indications for further exploring the potential nutraceutical and pharmaceutical applications of the genus *Polyalthia*.

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