

Discrimination of *Syzygium polyanthum* Cultivars (Wight) Walp Based on Essential oil Composition

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ABSTRACT

Myrtaceae has been considered as the richest species with the genus *Syzygium* having approximately 120 species in Malaysia. The aerial part of *Syzygium polyanthum* is largely consumed as *ulam* with the aim of treating diverse ailments and enhancing appetite in Peninsular Malaysia. The richness of the species in the niche has resulted in the taxonomic misidentification which caused a serious problem in biodiversity management and development of natural products. The study was aimed to discriminate the two cultivars of *S. Polyanthum* (Serai Kayu and Serai Kayu Hutan) based on the chemical composition of their essential oil. The volatile compounds were distilled from the aerial parts using Hydrodistillation. Up to 0.4% and 1.6% (v/w) were percentages of the oil yield obtained from Serai Kayu and Serai Kayu Hutan leaves, respectively. The GC/MS analysis revealed the presence of 40 and 29 compounds amounting to 70.29% and 95.15% in Serai Kayu and Serai Kayu Hutan, respectively. Alpha-Pinene (38.46%), Octanal (21.01%) and (E)-Methyl-cinnamate (7.21%) were the main constituents of Serai Kayu while in Serai Kayu Hutan Z-Methyl-cinnamate (39.18%), Terpinolane (23.63%) and Spathulenol (3.98%) were the major constituents. Compounds below 1% were considered to be minor constituents in both the species. The study documents the first chemical composition of the cultivar Serai Kayu Hutan. The analysis of the chemical content has added taxonomic information to the genus *Syzygium* and had provided the baseline information for natural product development.

Keywords: Discrimination, essential oil, serai kayu, serai kayu hutan, *Syzygium polyanthum*

INTRODUCTION

Therapeutic potentials of medicinal plants have make them as one of the only sources that has been in utilisation for ages by human which give birth to the present contemporary world modern medicine (Tohid et al., 2017). World Health Organisation (WHO) have reported no fewer of more than seventy percent of the world population relied on medicinal plants to meets up their primary health care.

Plants with medicinal value as a result of the presence of the bioactive components in the plant parts or the whole plants have made them to be the principle guide in curing and improving human health all over the globe. Plants are found to have abundance of secondary metabolites with promising biological activities, the metabolites they occur in different form of structures each with different or similar functions (Sanei-Dehkordi et al., 2016). Due to the availability of volatile compounds and essential oil in aromatic plants it makes them to be frequently utilised (Sanei et al., 2016; Tohidi et al., 2017). Recently it has been observed much utilisation of essential oil from plants sources in pharmaceutical industries, food industries, cosmetics and many more industries (Sanei et al., 2016). It has been documented there are not fewer than three hundred thousand essential oil that has been reported from plant origin of which more than three hundred were found to have application in industries like food and beverages company, medicinal application etc (Amalina et al., 2013; Govindarajan & Benelli, 2016). Information with regard to the chemical composition of plants with therapeutic value is required not only for utilisation in pharmaceutical industries, food companies but rather the information will provide the basic information needed in other to fully identified the plants with respect to the environmental factors, cultivar or different species (Dhouioui et al., 2016).

The science of assigning, naming and describing of plants species to a group or class has been considered as plant taxonomy (Govindaraghavan, et al., 2012; Zervakis et al., 2012). In order to understand the evolutionary relationship that exists among plants, Morpho-anatomical features are considered classical. In order to place the plants properly to a particular taxon, chemical identification also need. However, with regard to taxonomic identification of the plants species there is still wide disagreement in respect to the grouping of the plants (Zervakis et al., 2012; Khairudin et al., 2014).

Myrtaceae is a pan tropical family of trees and shrubs with nearly about 55,000 species, classified into 2 sub-family, seventeen tribes and 124 genera. They are mainly found in the large amount of number in Central America, South America, Australia and southern hemisphere (Retamales et al., 2015). More than 200 species of Myrtaceae have been reported in Asia (Padovan et al., 2014; Vasconcelos et al., 2017). Soh and Parnell (2015) reported *Syzygium* as the most diverse genus raging from shrubs to trees found in diverse habitat. Genus *Syzygium* is a flowering plant from the Myrtaceae family with more than 120 species across tropical countries (Retamales et al., 2015; Govindarajan & Benelli, 2016). The evolution of *Syzygium* started in southern Asia, Malaysia, Australia and to African countries (Soh and Parnell, 2015). High diversity of genus *Syzygium* with extreme morphological similarities was found in Southern Asia (Soh and Parnell, 2015).

Syzygium they are densely foliaceous and large ever green plants with thick bark and grayish brown utilised in woody industry (Alerico et al., 2016). Wood from *Syzygium* is durable, close grained and whitish (Soh & Parnell, 2011). They have leathery leaves, obovate elliptic or oblong ovate shape with six to twelve centimeters (Soh & Parnell, 2011; Retamales et al., 2015). There is wide variation in shape, presence of primary, secondary and tertiary vein, shining, smooth and the tips of the leaves are less acuminate and broad (Retamales et al., 2015). Most members of the genus *Syzygium*, their fruits are oblong ranging from 0.5 to 3.5 centimeters long which are black or dark purple, edible and flesh and almost all species bear seed within the fruits (Gamage et al., 2003; Hussin et al., 1992; Retamales & Scharaschkin, 2015). Fruits in this family are generally fresh or dry (Retamales & Scharaschkin, 2015).

Syzygium polyanthum is locally known as Serai Kayu, Serai Kayu Hutan, Salam, Daun and Kelat among the people of Peninsular Malaysia (Ahmad et al., 2017; Mahmoud et al., 2017). Whilst in Indonesia, it is named as Indonesian laurel or Bay leaf. *S. polyanthum* is a plant known for its medicinal value among the Malay ethnic group for curing diverse ailments such as Diabetes, Malaria, Postpartum, Hypertension, Diarrhea and Endometriosis (Mahmoud et al., 2017). Other than its medicinal value it is also consumed as *Ulam* which ad flavours to the food and enhance appetite (Rahim et al., 2018). People from peninsular Malaysia (Malay) are in the habit of taking *Ulam* (traditional vegetables) every day in their meal (Bachok et al., 2014). They have leathery leaves, obovate elliptic or oblong ovate shape with six to twelve centimeters (Soh & Parnell, 2011; Retamales et al., 2015). There is wide variation in shape, presence of primary, secondary and tertiary vein, shining, smooth and the tips of the leaves are less acuminate and broad (Retamales et al., 2015). The present study aimed to

discriminate *S. polyanthum* based on the chemical constituents of their essential oil. To further support the findings of morphology, micromorphology and anatomical studies of the Serai Kayu and Serai Kayu Hutan, to serve as a guide for standardization in natural product development.

MATERIALS AND METHODS

Plants material

Leaves of *S. polyanthum* (Serai Kayu and Serai Kayu Hutan) were collected in Besut Terengganu state, Peninsular Malaysia from the wild at the same time. The collected medicinal plants were identified in the field by comparison with the existing literature and fully confirmed by Botanist in the Universiti Sultan Zainal Abidin (UniSZA) after which the identified herbarium specimen was further taken to the University Kebangsaan Malaysia (UKM) and University of Malaya (UM) for further identification and finally deposited in the Unisza, UKM and UM herbariums respectively.

Essential oil distillation

Collected fresh leaves were washed gently in order to remove the stain in the plant leaves, arranged them accordingly and dried them under shade at room temperature, finally grind them to fine powder using blade mill. A total of 100 g of the leaves were immersed in water in a round bottom flask (2L) using Clevenger type apparatus. The Hydrodistillation was completed in 5hrs, obtained essential oil was dried using anhydrous sulphate, put in a dark bottle and stored at -4 °C till further analysis (Lucchesi et al., 2004; González et al., 2016).

Instrument and GC-MS (Gas chromatography Mass spectrometry) Conditions

The GC/MS was carried out using Agilent (Series MSD) gas chromatography 7890A/5975C coupled mass spectrometer Agilent Technologies (Malaysia). The compound mixtures were separate on Column: HP-5MS 30 m x 0.25 mm, 0.25 mm film thickness in a Temperature programme: Initially at 60 °C for 10 min, then to 230 °C for 1 min at 3°C/min with 1 min hold. The injector was at 245 °C and the flow rates of the carrier helium gas 1 mL/min. Volt 70 e V was used for the MS with the ion source and analyzer temperature at 260 °C (Rahim et al., 2018).

RESULTS AND DISCUSSION

Voucher numbers of the collected specimen were given in the deposited herbarium of Universiti Sultan Zainal Abidin (UniSZA), Universiti Kebangsaan Malaysia (UKM) and Universiti Malaya (UM) (Table 1)

Table 1: Voucher numbers of deposited specimen in respective Herbarium

S/N	Specimen	Herbarium No
1	<i>Syzygium polyanthum</i> (Serai Kayu)	UniSZA 00395
2	<i>Syzygium polyanthum</i> (Serai Kayu Hutan)	UniSZA 00396
5	<i>Syzygium polyanthum</i> (Serai Kayu)	UM-KLU49443
6	<i>Syzygium polyanthum</i> (Serai Kayu Hutan)	UM-KLU49444
7	<i>Syzygium polyanthum</i> (serai kayu)	UKMB 40352
8	<i>Syzygium polyanthum</i> (Serai Kayu Hutan)	UKMB 40353

The yields of the distilled oil from the leaves part of the Serai Kayu and Serai Kayu Hutan were expressed in (v/w) as 0.4% and 1.6%, on the basis of dry weight, respectively. The results revealed the aerial part of the Serai Kayu Hutan contained more essential oil when compared to the Serai Kayu. The abundance

of the essential oil in the leaves of Serai Kayu Hutan might be in connection with the abundance trichome in the leaves, from morpho-anatomical studies. This further validate the findings of Abdulrahman et al. (2018) Morpho-anatomical studies of *S. polyanthum* cultivar (Serai Kayu and Serai Kayu Hutan), reported the abundance of oil secretion glands and trichome anatomically in Serai Kayu Hutan leaves. Low yield of essential oil reported from the leaves of Serai Kayu is in agreement with Rahim et al. (2018) in their studies of phytochemical compounds derived from *S. polyanthum* leaves (Serai Kayu) where low yield of essential oil was reported. Also concurred with the findings of Hamad et al. (2017) where low yield was also observed from Serai kayu leaves 0.075 %.

Qualitatively (40) compounds were identified with the total composition of 70.29% from Serai Kayu with Alpha-Pinene (38.46%), Octanal (21.01%), (E)-Methyl-cinnamate (7.21%), Valencene (4.54%), limonene (1.98%), Alpha-Copaene (1.88%), Humulene epoxide II (1.68%), Beta-Salinene (1.57%), Decenal (1.38%), and (E)-Nerolidol (1.08%) as the major constituents (Fig. 1 and Table .2). While (29) compounds were identified with the total composition of 90.93% in Serai Kayu Hutan (Fig.2 and Table 2). Z-Methyl-cinnamate (39.18%), Terpinolane (23.63%) and Spathulenol (3.98%) are the major constituents are major constituents. The study completely disagree with Rahim et al. (2018) where the reported different compounds from the leaves of *S. polyanthum* (Serai Kayu) collected from Kelantan similarly disagree with Amalina et al. (2013) where they report alpha- pinene (30.88), Octanal (18.30) and alpha-caryophyllene (6.22) % respectively and Hamid et al. (2017) on their analysis of essential oil of *S. polyanthum* (Serai kayu) and *Syzygium aromaticum* from Indonesia where they report cis-4-decanal (43.489%) as the major constituents, follow by 1-decyl aldehyde (19.75) and Capryl aldehyde (14.09) % respectively. Therefore, the variation in Serai Kayu might be as results of the enviromental factors which strongly have effect on the yield and chemical composition of the essential oil. Compounds with less than 1% in both Serai Kayu and Serai Kayu Hutan are considered to be minor constituents (Table 2).

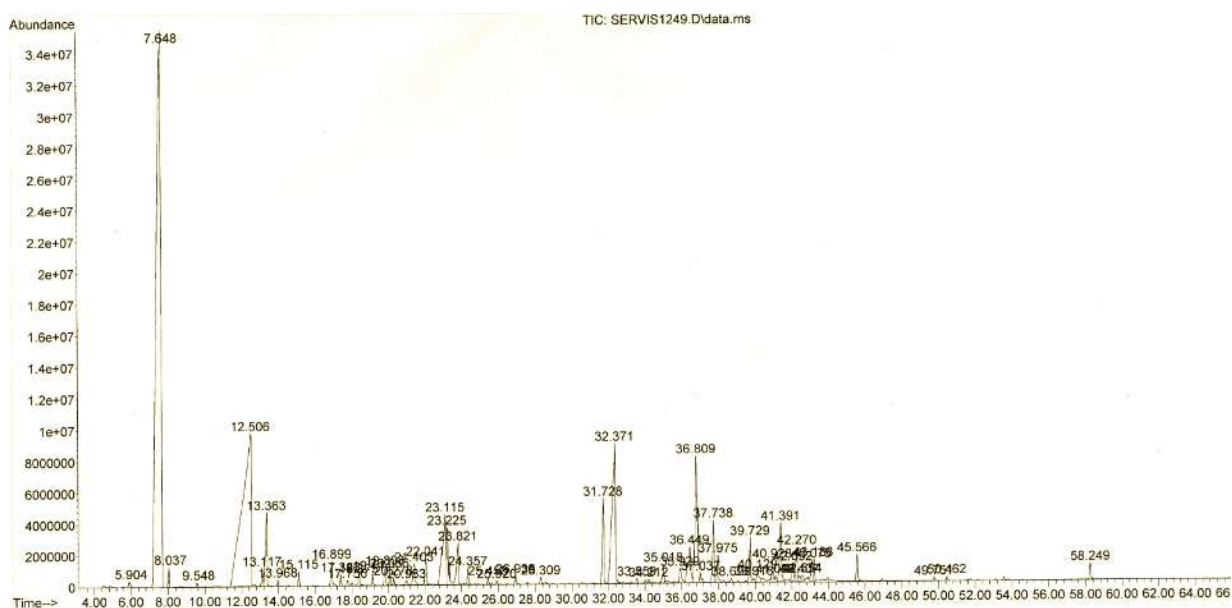


Fig. 1. Chromatogram of *Syzygium polyanthum* (Serai Kayu)

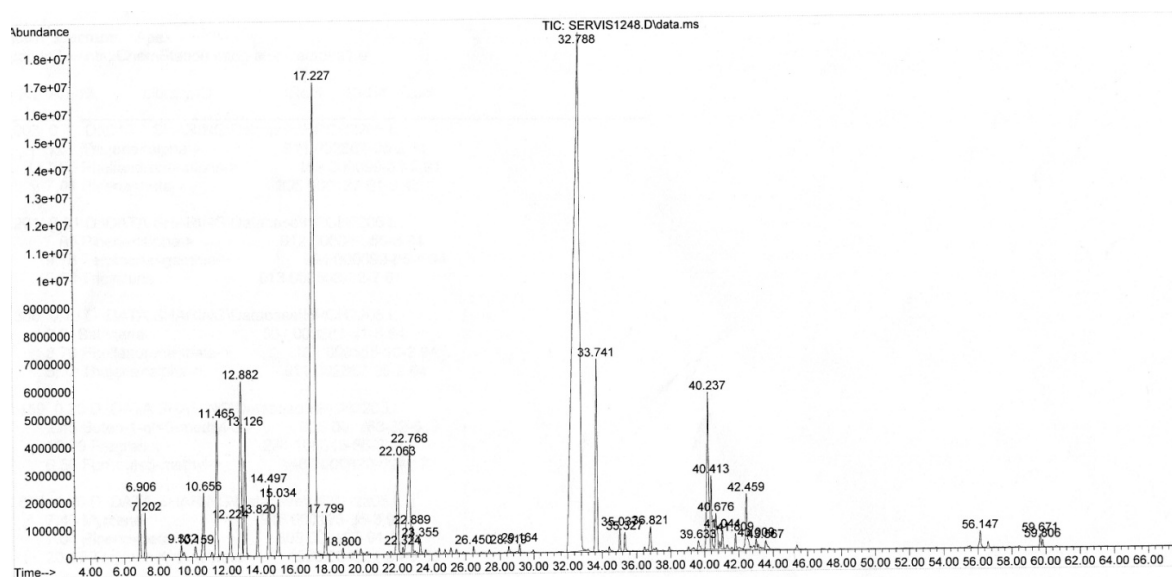


Fig. 2. Chromatogram of *Syzygium polyanthum* (Serai Kayu Hutan)

Table 2: Class composition of the Compounds in Serai Kayu and Serai Kayu Hutan

S/N	RT	Serai Kayu		RT	Serai Kayu Hutan	
		Compounds	Area (%)		Compound	Area (%)
1	5.904	Heptanal	0.17	6.906	Thujene alpha	0.73
2	7.648	Alpha-Pinene	38.46	7.202	Terpinene gamma	0.51
3	8.036	Camphene	0.31	9.330	Sabinene	0.16
4	9.547	Beta-Pinene	0.09	10.656	Myrcene	1.20
5	13.118	p-cymene	0.36	11.465	Phellandrene alpha	2.72
6	13.364	Limonene	1.98	12.225	Careen-delta-2-	0.63
7	13.970	Z-beta-ocimene	0.10	12.882	Cymene -ortho	4.53
8	15.114	Gamma-Terpinene	0.27	13.820	z-beta-ocimen	0.59
9	16.900	Terpinolane	0.59	14.497	e-beta-ocimen	1.20
10	17.386	Alpha-Pinene-oxide	0.22	15.034	Terpinene gamma	0.89
11	18.376	Endo-Fenchol	0.24	17.227	Terpinolane	23.63
12	19.114	Alpha-Campholenal	0.24	17.799	Linalool	0.65
13	19.807	Trans-Pinocarveol	0.44	18.799	Terpinene gamma	0.09
14	20.064	Camphor	0.27	22.061	Terpinen-4-ol	2.16
15	20.276	Camphene hydrate	0.16	22.324	Para-methyl acetophenone	0.08
16	20.985	Trans-Pinocamphone	0.12	26.450	Methyl citronellate	0.08
17	21.403	Borneol	0.72	26.450	E-methyl-cinnamate	0.10
18	22.038	Terpinen-4-ol	0.64	29.162	Eurcarvone	0.11
19	23.832	Decanal	1.31	32.790	Z-methyl-cinnamate	39.18
20	25.44	Neral	0.12	33.739	E-carryophyllene	4.19
21	31.725	Alpha-Copaene	1.88	35.038	Alpha-humulene	0.31
22	32.372	(E)-Methyl-cinnamate	7.20	35.327	Allo-aromadendrene	0.27
23	33.562	(E)-Caryophyllene	0.08	36.824	Bicyclogermacrene	0.46
24	35.021	Alpha-Humulene	0.42	39.633	Selina-3,7(11)-die	0.21
25	36.452	Beta-Salinene	1.57	40.240	Spathulenol	3.98
26	36.806	Valencene	4.54	40.411	Gamma-gurjunene	1.07
27	37.035	Alpha-Muurolene	0.27	40.675	Viridiflorol	0.75
28	37.739	7-epi-alpha-selinene	1.32	41.046	Rosifilol	0.29
29	37.974	Delta-Cadinene	0.56	43.009	Alpha-cardinol	0.16

30	38.700	Beta-Calacorene	0.12
31	39.730	(E)-Nerolidol	1.08
32	40.131	Spathulenol	0.39
33	41.390	Humulene epoxide II	1.68
34	42.054	Muurola-4,10(14)-dien-1 β -ol	0.44
35	42.408	Premnaspirodiene	0.15
36	42.551	Dauca-5,8-diene	0.18
37	43.078	selin-11-en-4-alpha-ol	0.49
38	43.186	Intermedeol	0.45
39	45.567	(2E,6E)-Farnesol	0.60
40	50.459	Benzyl salicylate	0.10

Relative concentration of the essential oil in Serai Kayu and Serai Kayu Hutan varied in terms of Monoterpene (53.8%), (79.16%) and Sesquiterpene hydrocarbons (16.22%) and (11.69%) (Table.3) respectively. The study is in agreement with Rahim et al. (2018) from genus *Syzygium*. The results of the study further supported the studies of morpho-anatomical features and chemical composition of *S. polyanthum* Cultivars (Serai Kayu and Serai Kayu Hutan) based on FTIR data sets. The cultivars were established to be two different cultivars but exhibit a strong relationship in terms of morphology, anatomical features and the finger prints present in the ethanolic and aqueous extract. In addition, Linalool compound was present in both Serai Kayu and Serai Kayu Hutan which is known compound with antidiabetic potential as it has been previously reported by Rahim et al.(2018). This also support the claim of traditional healers, that the plants are source of antidiabetic (Mahmoud et al. 2017). The study further supported the findings of Zhang et al. (2018) where they discriminate medicinal plants species from chinese known as daqingye from anatomy and chemical composition of the leaf. Further in agreements with Saulle et al. (2018) in taxonomic identification of *Eucalyptus saligna* from anatomy and volatile analysis. Aladdin et al. (2016) also used chemical composition to discriminate fully a commercialize medicinal plant in Malaysia known as *Marantodes pumilum*. The chemical content of both the two cultivars have completely discriminate the two plants to be entirely two different cultivars despite their relationship in the presence of high content of Monoterpene but also differ in terms of Sesquiterpene hydrocarbons (Table 3).

Table 3 Class composition of the compounds of Serai Kayu and Serai Kayu Hutan

Class composition	Serai Kayu	Serai Kayu Hutan
Monoterpene %	53.8	79.16
Sesquiterpene %	16.22	11.69
Others%	0.27	0.08
Total %	70.29	90.93

CONCLUSION

The study has analysed the chemical constituents of the *Syzygium polyanthum* cultivars (Serai Kayu and Serai Kayu Hutan) based on their essential oil composition. The volatile oil of the two cultivars was both rich in monoterpene hydrocarbons, though high constituent of sesquiterpene was reported in Serai Kayu Hutan. The study provides the first chemical composition on the essential contents of cultivar Serai Kayu Hutan. The findings further supported the variation between Serai Kayu and Serai Kayu Hutan in the Morpho-anatomical features and chemometric discrimination using multivariate analysis. The findings have added taxonomic information to the genus *Syzygium* which also provided the baseline information for natural product development.

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