DETERMINATION OF YIELD AND CHEMICAL COMPOSITION OF EUCALYPTUS OIL FROM DIFFERENT SPECIES AND LOCATIONS IN INDONESIA

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ABSTRACT: **There are many factors that influence the biosynthesis of eucalyptus oil. Determination of these factors are important to increase the productivity and quality of eucalyptus oil to create a sustainable eucalyptus-based bioindustry. Therefore, this study aims to explore and determine the yield and chemical composition of essential oil extracted from various species of eucalyptus in Indonesia. Three different species of eucalyptus from various locations in Indonesia were studied particularly *E. citriodora*, *E. urophylla*, and *E. viminalis*. The essential oil was extracted from the leaf using a steam–distillation technique. The yield of eucalyptus oil varied from 1.6% to 3.3% (w/w, dry weight) which depends on the species, plant growing location, and physiological age of leaf. The chemical composition was analyzed using a gas chromatography–mass spectrophotometry (GC-MS). The major components of eucalyptus oil were determined to be 1,8-cineole (18.2%–45.5%) and citronellal (69.27%–82.81%). The highest composition of 1,8-cineole was obtained from the eucalyptus oil extracted from *E. urophylla* whereas the highest composition of citronellal was obtained from eucalyptus oil extracted from *E. citriodora*.**

KEYWORDS: *Eucalyptus sp.: Essential oil; Yield; 1,8-cineole ; Citronellal.*

1. INTRODUCTION

Eucalyptus spp. belongs to the Myrtaceae family originating from Australia that was introduced into Indonesian archipelago around the Dutch colonial period. The plant is an evergreen tree and commonly used to produce various products; the leaf is used to produce essential oil; the stem is heavily used for pulp industry and furniture whereas the flower may be used as the source of nectar for honey production, (Zrira *et al.*, 1992 and Zrira & Benjilali, 1996).

One of the earliest usages of eucalyptus plant is for its essential oil that is extracted from the leaf. In general, essential oil may be utilized for various industries such as pharmaceutical, aromatherapy, chemicals, perfume, fragrances, and cosmetics. In eucalyptus oil market, the price is designated based on its component especially 1,8-cineole, citronellal, and piperitone. There are several species of eucalyptus that contain 1,8-cineole as the main component such as *E. globulus*, *E. polybractea*, *E. kochii*, *E. urophylla*, and *E. viminalis* while other species like *E. Citriodora* have citronellal as the main component. Eucalyptus oil with 1,8-cineole as the main component is used as a raw material in pharmaceutical industry for production of decongestants, aromatherapy oils, and disinfectant.

The yield and chemical composition of eucalyptus oil are influenced by several factors. Previous studies have shown that there were multifaceted factors between species or in-species that can be divided into external and internal factors. Several external factors such as altitude, soil nutrient, light irradiation, and climate are very strong factors. Meanwhile, internal factors such as tree age and physiological age of leaf (mature and juvenile) were identified as factors that affected yield and chemical composition greatly, (Cheng *et al.*, 2009; Coffi *et al.*, 2012; Cimanga *et al.*, 2002; Dagne *et al.*, 2000; De Oliveira *et al.*, 2008; Singh *et al.*, 2012; Su *et al.*, 2006 and Russo *et al.*, 2015).

Pretreatment and extraction methods of the essential oil are also crucial to the yield, productivity, and chemical composition of the eucalyptus oil. Previous studies have demonstrated that the most effective method in extracting essential oil is by performing a pretreatment that combines shade drying and size reduction prior to steam–distillation, (Ketaren, 2002). The pretreatment can help generate higher yield during the distillation process. However, prolonged drying time would result in oil loss due to evaporation and oxidation, (Ketaren, 2002). Therefore, controlled drying time and water content that result in optimum yield must be determined beforehand so that screening process can be solely focused on differentiating yield and chemical composition among various species and its growing environment. As such, further optimization can be carried out to produce a higher amount if the eucalyptus oil containing the desired chemical composition.

The aims of this study were to investigate the effect of different growing locations of *E. urophylla* and *E. citriodora* with respect to oil yield and chemical composition and the effect of leaf physiological age on essential oil yield from *E. viminalis* and its chemical composition. This study was conducted using leaf with an optimum water content for oil extraction. The analysis of microclimate and the physicochemical properties of soil were also conducted to support this study.

2. MATERIALS AND METHODS

2.1 Materials

Leaf samples of eucalyptus species were gathered from various places. Leaf of *E. citriodora* was obtained from Juanda botanical forest park (Tahura Juanda), Bandung City, West Java and Manoko research plantation (KP Manoko), Lembang, West Bandung Regency, West Java. Leaf samples of *E. urophylla* were taken from Cisanti research plantation (KP Cisanti), Pangalengan, Bandung Regency, West Java and ITB Jatinangor arboretum, Sumedang Regency, West Java. The selections were based on the difference of altitude and climate between the two areas. Leaf samples of *E. viminalis* with different physiological age were obtained from Dieng plateau, Central Java from December 2015 to July 2016. Soil samples were taken 60 cm below ground surface of each tree for further analysis. Sodium sulphate (Na_2SO_4) anhydrate and whatman filter paper were obtained from Brataco Chemicals.

2.2 Methods

2.2.1 Pretreatment of samples

Leaf samples were harvested and the total water content was measured by an HB43-S Halogen Moisture Analyzer Mettler Toledo or drying in an oven at a temperature of 105°C overnight until a constant weight was achieved. Leaf samples were then dried at room temperature without exposure to direct sunlight with a relative humidity of 70%–90% in a packed box of 200 g for each sample. Water content (% wt) of the samples were measured daily for five days using the following equation:

$$WC\ (wt\ \%) = \frac{M_{initial}\,(g) - M_{final}(g)}{M_{initial}\,(g)} \times 100\% \qquad (1)$$

where WC is the water content (wt %) and m is the mass of the sample (g).

2.2.2 Extraction and yield of essential oils

Leaf samples at optimum water content were chopped until the size were about 1.5 × 1.5 cm. Leaf samples were distilled with steam distillation at a boiling temperature of 96.5°C at 0.92 atm pressure and 780 m above sea level for 4 hr, with the condenser temperature was set at room temperature (25°C). The distillate collected had two phases where the lighter phase was eucalyptus oil. The lighter phase was then purified with Na_2SO_4 anhydrous. The purified essential oil was then weighed to determine the yield. The essential oil yield (Y) was quantified with this expression:

$$Y\ (wt\ \%) = \frac{M_{essential\ oil}\,(g)}{M_{sample}(g).(100\% - WC_{(wt\%)})} \times 100\% \qquad (2)$$

Subsequently, the composition of the chemical compounds in the essential oil was analysed using gas chromatography–mass spectrometry (GC-MS).

2.2.3 Analysis of chemical composition

Chemical composition of the essential oil was analysed using a GC-MS Ultra GCMS-QP 2010 (Shimadzu) with a capillary column HP-1 (cross-linked methyl silicone gum) measuring 50 m × 0.32 mm × 0.52 µm.

For the analysis of essential oil extracted from *E. urophylla* and *E. viminalis*, helium was used as the carrier gas with flow rate of 1.3 mL/min. Injector and interface temperatures were 250 and 280°C with a split ratio of 100:1 at 86.1 kPa (12.48 psi). The initial temperature was 50°C and maintained for 10 mins, then increased up to 280°C at a rate of increment that was programmed.

For the analysis of essential oil extracted from *E. citriodora*, helium was used as the carrier gas with a split ratio of 50:1 and a linear speed of 40.8 cm/s. The initial temperature was 40°C, which was maintained for 4 mins, then the temperature was raised steadily with 4°C/min increment rate until 220°C which was maintained for 15 mins. The temperature of source mass spectrometer was 200°C, the interphase and injector temperatures were 220°C. Both GC-MS compound identification were done using the NIST library.

3. RESULTS AND DISCUSSION

3.1 The Yield of Essential Oil

The characteristics of growing locations for *E. urophylla* and *E. citriodora* are summarized in Table 1 while fresh leaf water content for *E. urophylla*, *E. citriodora*, and *E. viminalis* are tabulated in Table 2. Soil structure and properties were analyzed at Soil Fertility and Plant Nutrition Lab at Padjajaran University. Based on Tables 1 and 2, the range of fresh leaf water content among different locations and the peak of optimum water content between species varied greatly.

The water content of the fresh leaf was measured directly upon receival. This is because the level of water content at the moment was the highest and reflected the original state of water holding capacity of the leaf. The wide range of fresh leaf water content is probably caused by the weather condition especially rain and daily average temperature prior to sample collection, (Figueiredo-Lima *et al.*, 2018). It is especially clear when comparing the leaf fresh water content of *E. citriodora* from two different locations. At Tahura Juanda, the leaf was harvested during rainy season which resulted in water content with larger deviations in comparison to the leaf

Table 1. Environmental characteristics at Tahura, KP Manoko, Jatinangor, Pangalengan.

Species *Eucalyptus*		***E. citriodora***		***E. urophylla***	
		Tahura	**KP Manoko**	**Jatinangor**	**Pangalengan**
Altitude (masl)		±825	±1.210	± 735	± 1565
Humidity (%)		70-80	82.7	68-79	70-85
Average temperatures (^{o}C)		22-24	15-25	23-30	12-24
Rainfall (mm/year)		2.500-3.000	3,047	1800 4000	1.500 – 4.000
Soil texture		Sandy-silt loam	Silty Loam	Clay	Loam
Composition of the soil (%)	Sandy	14	18	15	36
	Silt	51	55	40	46
	Clay	35	27	45	18
Soil properties	C-organic (%)	7.48	8.53	2.86	0.02
	N-total (%)	0.29	0.56	0.50	0.01
	P-total (mg/100g)	93.14	172.9	43.77	141.13
	K-total (mg/100g)	79.87	55.14	49.62	19.31

harvested at Manoko research plantation during a dry season (Table 2). As for the leaf water content of *E. urophylla*, it seems that temperature is playing a large role. From Table 1, KP Cisanti, Pangalengan (±1565 masl) has an altitude that is almost twice of the altitude at Jatinangor (±735 masl) and the general climate is much cooler. This condition promotes higher water content through balancing transpiration rates between leaf and ambient air since lower ambient moisture pushing water gradient leaf-air to be steeper, (Gale, 2004).

Upon receival, the leaf water content was decreasing during the shade drying process until it reached an equilibrium with the atmospheric water vapor pressure, (Wei *et al*., 2018). Within this range of moisture content, the yield of essential oil increased up to an optimum moisture content for the extraction of oil using a steam distillation technique. Beyond the optimum moisture content, the essential oil yield decreased due to the volatility and thermolabile properties of the oil. The relationship between water content and oil yield are presented in Figures 1 and 2.

Table 2: Total leaf water content of *E. citriodora*, *E. urophylla*, and *E. viminalis.*

Total leaf water content (wt %)	
E. citriodora	
Tahura	42,69±3,69
KP Manoko	40,79±0,35
E. urophylla	
Jatinangor	58,29±1,80
Pangalengan	65,25±0,97
E.viminalis	
Juvenile leaf	57,53±2,93
Mature leaf	53,32±4,11

Figures 1 and 2 show that every species investigated in this study has a varied optimum water content. The optimum yield of *E. citriodora* was 3.3 wt % at both locations with the water content of the leaf around 14 and 20 wt % for KP Manoko and Tahura Juanda, respectively. For *E. urophylla*, the optimum yield reached at 1.75 and 1.93 wt % with the water content for both locations around 32 wt %. While for *E. viminalis,* it was found that juvenile leaf generated higher oil yield (3.25 wt %) compared to the mature leaf (2.25 wt %) at water content around 35.45 and 24.80 wt % for juvenile and mature, respectively. Noted that moisture content was measured daily and represented through dots in the figure.

Based on the comparison of oil yield and its geographical location and environment, another trend appeared. This trend is the tendency of higher altitude plant to produce higher oil yield compared to its counterpart from the lower altitude. This trend is noticeable for *E. urophylla* while for *E. citriodora* the differences in yield is not significant enough. Similar trend has also been reported in previous studies on the extraction of essential oil from eucalyptus species, (Singh *et al.*, 2012; Su *et al.*, 2006 and Figueiredo-Lima *et al*., 2018).

A previous study has reported that the oil yield extracted from *E. urophylla* that was planted in Wetar Island showed that the highest oil yield came from the plants cultivated at a higher altitude (550 m) compared to the other plants from a lower altitude (350 m). This study also compared the effect of altitude for *E. urophylla* that was planted in Queensland, Australia which resulted in the similar conclusion. The study concluded that the highest yield was obtained from the plant at an altitude of 450 m and the lowest came from an altitude of 30 m, (Figueiredo-Lima *et al*., 2018).

The trend is possibly due to the physiological adaptation for plants that are cultivated at high altitude environments. This adaptation includes smaller leaf, stomata, or slower growth rate. It is also understandable that higher altitude locations receive a higher irradiation intensity especially UV rays. These adaptations and harsher nature of the location create stress

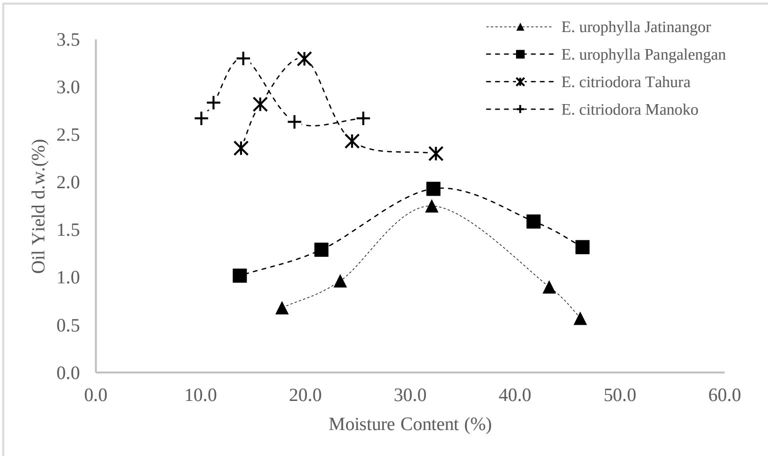

Figure 1. The influence of leaf moisture content of *E. urophylla* and *E. citriodora* towards oil yield.

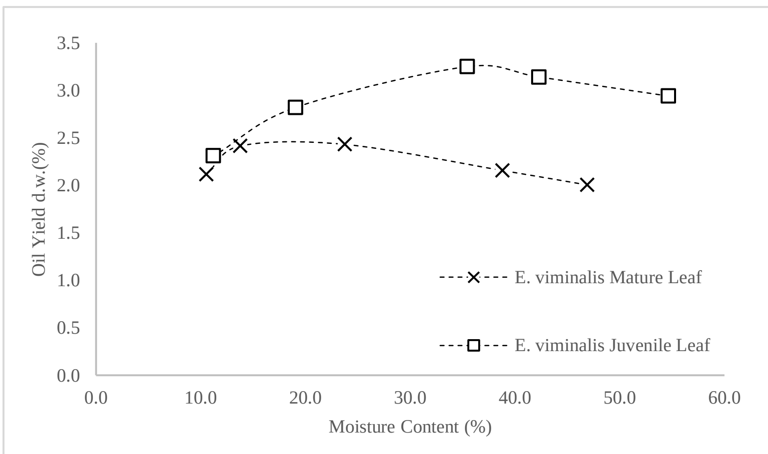

Figure 2. The influence of moisture content of mature and juvenile leaf of *E. viminalis* towards oil yield.

for the plants. Hence, the stress is one of the possible reasons of higher accumulation of secondary metabolite including essential oil, (Yang *et al.*, 2018).

Another interesting finding that has been summed up beforehand is that *E. viminalis* oil yield for juvenile leaf was greater than the mature leaf (Figure 2). This finding supported the results reported in other studies for *E. viminalis* and *E. Globulus*, (Li *et al.*, 1996 and Russo *et al.*, 2015). The possible explanation for a higher oil accumulation in juvenile leaf is due to the complex internal and external factors intertwined together.

The complexity of internal and external factors exists as an effect from the development of biosynthesis pathway, especially the increasing rate of catabolism and decreasing rate of terpenes production in the leaf during maturity, (Mihaliak & Lincoln, 1989). Another internal factor is the leaf growth in general. Leaf growth causes the leaf surface area to expand and this is inversely correlated with oil yield through the distribution of oil glands, (King *et al.*, 2006). These internal factors coupled with external factors especially location at ambient temperature and air moisture create a profound change in transpiration and evaporation ratex that accelerate the flow of photosynthesis product, (Taiz & Zeiger, 2002). The larger the surface area of leaf, which is the signs of leaf maturity creates a larger interface that promotes oil loss from the leaf, (Mihaliak & Lincoln, 1989).

Another factor that also contributes to the oil yield is the effect of medium growth towards the accumulation of oil in the leaf. Generally, nutrient content and water retention capacity of the soil are a good confirmation for proper growth of the plant, (Yang *et al.*, 2018). From Table 1, it can be seen that the soil properties and characteristic between locations were quite different from one another such as altitude, soil texture, composition of the soil, and soil properties. Nevertheless, the soil may not be the sole contributing factor that caused the difference in yield of essential from both locations, (Wei *et al.*, 2018).

3.2 Chemical Composition of Essential Oil from *Eucalyptus sp.*

The chemical composition of the essential oil from *Eucalyptus sp*. was analyzed using GC-MS. *E. urophylla* and *E. viminalis* contained 1.8-cineole as their main composition while for *E. citriodora* is citronellal. Other compounds that constituted eucalyptus oil from the three species are shown in Table 3.

Table 3 shows that there was a strong correlation for different locations among the species of eucalyptus towards the chemical composition. For instance, the eucalyptus oil from *E. urophylla* contained 45.47% 1,8-cineole for the sample taken from Jatinangor whereas the sample from Pengalengan contained only 18.19% 1,8-cineole.

The composition of citronellal in the eucalyptus oil obtained from *E. citriodora* planted at Tahura Juanda was higher (82.81%) compared to the composition of citronellal in the eucalyptus oil obtained from *E. citriodora* planted at

Table 3. Composition of eucalyptus oil extracted from *E. citriodora*, *E. urophylla*, and *E. Viminalis.*

Chemical Compound Name	***E. citriodora***		***E. urophylla***		***E. viminalis***	
	Percentage (%)					
	Tahura	**KP Manoko**	**Pangalengan**	**Jatinangor**	**Juvenile leaf**	**Mature leaf**
α-Pinene	0.05	nd	15.82	8.56	17.53	15.33
β-Pinene	nd	0.69	nd	nd	nd	nd
p-cymene	nd	nd	18.44	0.75	nd	nd
β-Myrcene	0.08	nd	nd	nd	nd	nd
1,8-Cineole	0.12	Nd	18.19	45.47	37.39	42.33
Isopulegol	3.75	0.61	nd	nd	nd	nd
Citronellal	82.81	69.27	nd	nd	nd	nd
β-Citronellol	nd	10.06	nd	nd	nd	nd
cis-Jasmone	0.22	nd	nd	nd	nd	nd
1-borneol	nd	nd	3.72	0.17	nd	nd
1-Phellandrene	nd	nd	nd	nd	8.07	7.08
dl-limomene	nd	nd	nd	5.54	nd	nd
veridiflorol	nd	nd	nd	3.75	11.44	13.77
Caryophyllene	nd	8.18	nd	nd	nd	nd
trans-Caryophyllene	2.89	nd	nd	nd	nd	nd
α-Humulene	nd	0.58	nd	nd	nd	nd
α-Gurjunene	nd	nd	nd	nd	2.54	2.43
β-Gurjunene	nd	nd	nd	nd	5.87	nd
Germacrene-D	nd	0.39	nd	nd	nd	nd
Bicyclogermacrene	0.43	0.9	nd	nd	nd	nd
other compounds	9.65	9.32	43.83	35.76	17.16	19.06

Note: nd: not detected, the compound is a trace (below 0,01%)

Manoko (69.27%). The different amount between locations may be caused by multiple factors that include internal and external factors. These factors are similar with the factors that affected oil accumulation in eucalyptus species. Internal factors that affected the chemical composition is the nature of oil biosynthesis in the plant. Each species has a slightly different biosynthesis matrix pathway that is governed by gene expressions. Gene expressions have a template that was inherited through generations. The template itself has some leeway to counteract outside influence to reach a satisfying equilibrium. The combinations of external factors affected internal mechanism of chemical biosynthesis through the interaction of gene expression that results in various compounds to be synthesized, (Rahim et al., 2014).

In this study, it can be inferred that internal factors that affected the chemical composition is the nature of oil biosynthesis in the plant. Each species has a slightly different biosynthesis matrix pathway that is governed by gene expressions. Gene expressions have a template that was inherited through generations. The template itself has some leeway to counteract outside influence to reach a satisfying equilibrium. The combinations of external factors affected internal mechanism of chemical biosynthesis through the interaction of gene expression that results in various compounds to be synthesized. Hence, climate and soil nutrients have a tremendous impact on chemical biosynthesis. In conjunction with data shown in Table 2, it is likely that phosphate as one of the soil nutrients plays an important role in changing certain chemical compound proportions because potassium diversifies compound biosynthesis. Apart from that, external conditions also affected the regulation mechanism itself.

The most glaring external factor is daily temperature. It was established that a higher ambient temperature promotes monoterpenes synthase, (Delfine *et al.*, 2000). This enzyme is responsible for the increase proportion of certain monoterpenes like citronellal and 1,8-cineole compared to other components. A higher ambient temperature coupled by soil nutrients and water availability creates chemical composition that make up the data as shown in Table 3.

Another finding in this study is the chemical composition make up between juvenile and mature leaf was not significantly different. A little detail that can be observed is that the mature leaf has higher 1,8-cineole (42.33%) compared to the juvenile (37.39%). The possible explanation for this situation is that 1,8-cineole accumulates in the leaf better than other compounds available. Another possible explanations rests on the age of oil glands. Oil glands is known to influence activity of terpenes synthase in the secretory cells, (King *et al.*, 2006).

4. CONCLUSION

The optimum essential oil and moisture content for various *Eucalyptus sp.* was investigated. An optimum essential oil yield of 3.3 wt % from *E. citriodora* was obtained when the leaf was extracted at a moisture content of 14 wt % for sample from KP Manoko and 20 wt % for sample from Tahura Juanda. For *E. urophylla*, an optimum yield of 1.75 wt % was obtained for sample from Jatinangor whereas for sample from Pengalengan, the optimum yield was 1.93 wt %. The optimum moisture content of the leaf sample from both locations was approximately 32 wt %. As for *E. viminalis*, juvenile leaf had a higher optimum yield of 3.25 wt % (moisture content of 35.45 wt %) compared to the mature leaf (2.25 wt % at a moisture content of 24.8 wt %). The chemical composition of the essential oil from the various species had also been determined. Essential oil extracted from the leaves of *E. urophylla* and *E. viminalis* primarily consisted of 1,8-cineole (18.19%–45.47%) while *E. citriodora* contained citronellal as the major component (69.27%–82.81%). Future studies on the exploration of gene expression for the biosynthesis of eucalyptus oil is important for a high amount and quality of eucalyptus oil.

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