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Essential oil production of *Murraya paniculata* (L.) Jack at different harvest times

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Abstract: *Murraya paniculata* (L.) Jack has a fragrant flower, from which the fragrance is due to the essential oil. The study aimed to investigate the production of essential oil and its chemical compounds at different harvest times. The research was conducted at an organic experimental farm, Bogor Agricultural University, Bogor, Indonesia (6°30'-6°45' S, 106°30'-106°45' E) from October 2016 to February 2017 using randomized complete block design. The experiment consisted of one factor, namely the harvest times, comprised of harvest at 05.00-07.00 and 07.00-09.00 a.m. *M. paniculata* flowers were collected at three different flower ages, comprised of two days before anthesis, one day before anthesis and the day of anthesis (blooming). The different flower ages indicated by the flower size. Ethanol extraction method was used to extract the essential oil of the flowers from different harvesting times and then chemical compounds were analyzed by Gas Chromatography-Mass Spectrometry. The result showed that flower number and weight were not affected by harvesting times. The flower collected on the day of anthesis had the highest flower number and weight. Harvesting flowers at anthesis can be done at 05.00-09.00 a.m. The highest quantity and quality of essential oils were obtained by harvesting the flowers at anthesis. β-methylesculetin and murralongin were the primary compounds in *M. paniculata* flowers that harvested at 05.00-09.00 a.m.

1. Introduction

Murraya paniculata (L.) Jack well known as orange jessamine is an ornamental plant and belongs to family Rutaceae (Shah *et al*., 2014), it has white flowers with sweet fragrance (Gilman, 1999). The plants are native to Southeastern Asia, i.e. Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia, Malaysia, and Philippine (Dosoky *et al*., 2016). *M. paniculata* has been used in traditional medicine because the plant has anti-amnesic, anti-inflammatory, anti-diabetic, anti-fungal, anti-bacterial, anti-helminthic, anti-cancer, and anti-oxidative properties (Sharma and Arora, 2015). Beside as a source for perfumery, *M. paniculata* is also used as a source of flavors (El-Sakhawy *et al*., 1998) because the flowers are highly aromatic and contain sufficient amount of essential oil (Naseem *et al*., 2015). Plants essential oils are aromatic components that composed

of different chemical compounds (Younis *et al*., 2011).

Different compositions of chemical compound of *M. paniculata* essential oils have been studied. Rout *et al*. (2007) found that pentane extraction was the best method to obtain the highest amount of essential oil of *M. paniculata* flowers in India. This study also found that manool and (*E*)-nerolidol were the major component of essential oil (Rout *et al*., 2007). The chemical compounds of *M. paniculata* flowers can also be extracted with liquid CO_2 (Rout *et al*., 2010). Different compounds from *M. exotica* flowers were found, namely (*E,E,E*)-α-springene, (*E*)-nerolidol, (*E,E*)-α-farnesene, methyl palmitate and germacrene B (Raina *et al*., 2006).

The variation of chemical composition in the essential oil from *Murraya* flowers is affected by the place where the plants are planted (El-Sakhawy *et al*., 1998) and harvesting time. Concerning the latter, harvesting time may influence the quantity and quality of essential oils of *M. paniculata* flowers; this has been reported in four *Rosa* cultivars (Younis *et al*., 2009) and *Jasminum sambac* flowers (Younis *et al*., 2011). Younis *et al*. (2011) reported that the best time to collect *J. sambac* flowers was in the morning before sunrise because highly volatile of jasmine oil. Therefore, the purpose of this research was to investigate the effect of harvest time on the production and chemical compounds of *M. paniculata* essential oil.

2. Materials and Methods

Plant material

The field experiment was conducted from October 2016 to February 2017. The experiment used 30 (62 month-old) plants that were planted in 1 m x 1 m on latosol soil, at the organic experimental farm, Bogor Agricultural University, Bogor, Indonesia (6°30'-6°45' S, 106°30'-106°45' E) at 250 m above sea level. A voucher specimen was deposit at The Herbarium Bogoriense, Bogor, Indonesia. Type-A climate based on Schmidt-Ferguson with the average monthly rainfall, temperature and humidity of 305 mm, 26°C and 85%, respectively (MCGA, 2017). Before treatment, each plant was fertilized with 3.0 kg rice-hull ash, followed Eliazar and Aziz (2015).

Experimental design

The experiment was arranged in randomized complete block design, with single factor (Petersen, 1994) with harvest times as treatments, comprised of harvest at 05.00-07.00 and at 07.00-09.00 a.m. The flowers were collected at three different ages, comprised of two days before anthesis, one day before anthesis, and at the day of anthesis. The two ages of flower before anthesis were indicated by the size of flower buds which have been observed in the preliminary study. The anthesis of the flower bud with 1.00±0.06 cm length and 0.48±0.06 cm width will occur two days later; while flower bud with 1.16±0.06 cm length and 0.56±0.04 cm width will occur in the next day. The observations include developmental stages, number, fresh weight, the content of essential oils and chemical compounds of *M. paniculata* flowers. Data were analyzed using t-student with α= 5% (Petersen, 1994).

Essential oil extraction

The analysis of essential oils was conducted at Tropical Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia. All harvested flowers (from different harvesting times) were extracted with solvent extraction method of ethanol. The flowers were immersed in ethanol until all samples were submerged, for two days. This mixture was filtered, then, the extract was separated from the solvent by rotary evaporator with a temperature of 40°C. The oil yield percentage was calculated from the weight of extract (g) divided by weight of fresh flower (g).

Gas chromatography-mass spectrometry analysis

Chemical compounds of essential oils were analyzed at Health Laboratory of DKI Jakarta Province. The essential oils were injected into Gas Chromatography-Mass Spectrometry (GCMS). An Agilent Technologies 7890 Gas Chromatography instrument with an Auto Sampler and 5975 Mass Selective Detector and Chemstation Data System, equipped with a 30 m x 0.25 mm HP Ultra 2 capillary column with 0.25 µm film thickness. Helium was used as the carrier gas. The initial temperature was programmed at 80°C and then increased at the rate of 3°Cmin^{-1} to 150°C held for 1 min and finally raised to 280°C at the rate of 20°C min^{-1} held for 26 min. The injector and interface temperatures were 250°C and 280°C, respectively. The ionization voltage was 70eV and a sample injection volume 5 µL. The individual peaks were identified by retention times, compared with those of compounds in Health Laboratory of DKI Jakarta Province database. The percentage of each compound detected from samples was calculated according to the area of the chromatographic peaks.

3. Results

The results showed that the average of flower number (regardless the flower ages) harvested at 05.00-07.00 AM was not significantly different to those harvesting at 07.00-09.00 a.m. (Table 1). At both harvest times, it was found the following order of flower number based on flower stages: flowers at anthesis, flowers that would bloom two days later, and the least was flowers that would bloom in the next day. The differences in flower number between flower stages were not always significant. The difference of flower number between harvesting time was 8.96%.

Table 1 - Number of orange jessamine flowers at different harvesting times

Harvesting time	Average of flower number/plant	P-value	Percentage of flower number (%)
At 05.00-07.00 AM, flower ages	29.40 (k)		
2 days before anthesis	24.67	x: 0.3349 NS	27.97
1 day before anthesis	14.07	y: 0.1742 NS	15.95
At anthesis	49.47	z: 0.0397 *	56.08
At 07.00-09.00 AM, flower ages	31.11 (k)		
2 days before anthesis	35.53	x: 0.0150 *	38.82
1 day before anthesis	12.87	y: 0.5056 NS	14.06
At anthesis	44.93	z: 0.0282 *	47.12

If P-value>α; α= 0.05, then means between the treatments were statistically equal; (k) = average of flower number at three flower age criteria; x= comparison between the flower 2 days before anthesis with 1 day before anthesis; y= comparison between the flower 2 days before anthesis with anthesis; z= comparison between the flower 1 day before anthesis with anthesis.

The average flower weight (regardless the flower stages) of *M. paniculata* was not significantly different between the times of harvesting (Table 2). A similar trend as of flower number was also found in flower weight based on flower stages. The highest flower weight was blooming flowers (flowers at anthesis), and the lowest was flowers at the stage of one day before anthesis. The difference in flower weight between harvesting time was 16.59%.

Table 2 - Weight of orange jessamine flowers at different harvesting times

Harvesting time	Average of flower weight (g/plant)	P-Value	Percentage of flower weight (%)
At 05.00-07.00 AM, flower ages	3.43 (k)		
2 days before anthesis	2.05	x: 0.4572 NS	19.92
1 day before anthesis	1.34	y: 0.0884 NS	13.21
At anthesis	6.88	z: 0.0355 *	66.86
At 07.00-09.00 AM, flower ages	4.72 (k)		
2 days before anthesis	5.70	x: 0.0819 NS	40.35
1 day before anthesis	1.33	y: 0.4398 NS	9.38
At anthesis	7.13	z: 0.0029 **	50.27

If P-value>α; α= 0.05, then means between the treatments were statistically equal; k: average of flower number at three flower age criteria; x: comparison between the flower 2 days before anthesis with 1 day before anthesis; y: comparison between the flower 2 days before anthesis with anthesis; z: comparison between the flower 1 day before anthesis with anthesis.

The extraction of *M. paniculata* flowers at different harvesting times with ethanol resulted in a yellowish-brown solution called concrete. These results were in line with that of Paibon *et al*. (2011), that reported how the extraction of *J. Sambac* flowers with ethanol produced a solution of yellowish brown to reddish. On the other hand, *M. paniculata* flowers that extracted with pentane produced a deep yellow waxy residue (Rout *et al*., 2007).

There was an indication that the essential oil percentage between harvest times was different. The percentage of essential oils from flowers harvested at 07.00-09.00 was higher than that at 05.00-07.00 a.m., regardless the flower stages, the difference was 0.38% (Table 3). Comparing among flower stages, it was found that anthesis flowers harvested at 05.00-07.00 AM had the highest percentage of essential oils. On the other hand, the highest percentage of essential oils at 07.00-09.00 AM was obtained from flowers at the stage of one day before anthesis. From this calculation, the production of essential oils from flowers harvested at 07.00-09.00 was higher than that from 05.00-07.00 AM. Based on flower stages, blooming flowers (at anthesis stage) produced the highest amount of essential oils at both harvesting times, this related to the highest fresh flower weight.

Table 3 - The percentage and production of essential oil of orange jessamine flowers at different harvesting times

Treatment	Essential oil (%)	Production of essential oil (g/g fresh flower) *
At 05.00-07.00 AM, flower ages	3.13 (k)	4.83
2 days before anthesis	2.06	0.63
1 day before anthesis	3.40	0.69
At anthesis	3.94	4.07
At 07.00-09.00 AM, flower ages	3.51 (k)	7.47
2 days before anthesis	3.49	3.00
1 day before anthesis	3.80	0.76
At anthesis	3.25	3.47

Data were not analyzed statistically;
(k) = average of essential oils at three flower age criteria;
* = Production of essential oils based on the weight of the harvested flowers.

The analysis of chemical compounds showed 41 types that were contained in *M. paniculata* flowers at different harvesting times. The highest number of chemical compound types was found in flowers at anthesis when they were harvested at 07.00-09.00 AM. This result was in line with Younis *et al*. (2011), where *J. Sambac* flowers harvested at anthesis had

more chemical compounds than those in flower bud. The analysis on *M. paniculata* flowers revealed the presence of coumarins, esters, fatty acids, phenolics, triterpenes, sesquiterpenes and other compounds (Table 4). Coumarin was the most common compound found in all treatments. Harvesting time at 05.00-07.00 a.m. gave the highest number of coumarins, esters, fatty acids, and sesquiterpenes.

(Table 4). The percentage of triterpenes was higher than sesquiterpenes, this finding was different from the previous study which showed that sesquiterpenes were the main compound in essential oils. The current study showed that sesquiterpenes derivatives found in the essential oils of *M. paniculata* flowers and found at both harvesting times were α-zingiberene, α-bergamotene, and caryophyllene

Table 4 - Chemical compounds of orange jessamine essential oils at different harvesting times

Treatment	% Peak area						
	Coumarin	Ester	Fatty acid	Phenolic	Triterpene	Sesquiterpene	Other compound
At 05.00-07.00 am, flower ages	71.29 k	1.80 k	9.12 k	4.34 k	3.27 k	0.77 k	9.42 k
2 days before anthesis	69.68	3.16	12.39	5.52	1.55	0.47	7.24
1 day before anthesis	74.33	1.41	10.09	4.43	1.33	0.55	7.87
At anthesis	69.85	0.83	4.89	3.06	6.93	1.30	13.14
At 07.00-09.00 am, flower ages	68.07 k	1.60 k	10.40 k	5.09 k	1.93 k	0.72 k	12.20 k
2 days before anthesis	66.98	2.15	11.91	6.20	1.03	0.41	11.33
1 day before anthesis	70.52	1.21	5.87	5.13	1.21	0.71	15.34
At anthesis	66.72	1.44	13.41	3.94	3.55	1.04	9.92

Data were not analyzed statistically; k= average of chemical compounds at three flower age criteria.

Coumarin was the highest amount of bioactive compound found in *M. paniculata* essential oils, (Table 4) and the dominant compounds in the coumarin group were β-methylesculetin and murralongin (Table 5). The amount of β-methylesculetin from flowers harvested at 07.00-09.00 AM was higher than those from 05.00-07.00 a.m. Different flower stages had a different dominant compound. Harvesting flowers at the stage of one day before anthesis gave the highest percentage of β-methylesculetin at both harvesting times, but harvesting at anthesis delivered the highest percentage of murralongin.

The analysis of chemical compounds showed that terpenoid groups found in the essential oil of *M. paniculata* flowers were triterpenes and sesquiterpenes

Table 5 - Coumarins compounds of orange jessamine essential oils at different harvesting times

Treatment	% Peak area	
	β-methylesculetin	Murralongin
At 05.00-07.00 am, flower ages	60.65 (k)	7.36 (k)
2 days before anthesis	59.46	5.36
1 day before anthesis	61.59	7.76
At anthesis	60.89	8.96
At 07.00-09.00 am, flower ages	62.07 (k)	5.71 (k)
2 days before anthesis	61.00	5.12
1 day before anthesis	65.51	5.01
At anthesis	59.71	7.01

Data were not analiyzed statistically; k: average of chemical compounds at three flower age criteria.

(Table 6). Flowers harvested at 05.00-07.00 AM had a higher percentage of α-zingiberene, α-bergamotene, and caryophyllene compare to those harvested at 07.00-09.00 a.m. There was an indication that different flower ages have different compositions of chemical compounds, except caryophyllene that was found at the same flower age at both harvesting times.

Table 6 - Sesquiterpenes compounds of orange jessamine essential oils at different harvesting times

Treatment	% Peak area		
	α-ZBN	α-BGN	CP
At 05.00-07.00 AM, flower ages	0.75 (k)	0.55 (k)	0.27 (k)
2 days before anthesis	0.47	0.00	0.00
1 day before anthesis	0.00	0.55	0.00
At anthesis	1.03	0.00	0.27
At 07.00-09.00 AM, flower ages	0.71 (k)	0.41 (k)	0.17 (k)
2 days before anthesis	0.00	0.41	0.00
1 day before anthesis	0.57	0.00	0.15
At anthesis	0.85	0.00	0.19

Data were not analyzed statistically; k: average of chemical compounds at three flower age criteria. ZBN, zingiberene; BGN, bergamotene; CP, caryophyllene.

4. Discussion and Conclusions

The above results showed that there were no significant differences between the number and weight of flowers harvested at 05.00-07.00 and 07.00-09.00 AM. The different harvesting times reflected the posi-

tion of sunrise where higher light intensity was found at 07.00-09.00 AM. The current study showed that the anthesis of *M. paniculata* flowers occurred before 05.00 AM, therefore there was no increase in the number and weight of flower after that time. De Souza *et al*. (2004) reported that the anthesis of *Metrodorea nigra* St. Hill. flowers, belonging to Rutaceae family, occurs in the morning. Time of harvesting is important because it is related to the amounts of essential oils produced. Filho *et al*. (2006) reported that harvest at 08.00 AM resulted in the highest yield of the essential oil from fresh leaves of basil (*Ocimum basilicum* L.). The importance of harvesting time is also shown by Dobreva and Kovacheva (2010) where the essential oils content of *Rosa damascena* Mill. and *R. alba* L. drops dramatically when the flowers collected after noon.

Besides investigating the effect of harvesting time, this study also observed the essential oil production at different flower developmental stages of *M. paniculata*. The results showed that harvesting flowers at anthesis stage (blooming flower) yielded the highest percentage of essential oils. Flowering plants released diverse blends of volatile to attract pollinator and seed disseminators. The floral scent is a signal, which pollinators can use to discriminate a particular flower. It may contain from one to 100 volatile substances, but most species emit between 20 and 60 different compounds, so there won't be any identical floral scents (Dudareva *et al*., 2006). Therefore, the presence of essential oil at anthesis will ensure the reproductive success. Azam *et al*. (2013) also reported that the highest amounts of volatile compounds were present in fully opened flowers of *Citrus reticulata* Blanco, *C. unshiu* Marc., *C. sinensis* (L.) Osbeck, *C. limon* (L.) Burm., *C. medica* (L.), and *C. changshanensis* Chen et. Fu.

In general, essential oils are a mixture of compounds belonging to different chemical entities such as terpenes, phenols, aliphatic compounds, benzenoid, and heterocyclic compounds (Shakeel-u-Rehman *et al*., 2018). Chemical compounds found in *M. paniculata* flowers in India were monoterpenes, sesquiterpenes, benzenoids, diterpenes, and fatty acids (Rout *et al*., 2007). Different from those study, the current experiment showed that coumarins were the dominant compounds in *M. paniculata* flowers. Coumarins are also present in leaves of *M. paniculata* in Indonesia (Kinoshita and Firman, 1996) and Taiwan (Kinoshita *et al*., 1996). Furthermore, from the current study, it was found the presence of two coumarins derivatives in essential oils of *M. paniculata*, β-methylesculetin and murralongin. β-methylesculetin compounds can function as antioxidants (Kontogiorgis and Hadjipavlou-Litina, 2005) and anti-inflammatory (Kontogiorgis and Hadjipavlou-Litina, 2005; Zuoqi *et al*., 2008). Murralongin is thought to be a chemical compound identifier of *M. paniculata* essential oils. Harvesting at anthesis produced the highest percentage of murralongin. The previous study reported that murralongin was found in essential oils from leaves and flowers of *M. paniculata* (Gill *et al*., 2014), and leaves of *M. omphalocarpa* in Taiwan (Chen *et al*., 2003).

Terpenoids compounds that were identified in this study were triterpenes and sesquiterpenes. In general, terpenoids are the dominant compounds in essential oils (Sangwan *et al*., 2001). The current study showed that the percentage of triterpenes compounds is higher than sesquiterpenes (Table 4). This was not in line with Butu *et al*. (2014) who reported that the basic compound in the essential oil was sesquiterpenes. Terpenoids have many volatile compounds that have high enough vapor pressures at normal atmospheric conditions to allow significant release into the air (Dudareva *et al*., 2004). Therefore, despite the same plant, may have different types of compounds. Sesquiterpenes compounds that could be identified in this current study were α-zingiberene, α-bergamotene, and caryophyllene. There were similarity and difference between this finding and the previous study. The similarity was reported by Raina *et al*. (2006) where those three compounds were also found in *M. exotica* essential oils from flowers. Raina *et al*. (2006) found that caryophyllene had the highest percentage, on the contrary, the current study showed that caryophyllene had the lowest percentage. The different finding indicates that the chemical composition and yield of essential oils are affected by many factors, such as provenance, weather, soil conditions, time of harvest, and the extraction method (Boira and Blanquer, 1998). Caryophyllene is one of the compounds in perfume ingredient (Salvador-Carreno and Chisvert, 2005), but it also used as a mixture of spices, citrus scents, soaps, detergents, lotions as well as in various food products (Sabulal *et al*., 2006). Flamini *et al*. (2007) and Darjazi (2012) reported that caryophyllene is also present in flower of *C. limon* and *C. nobilis* Lour var. deliciosa swingle.

It can be concluded that the harvest of *M. paniculata* flowers can be done at 05.00-09.00 AM to obtain the highest quantity and the best quality of essential oil. Flowers must be harvested at anthesis stage to reach the highest production of essential oil with β-

methylesculetin and murralongin, as the main compounds of *M. paniculata* flowers.

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