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Research Article

## Effect of the growth environment on Patchouli (*Pogostemon cablin* Benth.) oil character at Southeast Sulawesi, Indonesia

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### ABSTRACT

Patchouli (*Pogostemon cablin* Benth.) is a source of essential oils and widely used as an aromatic medicinal plant and used in flavour and fragrance industries as well as in the pharmaceutical industry. The essential oil constituents of *P. cablin* leaves collected from two different altitude habitats, highland area (A, 746 MAMSL) and coastal area (B, 17 MAMSL) of Southeast Sulawesi has been investigated. The characterisation was performed on its physico-chemical properties such as organoleptic, refractive index and other chemical constituents using GC-MS spectroscopy. The result showed that the essential oil content of patchouli in the highland area was higher (0.47%) than that in the coastal area (0.31%). The oil of highland area was lightly yellow with a refractive index of 1.507, while the coastal area was orange colour in a refractive index of 1.502. Analysis the main chemical constituent showed the highland area have a higher concentration of patchouli alcohol than coastal area. The results indicated that both plants were collected in different altitude habitats and have different essential oils characters.

**Keywords:** Patchouli, *Pogostemon cablin* Benth., altitude habitat, essential oil, patchouli oil

### INTRODUCTION

Essential oils are one of the natural products from plant. Its highly used as traditional medicine, fragrance and flavouring agent. Recently, the essential oil is using as nature aromatherapy. It has a character of volatile at room temperature, without any decomposition, with a pungent taste, with the odour as the plant itself, mostly soluble in an organic solvent and miscible with water. Production of secondary metabolite in essential oil was influenced by external and internal factors of habitat (Radusiene *et al.*, 2012).

The altitude, soil, temperature, rain intensity, moisture and sun appearance are the factor that can influence difference production of secondary metabolite on the same species of plants. These factors are also influencing the

morphologic and physiologic of the plant. Lack of habitat support will change the morphology and physiology of the plant and respect to its production of secondary metabolite (Buchbauer, 1993).

Patchouli (*Pogostemon cablin* Benth.) is one of the sources of essential oils in Indonesia. This plant is important as the export commodity and contributed at ~60% of the total export of the national essential oil. Mostly the patchouli essential oil was produced for cosmetics such as soap, toothpaste, shampoo, lotion and deodorant and for food industry such as essence and flavouring agent and also for medicine such as anti-inflammation, anti-fungi, anti-ant and anti-depressant and other industrial purposes. The good quality of patchouli oil in Indonesia was the oil distilled from the leaf of *P. cablin* cultivated in Aceh Province also known as Aceh Patchouli (Mangun, 2009).

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More than 140 compounds have been isolated from patchouli oils including terpenoids, phytosterols, flavonoids, organic acids, lignins, alkaloids, glycosides, alcohols and aldehydes. The main phytochemical compounds are patchouli alcohol,  $\alpha$ -patchoulene,  $\beta$ -patchoulene,  $\alpha$ -bulnesene, seychellene, norpatchoulene, pogostone, eugenol and pogostol. *In vitro* test on patchouli alcohol showed antibacterial activity against 127 bacteria strains [inhibit both Gram-negative bacteria (25–768  $\mu\text{g/mL}$ ) and Gram-positive bacteria (1.5–200  $\mu\text{g/mL}$ )] and active against some drug-resistant bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA). Patchouli alcohol also exhibited *in vivo* anti-MRSA activity in mice via intraperitoneal injection. Other *in silico* study using molecular docking technology showed potent antibacterial activity of the constituents reported from patchouli oils (Swami and Sinniah, 2015; vanBeek and Joulain, 2017; Wan *et al.*, 2016; Yang *et al.*, 2013).

Over the years, many biological activities have been reported such as *in vitro* and *in vivo* leukocytes behaviour in acute inflammatory response, prebiotic-like effect, immunomodulatory, anti-inflammatory, antioxidative, antitumor, antimicrobial, insecticidal, antatherogenic, antiemetic, whitening and sedative activity (Hu *et al.*, 2017; Leon *et al.*, 2019; Silva-Filho *et al.*, 2016).

This plant is also extensively cultivated in District Kolaka Utara, Southeast Sulawesi Province, Indonesia, as one of their people activity for essential oil products. Their geographical landscape is variable and covered the area of plantation of patchouli with the difference of habitats. In our interest to this plant, we have carried out the character of patchouli essential oil which was collected from two different area, highland and coastal area in Celebes Island (Southeast Sulawesi Province).

## MATERIAL AND METHODS

### Material

Refractometer (ABBE), GC\_MS QP2010 ULTRA (Shimadzu), distillator (Stahl), dichloromethane, sodium sulphate anhydrite.

### Plant material

Aceh patchouli leaves were collected from highland area with the altitude of approximately 746 MAMSL (Bangsala Village) and coastal area (approximately 17 MAMSL) at Porehu Village, Kolaka Utara District, Southeast Sulawesi

Province, Indonesia, in January 2019. The collected plant was determined by Sri Suhadiyah, Keanekaragaman Hayati Sulawesi Selatan Foundation. The leaf was dried and cut into small pieces before distillation. The research was performed from January to August 2019 at laboratory of Biofarmaka, Hasanuddin University.

### Distillation methods

Dried patchouli leaves were distillate with water (Stahl method) at 40°C–50°C for 5–6 hours.

### Refractive index

Refractive index was determined by refractometer. Firstly, the oil was removed from water content using anhydrate  $\text{NaSO}_4$ . Then, refractive index was measured at the temperature of 20°C.

### Gas chromatography-MS

Approximately 1  $\mu\text{l}$  of patchouli oil was dissolved with 1 ml dichloromethane then injected to GC-MS with injector temperature of 250°C, column temperature of 70°C, interface temperature of 300°C with total time of analysis of 50 minutes. Mass spectra data were compare to the library data like Wiley and National Institute of Standards and Technology (NIST) that has been integrated with the instruments. The similarity of the spectra of the compounds to the library data was 90% of similarly index (SI) of the peaks.

## RESULTS AND DISCUSSION

Volatile oils from plant mostly used in traditional medicines for treatment many diseases. The local wisdom of the use natural resource for daily life including on prevention and treatment of the diseases that commonly spread in the area. Various typical geography of Indonesian landscape enhanced their biological diversity in a numerus of species plant. However, some species can survive on this situation. On the survival condition, the same plant species can produce a different type or quantity of secondary metabolite than the suitable condition. On the highland, mostly temperature and moisture are lowest than that in the lowland area with that same of rain intensity. In our interest to these conditions, we have studied the different of volatile oil of patchouli leaf growth in high and coastal area of Southeast Sulawesi Province, Indonesia.

Patchouli is known as 'nilam' in Indonesia, the oils from leaves are the main target of plantation with the high economical values. In Celebes Island, patchouli widely planted in the centre and southeast area of the island and growth in any altitude and various typical geographic which will effect to the chemical constituents content of the plants. In our interest to this study, we have selected two areas in Southeast Sulawesi Province which was known as a source of patchouli oil in a different altitude. The first sample (patchouli leaves A) was collected from Bangsala Village, with the altitude of approximately 740 MAMSL, and the second one (B) from Sapaha Village with the altitude of approximately 17 MAMSL. The morphological environment of the plant patchouli growth was collected and is presented in Table 1.

The place of plant growth influences production of secondary metabolic such as altitude, temperature, rain intensity, humidity, wind blow and intensity of the sun exposure. The plant growth in highland can adapt to weather with low temperature, high humidity and low of sun exposure. While in coastal area can survive in high temperature, low humidity and high sun light exposure. In a high temperature, production of secondary metabolite is influenced by photosynthesis and respiration of plant. In low temperature, plant evaporation (transpiration) decreases, otherwise the root will absorb more water for metabolism (Uddin, 2019).

Oil from patchouli leaves was collected from leaves which were dried in room temperature for 3–4 days to reduce oxidation of the oil and prevent of the loss of volatile oils. The oil was collected from the leaves by distillation to give oil as in Table 2. The oil was collected from Location A

**Table 2:** Yield of patchouli oil distillation

| Location |   | Dried leaves (g) | Oil yield (%) | Average (%) |
|----------|---|------------------|---------------|-------------|
| A        | 1 | 90.05            | 0.34          | 0.31±0.03   |
|          | 2 | 90.01            | 0.31          |             |
|          | 3 | 90.02            | 0.27          |             |
| B        | 1 | 90.02            | 0.58          | 0.47±0.10   |
|          | 2 | 90.01            | 0.45          |             |
|          | 3 | 90.01            | 0.38          |             |

(0.47%), which showed higher yield compare with Location B (0.31%). The average oil percentage in dried *P. cablin* has been reported with the range of 0.54–5.2% (vanBeek and Joulain, 2017). Indian patchouli leaf has been reported contain oil with a concentration of 2.8% (Swami *et al.*, 2015). Short time of distillation gives low concentration of oil and some study also reported the fermented leaf gives oil in high concentration (Raharjo *et al.*, 2012). However, the aim of the study to compare the oil content between two sources of patchouli in a different growing altitude. Difference growing area of the same species plant affects its oil content since the growing environment affecting production of plant secondary metabolite (Uddin, 2019).

Physical characteristic was determined by its organoleptic characteristics and identification of oil refractive index. Organoleptic analysis was determined with the colour of yellowish (A) and orange for oil B. Both oils have a bitter taste and specific aromatic odour for patchouli. The number and type of compounds in oil influence refractive index value of oils. Both oils showed similarity refractive index value of 1.507 and 1.502. Based on the Indonesian national standardisation of patchouli oils, the colour should be in yellowish with their refractive index of 1.507–1.515. The lowest values indicated the possibility of the high water content and the differences of constituents. High of chemical constituent's content increase the density of volatile oil and increase of refractive index values. The similarity of the refractive index oil was isolated from Java Island with the value of 1.5095 (Raharjo *et al.*, 2012).

Identification of chemical content of the oil was determined with GC-MS resulted of 20 compounds was identified (Table 3). Among them, d-guaiene, kauran, clovene and patchouli alcohol was identified more than 20% each compare to other compounds, and patchouli alcohol is the main constituents from this plant. This result showed the

**Table 1:** Location of the collected sample

| No. | Parameters               | Sample                          |                                 |
|-----|--------------------------|---------------------------------|---------------------------------|
|     |                          | Highland (A)                    | Coastal (B)                     |
| 1.  | Location                 | S 03°03'30.4"<br>T 121°09'52.6" | S 03°17'52.1"<br>T 120°58'02.8" |
| 2.  | Altitude (MAMSL)         | 746                             | 17                              |
| 3.  | Soil                     | Andosol                         | Inseptisol                      |
| 4.  | Temp. (°C)               | 14–25                           | 26–33                           |
| 5.  | Humidity (%)             | 78                              | 85                              |
| 6.  | Rain intensity (mm/year) | 1.807                           | 1.1                             |
| 7.  | Sun light (%)            | 70–85                           | 75–90                           |
| 8.  | Wind                     | –                               | +                               |

**Table 3:** Chemical constituents of patchouli oil identified by GC-MS

| No. peak | A        |                   | B        |                      |
|----------|----------|-------------------|----------|----------------------|
|          | Area (%) | Compd.            | Area (%) | Compd.               |
| 1        | 1.93     | Calarene          | 1.81     | Calarene             |
| 2        | 1.11     | Diisopropenyl     | 1.29     | Cyclohexane          |
| 3        | 0.48     | Patchulane        | 0.44     | Patchulane           |
| 4        | 3.35     | t-Caryophyllene   | 3.29     | t-Caryophyllene      |
| 5        | 22.62    | d-Guaiene         | 22.46    | d-Guaiene            |
| 6        | 0.44     | a-Humulene        | 0.51     | a-Humulene           |
| 7        | 6.77     | Alloaromadendrene | 6.15     | Alloaromadendrene    |
| 8        | 0.34     | Germacone         | 0.36     | b-Element            |
| 9        | 0.53     | b-Selinene        | 0.47     | b-Selinene           |
| 10       | 0.22     | Ledane            | 0.25     | Widdrene             |
| 11       | 0.23     | Octadecatrienal   | 2.79     | Sativen              |
| 12       | 2.62     | Kauran            | 21.28    | Clovene              |
| 13       | 17.51    | Dihydrocarvyl     | 0.43     | Junipene             |
| 14       | 0.58     | Cycloprop         | 0.22     | Tetramethyl          |
| 15       | 0.38     | Longipinenepoxide | 0.33     | Longipinenepoxide    |
| 16       | 0.25     | Cyclododeca       | 0.19     | Dihydrocarvylacetate |
| 17       | 0.31     | Nitrobicyclo      | 0.31     | Octatriene           |
| 18       | 9.21     | Globulol          | 9.02     | Cyclodeca            |
| 19       | 29.88    | Patchouli alcohol | 25.70    | Patchouli alcohol    |
| 20       | 1.22     | 3-Undecen-2-one   | 2.69     | 3-Undecen-2-one      |

same main compound has been previously reported (vanBeek and Joulain, 2017) with the molecular formula of  $C_{15}H_{26}O$ , a sesquiterpene, tertiary alcohol tricyclic with a hydroxyl group and 4 methyl group. GC-MS spectra show that the oil from the highland showed higher concentration compared with the coastal area with a concentration of 29.9% than 25.7%, respectively.

### CONCLUSION

The result showed that the quality and quantity of patchouli oil extracted from the leaves of *P. cablin* was influenced by altitude of plant growth.

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