# Mini-Review: Extraction of Patchouli Oil from *Pogostemon cablin* Benth. Leaves

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> Abstract. Pogostemon cablin Benth. var 'Sidikalang' leaves are the main source of patchouli oil which is used in the cosmetics and perfume industry because of its better fixative nature and provides the foreign exchange of around 60 % of Indonesia's essential oil export revenues and is the largest patchouli oil supplier in the world with a contribution of 90 %. But apparently, several studies found that patchouli oil also has pharmacological activity such as aromatherapy, anti-influenza, antioxidants, and anti-microbial. This proves that leaves patchouli oil can be used as alternative medicine. Patchouli oil is composed of 20 main constituents, but the most important role as a determinant of the quality of patchouli oil is sesquiterpene patchouli alcohol (PA). The right extraction method is the most important factor so that the quality of patchouli oil is well maintained during the extraction process. Some methods that have been used for patchouli oil extraction include soxhlet, hydrodistillation, microwave-assisted hydrodistillation, microwave-air hydrodistillation, and water-bubble distillation. Microwave-assisted hydrodistillation and waterbubble distillation methods are more efficient methods for patchouli oil extraction. The microwave-assisted hydrodistillation method produces a yield of 1.9437 to 2.7227 % with a PA content of 26.32 % within 120 min to 126 min, and the water-bubble distillation method produces a yield of 2.40 % with a PA content of 61.53 % within 360 min.

**Keywords:** Extraction efficiency, microwave-assisted hydrodistillation, water– bubble distillation

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### 1 Introduction

At present, the highest consumption level of essential oil is patchouli oil from Aceh patchouli (*Pogostemon cablin* Benth.) leaves. Patchouli oil of superior quality has reached the international market at a price of USD 63 to 150 kg<sup>-1</sup>, so patchouli oil sales in 2020 are predicted to generate around USD 75 000 000. A study explained that patchouli oil provides the foreign exchange of around 60 % of Indonesia's essential oil export revenues and is the largest patchouli oil supplier in the world with a contribution of 90 % [1]. Patchouli oil has an important role in the cosmetics and perfume industry because it has fixative properties (scentbinders) so it is used as the main raw material for these products [1, 2].

Several studies also stated that patchouli oil also has pharmacological activity as aromatherapy, anti-influenza, antioxidants, and anti-microbial. This proves that leaves patchouli oil can be used as alternative medicine. Patchouli oil has the potential as aromatherapy because the aroma that comes from its main constituents namely patchouli alcohol (PA) can affect brain function, reduce physiological responses such as blood pressure, pulse, and stress index. Therefore the aroma of patchouli oil can help reduce anxiety, insomnia, and tension. Polyphenol compounds contained in patchouli oil have antiinfluenza activity because they have the potential to be neuraminidase inhibitors of influenza viruses. Patchouli leaves ethanol extract (PA) has antioxidant activity, where the extract is able to inhibit superoxide based on DPPH and ABTS scavenging tests. Patchouli oil also has antibacterial properties against Escherichia coli, Staphylococcus aureus ATCC 25923, and Streptococcus pyogenes with inhibition zones respectively 1.85 mm, 0.44 mm to 3.92 mm, and 1.55 mm. Antimicrobial activity of patchouli oil is then utilized in the development of biocompatible wound dressing containing Fe<sub>3</sub>O<sub>4</sub> nanoparticles by making bioactive layers and the results obtained indicate the presence of anti-biofilm activity for 72 h and low-level soft toxicity in mammalian cells that have wound infection [3-7].

In order to get good quality patchouli oil, extraction methods are also needed in accordance with the nature of patchouli oil. A study suggested that patchouli oil extracted from Aceh patchouli leaves was composed of several main constituents namely patchouli alcohol (PA) (42.75 %), delta–guanine (28.30 %), azulene (20.48 %), transcaryophellene (11.84 %), seychellence (10.77 %), naphthalene (8.02 %), cycloheptane (6.02 %), dan caryophyllene (5.73 %) [8]. The constituents with the highest levels are PA, soth at the quality of patchouli oil is largely determined by the levels of PA contained. PA content is more abundant in the leaves than in the stem or roots [9], and PA contained in patchouli oil can reach 30 % to 40 % (v v<sup>-1</sup>) [10]. The best quality of patchouli oil based on Indonesian national standards is a minimum PA content of 31 % [11]. PA is able to bind and prevent aroma evaporation because it is fixative so it can retain the aroma longer [12]. Another study report obtained a yield of patchouli oil of 36.55 g from 3 000 g of simplicia, with PA levels of 35.06 % and water of 0.56 % [13]. There is also according to Rosman [14] stated, the yield of patchouli oil obtained by 1.72 % contains a PA of 36.21 %.

Extraction is the initial stage in separating target natural products from raw materials. Extraction methods include extraction using solvents, distillation methods, pressing, and sublimation in accordance with the extraction principle. The choice of solvent for extraction is the most important part, with various considerations, namely selectivity, polarity, cost, and safety of the use of the solvent. In order to be able to extract good quality patchouli oil, the extraction method used must be chosen appropriately based on the nature of the material and the compound to be isolated. Various extraction methods are generally used, such as maceration, percolation, reflux, steam distillation, hydrodistillation, soxhlet, pressure liquid extraction (PLE), supercritical fluid extraction (SFE), ultrasound-assisted

extraction (UAE), microwave-assisted extraction (MAE), pulsed electric field (PEF), dan enzyme-assisted extraction [15].

This mini-review article aims to provide information by comparing the efficiency of several methods of extraction of patchouli oil from *P. cablin.*, as well as outlining the factors that influence the quality of PA.

### 2 Several factors affecting the quality of Aceh patchouli oil

In order to obtain higher quality patchouli oil extraction results and more optimal extraction, several supporting factors need to be considered, namely cultivation, fertilization, harvest time, drying and sample size reduction, patchouli leaf extraction techniques, and identification and analysis of volatile chemical compounds contained in patchouli oil [2].

#### 2.1. Fertilization and cultivation conditions

Fertilization is done by giving micro or macro elements to the soil. Application of fertilizer and rice straw mulch can reduce stress due to drought on patchouli plants by providing the best growth effect on each parameter measured (plant height, number of branches, number of leaves, root length, plant wet weight, root canopy ratio, total chlorophyll, temperature soil, and soil moisture). The use of mulch rice straw as fertilizer on patchouli plants is able to maintain soil temperatures so that it remains low maintaining high levels of moisture so that nutrient uptake, water, and metabolic processes can run well. Weathering rice straw mulch is able to provide additional nutrients for plants, especially increasing organic C content and improving soil structure [16].

A study makes appropriate cropping patterns to determine the effect on patchouli productivity. In the treatment of 100 %, the light intensity can produce the highest patchouli oil yield, but in the treatment of 80 %, light intensity is able to produce the highest PA value of 37.99 % [14]. Patchouli plants can grow in tropical areas at an altitude of 1 000 m asl but will grow well and produce high in an altitude area of 100 to 400 m asl, temperatures around 24 °C to 28 °C, and relative humidity between 70 % to 90 %. In the lowlands, patchouli oil content is more than 2 %, but patchouli alcohol (PA) levels are low. Conversely, in the highlands patchouli oil content of less than 2 %, but high PA levels. Therefore 80 % light intensity treatment produced patchouli oil with PA levels higher than 100 % light intensity treatment. Too high light intensity causes patchouli leaves to undergo chrome pigment photodegradation (yellowing of the leaves). Chromopigment photodegradation resulted in the process of photosynthesis and patchouli plant growth being inhibited so that patchouli oil production was also low [14, 17].

In addition, intercropping with corn and green beans on patchouli plants resulted in lower plant height and number of branches compared to patchouli monoculture plants. Intercropping with maize and mung beans can cause patchouli plant growth to be hampered due to competition of nutrients from the soil so that plant height and number of branches are lower when compared to monoculture patchouli plants [14].

#### 2.2. Harvest time

The year of harvest includes factors that can affect patchouli oil levels in the harvest each year. Patchouli leaf harvesting is carried out about 6 mo, 9 mo, and 12 mo after planting. The first year yielded 4.1 %, the second year yielded 3.5 %, and in 3<sup>rd</sup> yr the yield was

2.7 %. Based on these data patchouli oil yields have decreased each year but not significantly. Patchouli plants that have been harvested in the first year of oil content will decrease when harvesting again in the following year where the decrease in oil content is also influenced by the functional age of the leaves. Stages of leaf maturity also have an effect on patchouli oil yield. The percentage of oil content in semi-mature patchouli leaves is the highest (4.5 %), compared to the oil content in vegetative patchouli leaves (4 %) and fully mature (3.8 %) This is because, in the semi-mature stage, plant activity in carrying out secondary metabolite biosynthesis is very high [18].

#### 2.3. Drying and sample size-reduction

The process of drying and sample size reduction is an important process in sample extraction preparation. Drying can be done directly in the sun or indirectly drying, which is stored in a cool and dry room (air-conditioned room). Patchouli oil consists of several volatile components and is present in the leaf epidermal gland trichomes. At least one leaf contains 60 000 trichomes, and each trichome contains 2 ng patchouli oil. The oil contained in the patchouli leaves can hardly pass through the intact oil gland membrane due to its internal structure which sinks too deep so it requires a drying process. Direct drying in the sun can cause some essential oils, especially in the extraction of patchouli oil, the oil will also evaporate, and drying too quickly causes the leaves to become brittle and difficult to extract. However, when drying too slowly the leaves will become damp and have an unpleasant aroma due to mold so the quality of the oil will decrease. Extraction from fresh leaves is not good because the oil yield is too low, because the cells containing oil are partly on the surface and partly inside the leaves so that by drying the cell walls will open so that it is more easily penetrated by steam. Drying aims to vaporize some of the water in the material so as to facilitate the extraction process that takes place and is shorter [19–25].

Reduction of the sample size is carried out in order to increase the surface area of the simplicia and the oil glands in the patchouli trichomes that can be opened as much as possible so as to facilitate the removal of oil from the material, thus facilitating the extraction process and the resulting yield is maximized. There is also a sample size reduction technique that uses sonification so that the results obtained are in the form of nanopowder. The sonification technique is very possible to be applied in the preparation of patchouli leaf samples to be extracted because it will further expand the surface of the simplicia particles [12, 25, 26].

#### 2.4. Fermentation technique

Fermentation techniques have been applied to increase the yield of patchouli oil so that it can be considered as a factor affecting the acquisition of patchouli oil. Types of molds used for patchouli leaf fermentation are *Trichoderma viride* and *Rhizopus oligosporus*. Based on the results of GC–MS analysis of patchouli oil, the patchouli alcohol (PA) compound with the highest peak percentage of 39.94 % was obtained from the combination process of the fermentation–distillation method. The optimal time needed for fermentation of patchouli leaves is 4 d. The fermentation technique is able to increase patchouli oil yield due to the activity of mold as a fermentation agent that is able to decompose lignocellulose by breaking the  $\beta$ –1.4 glycosidic bonds by cellulase enzymes produced by molds. In addition to depolymerization, molds also transfer substrates into cells through the cytoplasmic membrane to decompose organic matter. Through this decomposition process, it is expected that the yield of patchouli oil can increase because the crystalline structure of the patchouli

leaves is open and the oil in the vacuole is easily isolated [13, 28, 29].

#### 2.5. The efficiency of the Aceh patchouli oil extraction method

Increased consumption of patchouli oil demands the industry to improve the patchouli oil production process. Currently, there have been many studies on patchouli oil extraction using a modified hydrodistillation (HD) method so that the patchouli oil extraction process becomes more effective and efficient. Syahputra. [30], compared the results of patchouli oil extraction using two different methods namely the microwave-assisted hydrodistillation (MHD) and soxhlet methods, with respective yields of 5.8252 % (3 h) and 7.4741 %, (8 h). An increase in energy consumption and efficiency makes an industry demand to improve the production process with time and cost that is more efficient but also able to improve the quality of patchouli oil.

Kusuma and Mahfud [1] compared the results of patchouli oil extraction using conventional hydrodistillation (HD) methods and microwave-assisted hydrodistillation (MHD), with respective yields of 2.722 7 % (126 min) and 2.613 2 % (417 min). However, based on GC–MS analysis of PA levels, the HD method occupies the highest percentage (27.78 %) than PA produced by the MHD method (26.32 %). The extraction method has a big influence on patchouli oil yield. A study explained that conventional hydrodistillation (HD) methods have drawbacks such as can cause a decrease in the amount of yield because when a sample is lacking in the water there will be overheating and plant material being damaged [31]. The length of time the extraction affects the yield obtained, the longer the extraction time can increase the amount of yield and tends to be consistent after 150 min. The existence of heat transfer from the heating process of water can change the permeability of leaf cells so that oil will come out of the cell to the surface.

Based on these problems, a conventional HD method, the microwave-assisted hydrodistillation (MHD) method, is used to utilize the heat energy produced to help break down the patchouli leaf cell wall so that it is expected to extract patchouli oil more effectively. If in terms of energy consumption, the MHD method can be referred to as green extraction method, in addition, MHD still requires further optimization to improve the quality of patchouli oil obtained because the quality of patchouli oil is good is measured based on the PA levels [1, 32].

Kusuma and Mahfud [32] reported a study of the microwave-assisted hydrodistillation (MHD) method which was further developed into the microwave air–hydrodistillation (MAHD) method with the aim of optimizing the patchouli oil extraction process. Patchouli oil yield produced by the MHD method was 1.943 7 % with 26.32 % PA levels (120 min), while MAHD produced 2.767 8 % with 25.23 % PA levels (120 min). Airflow added to the MHD method is expected to increase the yield and quality of patchouli oil. The airflow serves to help the extraction of patchouli oil components that are in the cell membrane or patchouli leaf tissue so that it does not easily diffuse out. This is because patchouli oil consists of several components in a heavy fraction that is difficult to extract without additional airflow. However, based on the analysis of PA levels, the quality of patchouli oil produced from the MHD method is still better than the MAHD method.

There is also the development of other distillation methods such as the water-bubble distillation (WBD) method as in a study reported by Fitri [33], which compares the quality of patchouli oil extracted through the water-steam distillation (WSD) method. Yields produced by the WSD method are higher (5.90 %) than WBD (2.40 %). However, the quality of patchouli oil produced by WSD is not in accordance with SNI because the higher specific gravity value is 0.991. PA levels contained in patchouli oil produced by WBD are quite high at 61.53 %. The yield from the WBD method is less when compared to the yield

from the WSD method, but the PA content in the yield from the WBD method is the highest. Patchouli oil yields from the WSD method also contain many heavy fractions or semi-polar components so that the specific gravity value is higher than the SNI requirements while the SNI specific gravity requirement is around 0.950 to 0.975. The results also showed that using the WBD method was able to remove contaminants in patchouli oil because only five minor compounds (under 1 %) were found, whereas in patchouli oil from the WSD method found 12 minor compounds.

#### 2.6. Identity and analysis of Aceh patchouli oil

Identification and analysis of essential oil scan use the Gas Chromatography-Mass Spectrometer (GC–MS) technique. The GC–MS functions to determine density, refractive index, molecular formula, retention time (RT), molecular weight (MW), and peak area which are presented as fingerprints in percentages and are very accurate for analyzing the purity and quality of the essential oils produced. The GC–MS technique has gone through several tests and is declared valid for use as a patchouli oil identification and analysis technique. Validation parameters tested include selectivity (Rs or separating value of 1.5 to 2.0), linearity ( $R^2 > 0.990$  or  $R^2$ value > r table value), precision of retention time (KV or RSD < 20 %), accuracy (% recovery of 70 % to 100 %), limit of instrument detection (LOD), and limit of instrument quantification (LOQ) [34].

Based on some of the studies above, patchouli oil extraction results are identified and analyzed using. Through various GC–MS validation parameters, the tests have proven to be valid and can be used in identifying and analyzing patchouli oil. Based on GC–MS analysis, the PA content in patchouli oil was found to be the highest (30 % to 40 %). Therefore, the quality of patchouli oil is measured based on the PA content in patchouli oil. The best quality of patchouli oil based on Indonesian national standards is with a minimum PA content of 30 % [2, 10–12].

Patchouli oil constituents can be identified by comparing retention times obtained through GC–MS analysis techniques. Retention time is the time required for a compound from the initial column to the detector. This is influenced by the length of the interaction of the compound with the stationary phase, the longer the compound interacts with the stationary phase, the longer the time needed to get to the detector by showing the maximum peak of a compound. Van Beek and Joulain presents a comparison of the retention times of patchouli oil extraction results for the identification of its constituent constituents (Figure 1) [2]. Through the retention time data, it can be seen that PA occupies the highest peak and long retention time. This explanation proves that PA is the most important constituent for patchouli oil, so PA levels are also used as a parameter for determining oil quality.

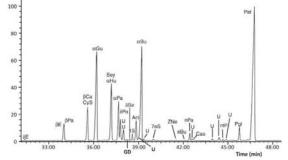


Fig.1. Partial gas chromatography (sesquiterpene region) of patchouli oil [2].

The MAHD method is quite short in the process but the quality of the patchouli oil is still lower than the quality of the patchouli oil using the MHD method. Based on the results of these studies it can be concluded that the MHD and WBD methods are the most superior and efficient methods for extraction patchouli oil from *P. cablin* leaves, although both of them still require further optimization. Table 1. states the comparison between the two methods based on yield, the length of time needed for extraction, and PA levels.

Extraction methods	Yield (%)	Time (min)	Patchouli Alcohol (%)	Sources
MHD	1.9437 to 2.7227	120 to 126	26.32	1.32
WBD	2.40	360	61.53	33

Table 1. The comparison between MHD and WBD methods

### **3** Conclusion

The success of patchouli oil extraction from *P. cablin* leaves based on patchouli alcohol (PA) quality is influenced by fertilization factors and cultivation conditions, harvest time, fermentation, drying and scaling down, and extraction methods. The water–bubble distillation (WBD) and microwave-assisted hydrodistillation (MHD) are efficient methods for extracting good quality patchouli oil from *P. cablin* leaves.

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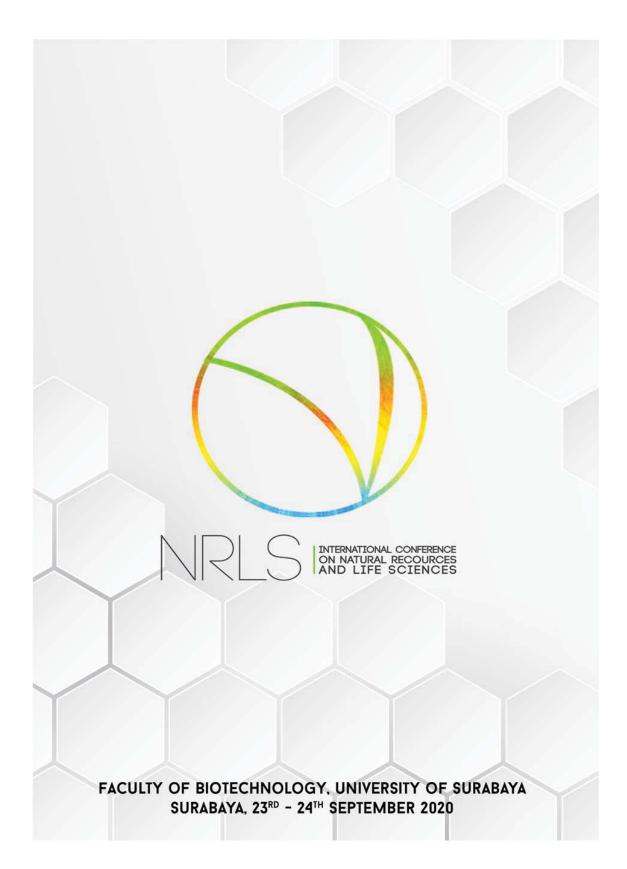
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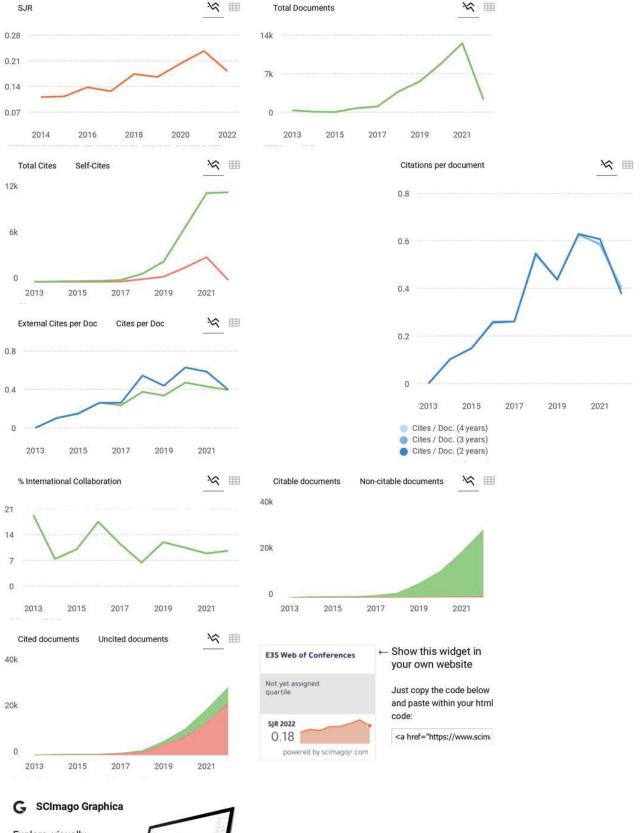
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# ORAL PRESENTER

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Dean of Faculty of Biotechnology University of Surabaya

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Dr.rer.nat Sulistyo Emantoko Dwi Putra, S.Si., M.Si.

Chairwoman Natural Resources and Life Sciences 2020

Dr.rer.nat. Theresia Desy Askitosari, S.Si., M.Biotech.