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Research Article

Phytochemical Identification and Antioxidant Activity of Essential oil of *Pogostemon cablin* Benth. cultivated in Java Island Indonesia

Hariyanti[1], Endang Hanani[2*], Desi Yoga Dayatri[2]

[1]*Department of Chemical Pharmacy,* [2]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. DR. HAMKA*

Abstract

Background: *Pogostemon cablin* belongs to the Lamiaceae family is an aromatic herb havingimmense commercial importance; is due to its oil (patchouli oil), which can be obtained by steam/hydro-distillation technique of the shade dried leaves.
Objective: This study was to compare the chemical composition and the antioxidant activities of patchouli oils extracted from *P. cablin* leaf that cultivated in 2 (two) different regions are Cibinong (A) and Batu (B) in Java Island, Indonesia.
Methods: The chemical composition of the essential oil was analyzed by GC/MS method, and the antioxidant activities were determined by evaluating their scavenging activities against DPPH.
Result: The major components of patchouli oil from Cibinong (A) have been identified as the patchouli alcohol 18.12% and gamma-curcumene 35.07%, whereas patchouli oil from Batu (B) were 26.31% and 34.79%, for patchouli alcohol and gamma-curcumene respectively. The antioxidant activities were found for patchouli oil from Cibinong and Batu, IC_{50}=22.45 ± 0.2974 and 19.87 ± 0.4051 µg/mL by DPPH scavenging assays, respectively.
Conclusion: The chemical constituents, concentration and antioxidant activity of patchouli oil vary depending on the origin and different derived from various cultivation regions of patchouli, affected by temperature, rainfall, aspect, length of the day, altitude and different climatic areas.
Keywords: Chemical composition, DPPH, GC-MS, Lamiaceae, Patchouli oil.

***Correspondence Info:**
Endang Hanani
Department of Pharmacognosy and Phytochemistry,
Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. DR. HAMKA

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1. Introduction

Patchouli (*Pogostemon cablin* Benth.) is belonging to the Labiatae family originating from Southeast Asia. Patchouli leaves contain an essential oil which is made up of patchouli alcohol (patchoulol) as a major component and several other minor components such as some mono- and sesquiterpene[1,2]. Essential oils(EO) are liquid mixtures of volatile compounds obtained from aromatic plants. Many essential oils have antioxidant properties, and the use of EO as natural antioxidants is a field of growing interest because some synthetic antioxidants such as BHA and BHT are now suspected to be potentially harmful to human health. Natural antioxidants (patchouli oil) have been proven safe and potent for the treatment and prevention of several human diseases. *Pogostemon cablin*, normally alluded to as "patchouli" is a local of the Philippines and develops wild in numerous South Asian nations, and is directly developed on a business scale in Indonesia, India, Malaysia, China, Singapore, and Vietnam [3]. Patchouli is a perennial herb and a fragrant plant that grows in the tropical region throughout the world. The leaves of *P. cablin* on steam distillation yield an essential oil called the patchouli oil. Volatile oils are a mixture of known and partially unknown compounds such as hydrocarbons, terpene alcohols,

aldehydes, ketones phenols and esters [4]. The fundamental essential oils containing these volatiles compounds have discovered application in pharmaceutical businesses, broadly utilized in the assembling of fragrances, body moisturizers, and cleansers[3]. Patchouli possesses biological activities to include on antimicrobial [5],antibacterial, antifungal, insecticidal, and antioxidant properties [6-8].

Antioxidative properties of fundamental oils and different concentrates from numerous plants are of incredible enthusiasm for the two scholastics, the nourishment and corrective industry since their conceivable use as regular added substances has risen up out of a developing pattern to supplant manufactured cell reinforcements by characteristic ones [9,10]. Antioxidant activity of *P. cablin* oil is to be explored to as a source of natural antioxidants. Patchouli oil is utilized in fragrance base treatment to alleviate misery, stress, quiet nerves, control craving and to improve sexual intrigue [2,10,11]. A portion of its other organic exercises incorporate antimicrobial, antioxidant, analgesic, anti-inflammatory, antiplatelet, antithrombotic, aphrodisiac, antiemetic, antidepressant, antimutagenic, fibrinolytic and cytotoxic activities[9]. Patchouli plants were collected from various cultivation regions and harvested at various occasions demonstrated contrasts in their volatile oil compositions [11-13].

In Indonesia, *P. cablin* is cultivated in some different region; with difference condition such as attitude, irrigation, temperature, etc. Up to date, *P. cablin* is widespread in Batu (East Java-province) and Cibinong (West Java- Province). The oil of plant has been used as Indonesian herbal medicine to stop vomiting, relieve summer-heat, as aromatherapy, perfumes as well as in soaps and cosmetic products. The essential oil of *P. cablin* collected by steam distillation mainly contributes to the pharmacological activities, and the therapeutic properties of the essential oils are directly correlated with their qualitative and quantitative composition which is differently derived from various cultivation regions of Patchouli [14-16]. This study will be focused to compare the patchouli oil components obtained by hydro-distillation and evaluation of their antioxidant activities; that cultivated in two various regions in Java island Indonesia.

2. Materials and Methods

2.1. Preparation the drug

Healthy and diseases free leaves of *P. cablin* (Blanco) Benth. were collected in March2019from 2 (two) different plantations area in Cibinong (West Province) and Batu (East-province), in Java Island, Indonesia. The raw medicinal herbs were identified at Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Sciences, Bogor and the voucher specimens were deposited at the Pharmacognosy Laboratory, Faculty of Pharmacy and Sciences, Universitas Prof. DR. HAMKA, Klender, Jakarta, Indonesia. These leaves were used for the preparation of patchouli oil. The harvested part consists of leaves, stems (aerial part). It has been dried in the air in the shade at ambient temperature for one day.

2.2. Extraction of patchouli oil

The extraction of the essential oil was performed through the hydro-distillation process using an extraction device called Clevenger. The 250 g dried plant has been introduced inside a large flask of 2litter; then we have added a quantity of distilled water corresponding to 2/3 of the volume of the flask. The extracting operation was carried out slowly for three hours after the beginning of boiling, and no more oily drops were collected. The oil was dehydrated with anhydrous sodium sulphate. The fundamental oil which is entrained by azeotropic refining was liberated. The vapor at that point went through a condenser outside the power warmer where it dense. The distillate is collected gathered continuously with a Clevenger-type apparatus [16,17]. Condensed water was returned to the flask and heating was continued at 100°C until no more essential oil was obtained. Finally, the collected oil has been kept in tinted and well-sealed bottles at a temperature of 5^{o}C until used.

2.2.1. Physicochemical properties

Using the Farmakope Herbal Indonesia (FHI) standard procedures [18]the physicochemical properties of patchouli oil were determined and compared with standard specifications. Measurement of the oil physicochemical properties including specific weight, refractive index, optical rotation, solubility in alcohol, and determination of patchouli alcohol content. All of these parameters are described in the Indonesian National Standard 2006 (SNI 06-2385-2006).

2.2.2. Determination of chemical composition

The components of patchouli oil have been conducted in the LabKesDA (Regional Health Laboratory) DKI Jakarta Indonesia, by gas chromatography, type HP 7890 coupled to a mass spectroscopy, type HP 5975B with ionization by electron impact (70 eV), equipped with a capillary column HP- innowax 30 x 0.25 mm, 0.5 µm film thickness. The oven temperature was programmed at 60–150°C at 2°C/min and then 150–210°C and maintained at 210°C for 10 minutes. The carrier gas was helium, the injector temperature was 230°C, at a flow rate of 0.6 mL/min with a split ratio of 1:250. The essential oil has been automatically injected via Split Mode. The device is controlled by a computer system managing a library of mass spectra and compared with standard published data.

2.2.3. Antioxidant Activity

The antioxidant activity of the Patchouli oil was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay as described by Braca *et al*. [19] with a slight modification. The essential oil was prepared by dissolution in absolute ethanol. The test has been carried out by mixing 1.0 ml of DPPH (0.25mM) in methanol taken in tubes, and 2.0 mL solution of various concentrations of patchouli oil was added. The reaction mixture was allowed to stand at room temperature in a dark chamber for 30 minutes. The change in colour from deep violet to light yellow was then measured at 517 nm in an ultraviolet spectrophotometer. The reference standard antioxidant or the positive control (ascorbic acid and vitamin E) was also prepared according to the same method with the same concentrations for the comparison. The test samples and reference standard were measured in three replicates, and a 95% methanol was served as blank. Percentage of DPPH scavenging activity was calculated as:

% inhibition of DPPH = [Abs blank- Abs sample / Abs blank] x 100.

The graph of the variation of the percentage of inhibition depending on the concentration of the patchouli oil allowed to determine the IC_{50} corresponding to 50 % of inhibition, which constitutes the antioxidant activity of the essential oil. This value was compared to the reference compound. The IC_{50} was calculated graphically using a calibration curve in the linear range by plotting the extract concentration vs. the corresponding scavenging effect. The antioxidant activity was expressed as the antioxidant activity index (AAI), calculated as follows as [20]:

$$AAI = \frac{\text{(final concentration of DPPH (µg /mL)}}{IC_{50}(\mu g/mL)}$$

3. Results

3.1.Physicochemical

The essential oil was obtained by hydro-distillation technique, the yield of extraction of the aerial part of the 2 kinds of *Pogostemon cablin* from Cibinong (A) was 0.75 % (w/w), it is lower compared to the other drug planted in Batu (B), were 1.7 % (w/w). The results of the physicochemical properties measurements of patchouli oil A and B including specific weight, refractive index, optical rotation, solubility in alcohol, patchouli alcohol content, and γ-curcumene content (Table 1). All the values obtained are in the range and the quality standard approved by the SNI, except the patchouli alcohol content.

Table 1: The Physicochemical properties of patchouli oil A (Cibinong) and B (Batu)

Parameters	Patchouli oil A (Cibinong)	Patchouli oil B (Batu)	SNI
Colour	Yellow	Light yellow	Yellow
Specific weight (25°C/25°C)	0,952g/ml	0,951g/ml	0,943 – 0,983g/ml
Refractive index (nD_{20})	1,5063	1,5054	1,5040 – 1,5140
Solubility in alcohol 90%	Clear solution in volume ratio 1:1	Clear solution in volume ratio 1:1	Clear solution in volume ratio 1:10
Patchouli Alcohol	18.12 %	26.31%	30%
γ-Curcumene	35.07 %	34.79%	No data

3.2. Chemical composition

The essential oil from the leaves of *P. cablin*, grown in two different regions in Jawa island, Indonesia was extracted by hydro-distillation and analyzed by GC/MS. The results of the analyzes by GC/MS of the essential oils are presented in Table 2.

The components of the patchouli oil from Cibinong were 18 and from Batu were 14 compounds. The major component of patchouli oil from Cibinong (A) are 35.07% Gamma-Curcumene and 18.12%patchouli alcohol, while from Batu (B) 34.79% and 26.31% respectively (Table 2 and Figure 1).

3.3. Antioxidant activity

The antioxidant activity of this patchouli oil was assessed by its capacity to search DPPH free radicals. The radical searching action of the mixes can be estimated by the decolorizing impact following the catching of the unpaired electrons of DPPH. The patchouli oil showed high antioxidant activity, with an IC_{50} value of 22.45µg/mL for Cibinong and 19.87 µg/mL for Batu; ascorbic acid and vitamin E produced an IC_{50} value of 5.37 µg/mL and 9.93 µg/mL, respectively (Table 3). Patchouli alcohol is a sesquiterpene alcohol compound that can participate potentially in antioxidant effect. The patchouli oil has also phenol and terpene components which are also having antioxidant effect.

Table 2: Chemical Composition of the Essential Oil of *Pogostemon cablin* Cultivated in Cibinong (A) and Batu (B)

No.	Cibinong (A) Components	Area (%)	Batu (B) Components	Area (%)
1	α-Pinene	2.37	trans- Caryophyllene	6.48
2	2-β -Pinene	6.18	β-Naphthalene	5.41
3	β-Patcloulene	1.30	Sesquiphellandrene	1.32
4	β-Curcumene	1.00	γ-Curcumene	34.79
5	Aromandendrene	6.09	β-Bisabolene	1.41
6	β-Selinene	3.45	β-Curcumene	1.40
7	Seychellene	3.35	ar-Curcumene	3.20
8	1,7-Diepi-β- Cedrene	1.22	Phenol,3,5-dimethyl	1.00
9	γ- Curcumene	35.07	Benzenemethanol,4-methyl	2,71
10	α-Bulnesene	2.32	Octahydroazulene	1.82
11	β-Bisabolene	1.53	Patchouli alcohol	26.31
12	β-Curcumene	1.64	γ-Cadinene	2.60
13	α-Curcumene	3.76	Isolongifolene	4.22
14	Eugenol	1.10	α-Farnesene	1.61
15	Patchouli alcohol	18.12		
16	Naphthalene	1.41		
17	Valerenol	3.30		
18	α -Farnesene	1.92		

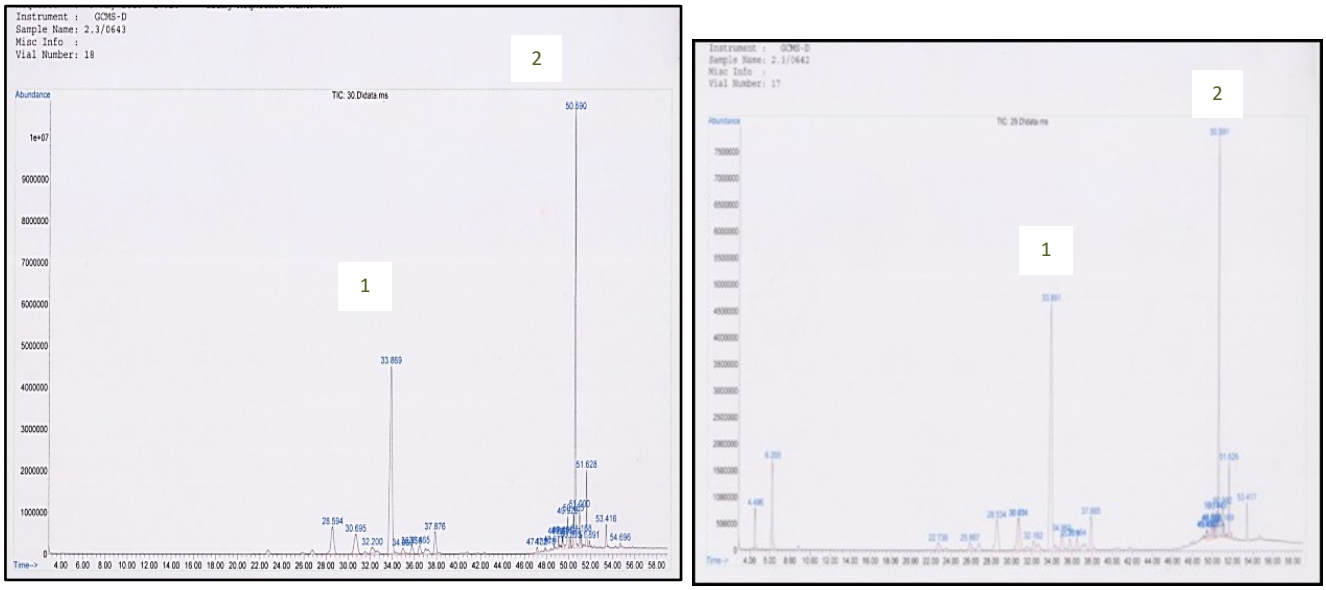

Figure 1: A. Chemical Components of Patchouli oil A from Ciibinong; B from Batu (1. Gamma-Curcumene; 2. Patchouli Alcohol).

3.3. Antioxidant activity

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Table 3: Antioxidant activity of the patchouli oil from Cibinong and Batu

Sample	DPPH IC_{50} (µg/ml)
Pachouli oil from Cibinong	22.45
Pachouli oil from Batu	19.87
Vitamin C	5.37
Vitamin E	9.93

4. Discussion

The results of physicochemical of the patchouli oil are summarized in Table 1; that there is great variation for the contents of investigated components in this oil from a different region of Cibinong and Batu, Indonesia. The biosynthesis, accumulation, and variations of secondary metabolites contents were affected by the biotic and abiotic factors. Different plant species vary enormously in their soil and nutritive requirements, and their aspect has received considerable attention with medicinal plants. Three important basic characteristics of soil are their physical, chemical, and microbiological properties. Plant growth and development, and often the nature and quantity of secondary metabolites are affected by temperature, rainfall, aspect, length of the day, altitude, and different climatic areas [21].

Regarding the formation of volatile oils, it was verified that hot days could result in an excessive loss of such metabolites; however, in general, their formation takes place in higher temperature. Nevertheless, the opposite has also been reported, as in the case of some essential oil, where a decrease in the concentration of some substance has been observed as temperature increased [21]. In Cibinong this plant is occurring at elevations of sea level to about 400 m as a weed along roadsides, in plantations, on damp ground in shady places near houses. The other region was Batu, these places where the plant is found in damp shady conditions plantation and living bare ground which is mostly sea level to 875 m. The rainfall was ± 152 mm/day and± 256 mm/day, respectively. The essential oil was obtained by hydro-distillation technique, the yield of extraction of the aerial part of *Pogostemon cablin* collected from Cibinong (A) were 0.75 % (w/w), it is lower compared to the other drug planted in Batu (B), were 1.7 % (w/w). Patchouli plants were collected from different cultivation regions and harvested at different times showed differences in their volatile oil compositions [15,17].

The gas chromatographic method is used almost exclusively for the qualitative analysis of the volatiles because the retention times were utilized as a primary criterion for the identification of the peaks. Generally, steam or partial refining is utilized to get patchouli oil despite the fact that these procedures cause the warm corruption of a couple of the concoction constituents present in the oil. The results of the analyse by GC of the essential oil extracted from the plant *Pogostemon cablin* are presented in Table 2. Patchouli oil is rich in sesquiterpenes, essentially the patchouli liquor (patchoulol), a sesquiterpene which is broadly utilized in perfumery items cleansers and other restorative products. Patchoulenes, guaiene, seychellene are not many other sesquiterpene hydrocarbons likewise describe the smell of patchouli oil [10]. The chemical profile of patchouli oil gathered from Cibinong and Batu were identified 18 compounds and 14 compounds respectively (Table 2). The results showed that there is great variation for the contents of investigated components in Patchouli from different areas of Java Island, Indonesia. Silva *et al.* [16] have examined by the GC/MS profile of the patchouli oils collected at different periods showed the presence of 13 compounds. Nonetheless, they did not present any noteworthy contrast as an element of the gather time. The concoction piece of patchouli oil shifts among tests gathered from various geographic areas. Lie *et al.* [22] revealed the huge impact of various living spaces, gathering periods, and handling strategies on the unstable oil yield and its fundamental constituents. Likewise, the oil yield is additionally impacted by various gathering times. It is reported that contents of the volatile oil obtained from leaves harvested from June to August and cultivated in Hainan, China was 0.8%, 0.7%, and 0.6%, respectively, while the patchouli alcohol content was highest in the month of June [3,23].

The decolorizing effect measured the DPPH free radicals scavenging activity of the patchouli oil following the trapping of the unpaired electrons of DPPH. The patchouli oil exhibited antioxidant activity with an IC_{50} value were 22.45 ± 0.2974 and 19.87 ± 0.4051 µg/ml of patchouli oil from Cibinong and Batu respectively, when compared to the IC_{50}value of vitamin E were 5.37 ± 0.5701µg/ml and ascorbic acid 9.93 ± 0.4161µg/ml. Patchouli alcohol is a sesquiterpene alcohol compound that can participate potentially in antioxidant effect. The patchouli oil has also phenol and terpene components which are also having antioxidant effect.

The antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron or hydrogen from substances to an oxidizing agent. Oxidation responses can deliver free radicals. Thus, these radicals can begin chain responses, when the chain responses happen in a cell, it can make harm or passing the cell [24]. The antioxidant activity of the patchouli oil was estimated by DPPH assay. The DPPH examine is an advantageous strategy for screening little cell reinforcement atoms in light of the fact that the response can be broke down by a basic spectrophotometric measure [25]. The graph of the variation of the percentage of inhibition depending on the concentration of the essential oil allowed to determine the IC_{50} corresponding to 50 % of inhibition, which constitutes the antioxidant activity of the essential oil. This value was compared to the one or two found for the reference compound. The IC_{50} is inversely related to the antioxidant capacity of a component since it shows the quantity of antioxidant required to decrease the concentration of the free radical of 50 %. The lower value

of IC_{50} means the higher antioxidant activity of a compound.

According to Scherer and Godo [20], the antioxidant activity was expressed as the antioxidant activity index (AAI). The AAI was calculated considering the mass of DPPH and the mass of the tested compound in the reaction, resulting in a constant for each compound, independent of the concentration of DPPH and sample used. The component was to show poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when AAI > 2.0. In this study, we considered the Cibinong and Batu patchouli oil were to show as moderate antioxidant activity (AAI = 0. 70 and 0.79 respectively), even though vitamin E (AAI=15.92) and ascorbic acid (AAI=29.44) to show very strong antioxidant activity.

5. Conclusion

In this study, we identified that *P. cablin* collected from different cultivation regions (Cibinong and Batu) and harvested at different times showed differences in their volatile oil compositions. As chemical constituents of *P. cablin* vary depending also on the origin and plant parts, a protocol needs to obtain the patchouli oil. We have shown that patchouli oils from this *P. cablin* showed moderate antioxidant activity.

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Conflict of interest

Authors declare no conflict of interest.

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