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Profiling and Phenolic Content of Patchouliioil (*Pogostemon cablin* Benth.)

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Abstract: *Patchouli oil* is an essential oil obtained from the distillation of leaves, stems, and branches of patchouli plants. Patchouli contains essential oils, patchouli alcohol and its derivatives, phenols, and a group of terpenoids in patchouli oil has antibacterial activity. The purpose of this study was to determine the total phenolic content of patchouli oil using KLT-Densitometry with a wavelength of 254 nm with gallic acid comparison. The test results of patchouli oil had a total phenolic content of 0.97619 gGAE/g extract or 97.619%. Validation of the Thin Layer Chromatography - Densitometry analysis method needs to be done to ensure that the method is suitable to be applied as a method of analyzing phenolic content in patchouli oil. Based on the test results of the validation parameters which include linearity, LOD (*limit of detection*), LOQ (*limit of quantifitation*), precision, and accuracy. The TLC-Densitometry method for determining phenolic levels in patchouli oil is said to be valid because it meets the validity test requirements, namely linear test with a value of r = 0.9909, LOD = 0.012 ppm; LOQ = 0.040 ppm; precision with a %RSD value of 0.059% and accuracy with a % recovery of 119.938%.

Keywords- *A Patchouli oil, Pogostemon cablin Benth., gallic acid, KLT-Densitometry*

I. INTRODUCTION

Essential oils can be produced from various parts of the plant, such as roots, stems, twigs, leaves, flowers, and fruits. Essential oils in plants play an important role for health. Essential oils can be obtained from plants, one of which is patchouli. Patchouli is a fragrant shrub plant with fine leaves and rectangular stems. The main function of patchouli oil is as a raw material for the binder (fixative) of its main content component, namely patchouli alcohol (15 26). *Patchouli oil* is used as a mixture of cosmetic products (including for making soap, toothpaste, shampoo, lotion, and deodorant), food industry needs (including for essence or flavor enhancers), pharmaceutical needs (for making anti-inflammatory drugs, antifungal, antinsect, aphrodisiac, anti-inflammatory, antidepressant, antiphlogistic, and decongestant), aromatherapy needs, compound raw materials and preservation of goods, and various other industrial needs (Waruwu, 2022). Because of the many health benefits of *patchouli oil*, people in the Barru area manage *patchouli oil* to be exported to industry as an ingredient for making products.

In addition, patchouli oil also contains phenolic and terpenoid groups that have antibacterial activity (Tahir *et al*, 2017). Phenolic compounds are secondary metabolite compounds derived from patchouli.

Based on the description above, this is the background of the researcher wants to know the Profile and Determination of Phenolic Content contained in *Patchouli Oil* (*Pogostemon cablin* Benth.) by KLT-Densitometry method.

II. METHOD

1. Materials and Tools

The tools used in this study are *Syngene Chromascan* TLC *Capture and Analysis System Densitometry* Tool, porcelain cup, KLT plate, chamber, vial, volume pipette, capillary pipette, micropipette, set of glassware, stirring rod, test tube, analytical balance. The materials used are gallic acid, distilled water, ethyl acetate, n-hexane, chloroform, methanol, □□□□3 reagent, □□□□3 reagent, Dragendroff reagent, Liberman Burchard reagent, methanol PA and Patchouli Oil (*Pogostemon cablin* Benth.).

2. Work Procedure

The work procedures in this study are:

2.1 Extraction

The sample was weighed 100 grams and then put into a round bottom flask, then added toluene and distilled water until all the simplisia was submerged, then heated until the distillate came out and was accommodated in an erlenmeyer that had been installed in a distillation device. The distillate obtained forms two layers, the oil layer is above while the water is below because the specific gravity of oil is smaller than the specific gravity of water. The distillate obtained was put into a separating funnel, shaken and then separated the water phase and oil phase Furthermore, sodium sulfate is added to bind the water that is still left in the oil phase so that water-free oil will be obtained (Nurdin, *et al*. 2022).

2.2 Qualitative Analysis with KLT Profile

a. Mobile phase optimization
Patchouli oil (*Pogostemon cablin* Benth.) was bottled on a KLT plate, then inserted into a chamber consisting of chamber 1 containing ethyl acetate: n-hexane eluent and chamber 2 containing chloroform: methanol (7:3). After the eluent reaches the mark, remove and dry. Then observe the spots in UV light at wavelengths of 254 nm and 366 nm then calculate the Rf value.

b. Identification of compounds
From the results of mobile phase optimization, then sprayed with reagents:
1) $FeCl_3$ 1% positive results when a black stain is formed (Kinam, *et al*, 2021).
2) $AlCl_3$ 1% The sample is said to be positive if a brownish yellow stain is formed (Kinam, *et al*, 2021).
3) Dragendroff is positive if an orange or red stain is formed (Kinam, *et al*, 2021).
4) Libermann Burchard (anhydrous acetic acid and concentrated sulfuric acid) 1 mL Samples containing terpenoid compounds will form green-blue stains (Kinam, et al, 2021).

2.3 Determination of Phenolic Content with KLT - Densitometry

a. Preparation of Gallic Acid Standard Solution
Standard solution of gallic acid 1000 ppm was made by weighing 100 mg of gallic acid dissolved with methanol p.a to a volume of 10 mL. From the stock solution was pipetted as much as 2.5 mL diluted with methanol p.a to a volume of 25 mL resulting in a concentration of 10000 ppm. From the solution was pipetted 1, 2, 3, 4, 5 mL and sufficed with methanol p.a up to 10 mL, resulting in concentrations of 1000, 2000, 3000, 4000 and 5000 ppm.

b. Preparation of patchouli oil extract solution (*Pogostemon cablin* Benth.)
Patchouli oil extract (*Pogostemon cablin* Benth.) was made by weighing 10 mg each of the sample extract then dissolved with 10 mL of n-hexane. Replication was carried out 3 times.

c. Analysis by KLT Densitometry
A 10 x 10 cm KLT plate was prepared, marked with 0.5 cm top edge and 1 cm bottom edge. From the standard solution, each concentration was localized using a micropipette. Then each sample of patchouli oil (*Pogostemon cablin* Benth.) which has been dissolved with n-hexan was bottled on the same KLT plate and replicated 3 times. The plate was eluted in space with the appropriate elution solution. Furthermore, observed under UV lamp 254 nm and 366 nm and measured by densitometry at a wavelength of 254 nm and analyzed the scan results.

d. Phenolic content calculation

$$\text{total phenolic content} = \frac{C \times V \times Fp}{M}$$

Description:

C = gallic acid concentration

V = Volume of Extract

M = Weight of extract

Fp = Dilution factor

e. Data analysis

The data collected is primary data obtained from the absorbance of gallic acid comparison solution and obtained a linear regression equation. The phenolic content of the compound is calculated by entering into the linear regression equation y = ax + b, then the data obtained is analyzed by linear regression analysis using Microsoft Excel calculation application.

f. Method validation

1) Accuracy test Determination of accuracy is bottled on a KLT plate, eluted with mobile phase then measured on a densitometer. The test results were calculated by the formula:

$$\%\ recovery = \frac{measured\ level}{true\ level} \times 100\%$$

2) Precision testis done by making gallic acid solution with a concentration of 1000 - 5000 ppm, diluted to the limit mark. Then measured on a densitometer. The test results were calculated standard deviation (SD) using the formula:

$$SD = \frac{\sqrt{\sum(xi - \overline{xi})^2}}{n-1}$$

$$RSD\ (\%) = \frac{SD}{\bar{x}} \text{ x } 100\%$$

3) A good linearity value is $0.997 \leq r \leq 1$

4) The LoD and LoQ equations are as follows:

$$LOD = \frac{3\ S\left(\frac{y}{x}\right)}{b} LOQ = \frac{10\ S\left(\frac{y}{x}\right)}{b}$$

III. RESULT AND DISCUSSION

Patchouli (*Pogostemon cablin* Benth.) belongs to a group of plants from the *Lamiaceae* family. This *Lamiaceae* family has around 200 genus, and one of them is *Pogostemon.* This *Pogostemon* genus has 40 species, and patchouli plants are one of them. Patchouli oil has a variety of uses both in industry and health. Such as cosmetic ingredients, soaps, perfumes, wound healing drugs, heat reduction, can eliminate scars, and have pharmacological effects. And one of the compounds contained in patchouli oil is phenolic compounds which have antibacterial, anti-inflammatory, antifungal, anti-inflammatory, antidepressant and decongestant activities (Waruwu, 2022).

This study aims to determine the phenolic content of patchouli oil using KLT-Densitometry with gallic acid comparison solution. Gallic acid is one of the natural and stable phenolics. Gallic acid is included in

phenolic compounds derived from hydroxybenzoic acid which is classified as a simple phenolic acid (Supriningrum, *et al*, 2020).

Qualitative tests of patchouli oil (*Pogostemon cablin* Benth.) were carried out, namely the identification of chemical compounds using Thin Layer Chromatography (KLT). This aims to prove previous research related to phytochemical compounds contained in patchouli oil. Identification of the compound group was carried out using KLT F_254 and using optimization of the mobile phase of the eluent n-hexan: ethyl acetate (9: 1). And chloroform: methanol (7:3). The results of mobile phase optimization can be seen in Figure 1

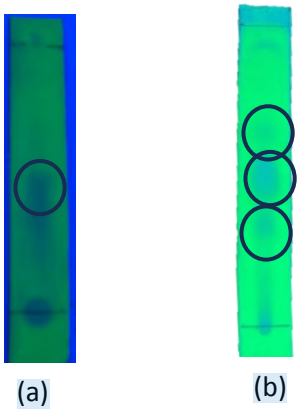


Figure 1. Mobile phase optimization results, (a) chloroform: methanol (7:3); (b) n-hexan: ethyl acetate (9:1)

Based on the optimization results, n-hexan: ethyl acetate (9:1) is the best mobile phase that can separate the compounds contained in patchouli oil. The reason for using the eluent is because it is a combination of two kinds of non-polar solvents and polar solvents, this is intended to achieve a level of polarity so that the eluent can elute stains with different levels of polarity. The ratio (9:1) was used because patchouli oil is soluble in n-hexane and when observed at UV lights 254 nm and 366 nm. The results of qualitative analysis of chemical compound content

TABLE 1. Qualitative test results of Patchouli Oil (*Pogostemon cablin* Benth.) by Thin Layer Chromatography method.

KLT plate	Number of spot stains formed	Rf Value		Color
		UV 254 nm	UV 366 nm	
Patchouli Oil Sample	3	0,50 0,63 0,69	0,50 0,63 0,69	Blackish brown
Gallic acid comparator standard	1	0,63	0,63	Blackish brown

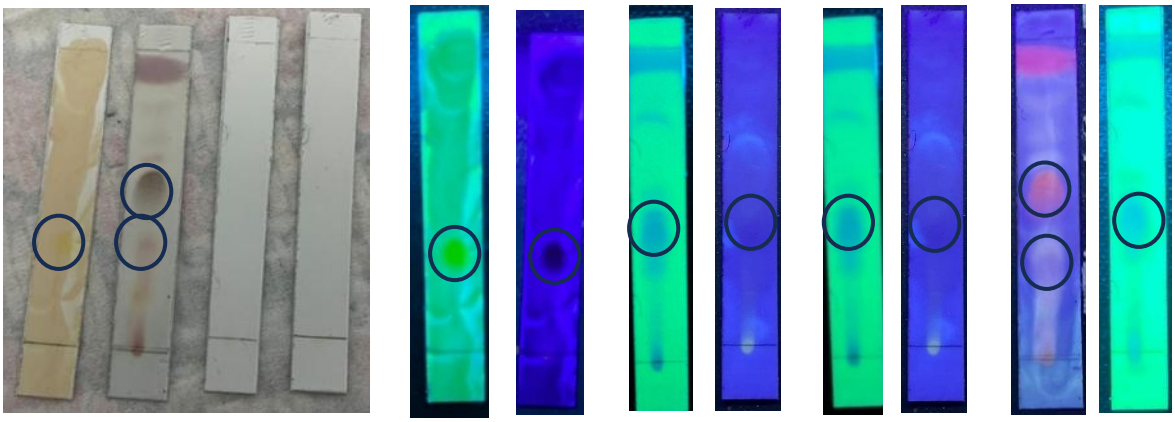
The results of the KLT plate obtained the number of spots formed for the sample as many as 3 spot stains and for gallic acid comparison standard as many as 1 spot after elution. Based on qualitative tests, the value of Rf1= 0.50, Rf2= 0.63 and Rf3= 0.69 has met the requirements of a good Rf value of 0.2 to 0.8 (Aritonang, et al. 2022). Then profiling was carried out using 4 reagents, the results of the patchouli oil chemical compound test can be seen in table 2 below.

TABLE 2. Test results of Patchouli Oil chemical compounds (*Pogostemon cablin* Benth.) with specific reagents.

Compound	Reagents	Results	Description

Phenolic	$FeCl_3$ 1%	+	Black stains formed
Flavonoids	$AlCl_3$ 1%	-	No color change occurs
Alkaloids	Dragendroff	-	No color change occurs
Triterpenoids	Lieberman-Burchard	+	Formation of brownish or violet stains

From the test results of chemical compounds that showed positive, namely phenolic compounds and triterpenoids, where the KLT plate dripped with reagent $FeCl_3$1% positive results in black color changes. KLT plates dripped with Liberman-burchard reagent are brownish or violet in color (Kinam, *et al*, 2021).



(a) (b) (c) (d) (e)

Figure 2. Qualitative test results of chemical compounds by thin layer chromatography (a) visible light; (b) phenolic compounds; (c) flavonoid compounds; (d) alkaloid compounds; (e) triterpenoid compounds.

Then proceed to measure the amount of phenol contained in the extract. The determination of phenolic content was measured using densitometry. KLT-Densitometry has the advantage of being able to separate the intended compounds from other components, and can determine the levels of several samples simultaneously (Fatimah, et al, 2020).

TABLE 3. Area measurement results of gallic acid standard stains and patchouli oil samples (*Pogostemon cablin* Benth.)

Replication	**Concentration (ppm)**	**Rf**	**Area**
Gallic acid standard	1000	0,89	1946577.1
	2000	0,87	2636447.5
	3000	0,75	3185712.5
	4000	0,79	3457476.3
	5000	0,86	4029260.3

y = 498.64x + 1555; R² = 0.982			
Patchouli oil	1000	0,87	445632.6
	1000	0,86	474899.9
	1000	0,89	544440.3

Based on the linear regression obtained from the gallic acid comparator is y = 498.64x + 1555; R² = 0.982, and on the patchouli oil sample is y = 49404x + 389517; R² = 0.9475. The equation was then entered into the equation y = bx + a, where y is the AUC and x is the sample replication value (Figure 3). Then calculate the total phenolic content and average phenolic content in patchouli oil samples. The results of the calculation of phenolic content can be seen in table 4 below.

TABLE 4. Calculation results of the average phenolic content in Patchouli Oil (*Pogostemon cablin* Benth.) using KLT-Densitometry

Sample	**Area**	**Total phenolic content (mgGAE/g)**	**Average phenolic content (gGAE/g)**
Sampel 1	445624.72	890,57	0,97619
Sampel 2	474892.02	949,27	
Sampel 3	544432.42	1088,73	

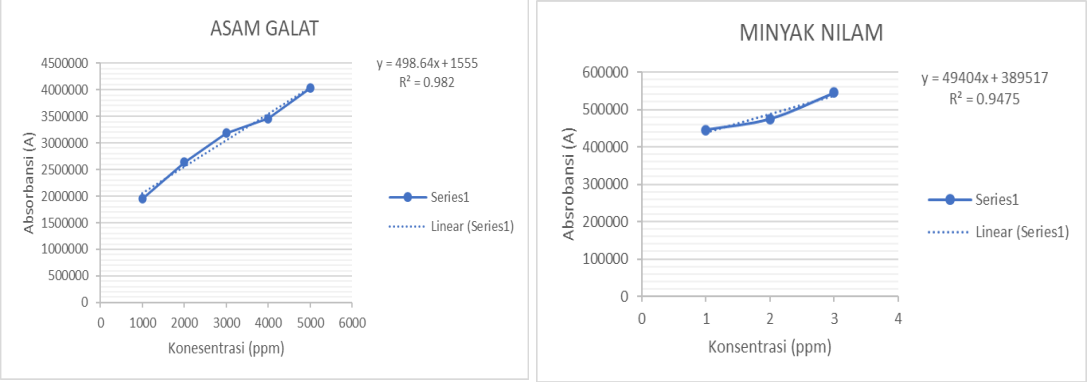


Figure 3. Standard curve graph of Gallic Acid comparator and Patchouli Oil (*Pogostemon cablin* Benth.) sample solution.

Gallic acid analysis by KLT densitometry gives Rf values for gallic acid comparison and samples can be seen in Table 3. Resolution is influenced by the space saturation factor and although the score is not very good, the difference is very small. To determine the phenol content in patchouli oil (*Pogostemon cablin* Benth.) from the equation y = 498.64x + 1555 the average content of 3 replicates can be seen in Table 4. is 0.97619 gGAE/g extract or 97.619%. The greater the content of phenolic compounds, the greater the antibacterial activity.

Analysis method validation parameters include linearity, precision, detection limit, quantitation limit, and accuracy. The KLT-Densitometry method is expected to be able to meet the validation parameters, which can produce good accuracy and precision and meet other criteria such as specificity, detection limit, quantitation limit, and linearity so that valid and reliable analysis results can be obtained (Fatimah, *et al*. 2020).

TABLE5. Linearity test results of gallic acid comparator solution

Concentration (ppm)	**AUC (mV)**

1000	1946577.1
2000	2636447.5
3000	3185712.5
4000	3457476.3
5000	4029260.3

The linearity of a method is indicated by the correlation coefficient (r) of the standard curve, where the r value shows the relationship between the concentration of gallic acid standard solution and the AUC value. The test results showed a linear relationship between the phenolic content and the AUC value on the densitometer, namely the value of r = 0.9909. The correlation coefficient above shows that the relationship between concentration and absorbance has a better value because it is closer to 1 in accordance with the literature which states the acceptance criteria, namely the correlation coefficient (r) value close to 1 ($0.990 \leq r \leq 1$) (Syafitri, *et al*. 2020).

Limit of detection (LOD) testing is carried out to determine the lowest concentration of analyte in the sample that can still be detected. While *Limit of quantification* (LOQ) testing is carried out to determine the lowest concentration of analyte in the sample determined from the precision and accuracy that can be accepted under the operational conditions of the method used (Fatimah, *et al*. 2020). The results of the linear regression equation obtained an LOD value of 0.012 ppm while for the LOQ value of 0.040 ppm.

TABLE 6. Results of LOD and LOQ test data

Concentration (ppm)	**Area (Y)**	**Yi**	$(Y - Yi)^2$
1000	1946577.1	500195	2.092
2000	2636447.5	998835	2.681
3000	3185712.5	1497475	2.85
4000	3457476.3	1996115	2.135
5000	4029260.3	2494755	2.354
		Total Average S(y/x) LOD LOQ	12.112 4.0373 2.009 0.012 0.040

Precision and Accuracy is an analytical method that is tested said to meet the precision parameters indicated by the value of the percent *coefficient of variation* (KV) or percent *relative standard deviation* (%RSD). Based on the data presented in Table 7, the accuracy and precision parameter data for testing phenolic levels with the KLT densitometry method include % recovery of 119.938%. The accuracy test is acceptable if the % recovery obtained is in the range of 80-120% (Rohmah, *et al*. 2021). SD 0.298, coefficient of variation (%KV) or %RSD 0.059%, RSD value that can meet the precision test criteria is ≤ 2% (Rohmah, *et al*. 2021). Thus, it can be concluded that the tested gallic acid standard analysis method meets the precision and accuracy parameters.

TABLE 7. Precision and accuracy of gallic acid standard using KLT-Densitometry

Gallic Acid	Area	Concentration	Average	%	Average %	SD	%RSD

Raw Concentration		(ppm)	Concentration (ppm)	Recovery	Recovery	(ppm)	
1000	1946577.1	1946573.9	2406.5	194.657	119.938	0.298	0.059
2000	2636447.5	2636444.3		131.822			
3000	3185712.5	3185709.3		106.190			
4000	3457476.3	3457473.1		86.436			
5000	4029260.3	4029257.1		80.585			

IV. CONCLUSION

Based on the results of the research that has been done, it can be concluded that the profile of patchouli oil (*Pogostemon cablin* Benth.) with KLT is done with the mobile phase of the eluent n-hexan: ethyl acetate (9: 1). Patchouli oil (*Pogostemon cablin* Benth.) contains phenolic with phenolic content of 0.97619 gGAE/g (97.619%). Validation of analytical methods performed on the test parameters of linearity, accuracy, precision, LOD and LOQ measured by KLT-Densitometry has met the requirements. The validation value of the analytical method of linearity results with a value of r = 0.9909, LOD = 0.012 ppm; LOQ = 0.040 ppm; precision with a % RSD value of 0.059% and accuracy with a % recovery result of 119.938%.

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