Volume 07, Issue 04 (July-August 2024), PP 01-08 www.ijmsdr.org
ISSN: 2581-902X

International Journal of Medical Science and Dental Research

STANDARDIZATION Of PATCHOULI OIL (*Pogostemon Cablin* Benth.)

Aktsar Roskiana Ahmad[1], Putri Utami Astuti[2] , Virsa Handayani[3].
[1,2,3]*(Department Farmakognosi-Fitokimia, Universitas Muslim Indonesia, Makassar)*
[1]*(Magister Pharmacy, Universitas Muslim Indonesia, Makassar)*

Abstract : *Patchouli oil* is one type of vegetable oil derived from patchouli plants, which is currently widely cultivated in Indonesia. Patchouli has essential oils, flavonoids, saponins, tannins, glycosides, terpenoids, steroids. The purpose of this study was to standardize the specific and non-specific parameter values of patchouli oil, as well as the analysis of Patchouli Alcohol levels with GC-MS instruments. The results of the study obtained the specific parameter values as follows; organoleptic test obtained oil in liquid form, brownish yellow in color, distinctive odor and tasteless; positive for phenolic and terpenoid; and non-specific parameter values as follows: specific gravity 0.968 g/mL; total plate count < 1.0 × 100 colonies/ml; mold and yeast count < 1.0 × 100 colonies/ml; lead metal (Pb) contamination 0.244 mg/L. PA analysis value 31.80%

Keywords - *A Patchouli oil, Pogostemon cablin Benth, Standardization, GC-MS*

I. INTRODUCTION

Patchouli oil is an essential oil obtained from patchouli plants by distillation. Patchouli oil distillation is a process of taking oil from dry material with the help of water (Bahri, 2021). The distillation results in brown oil, which is soluble in alcohol, volatile and can be mixed with other essential oils (Muna et al., 2021). Patchouli oil is one type of vegetable oil derived from patchouli plants, which is currently widely cultivated in Indonesia and is the main ingredient in the mixture of cosmetic products, food industry, medicines and other industrial needs (Jumardin et al., 2019).

Traditionally, *patchouli oil* has been utilized medicinally and is best known for its antiseptic qualities and its use to treat skin problems, scalp, athlete's foot, dandruff, acne, dermatitis, and to help heal wounds and scars. Patchouli oil is also used as a topical remedy for skin problems such as acne, eczema skin, inflammation, cracking, and irritation. For the nervous system, essential patchouli oil helps reduce tension, insomia, and also anxiety. It is also known as an uplifting fragrance, a calming aid and a food flavor carrier (Base & Syamraharji, 2018).

Based on the description above, this study was conducted to standardize *patchouli oil* (*Pogostemon cablin* Benth.) from Barru city. To find out whether *patchouli oil* (*Pogostemon cablin* Benth.) meets the quality requirements as a medicinal ingredient.

II. METHOD

1. Materials and Tools

The tools used in this research are analytical scales, aluminum foil, porcelain cups, petri dishes, chambers, funnels, erlenmeyers, incubators, UV lamps 254 nm and 366 nm, silica plates, ovens, capillary pipes, dropper pipettes, pycnometers, tube clamps, atomic absorption spectrophotometers, test tubes, and furnaces, a set of GC-MS tools. The materials used in this study are hot water, distilled water, N-Hexan, ethyl acetate, patchouli oil, FeCl3, HCl 2 N, concentrated HCl, filter paper, Libermann- Bauchard reagent, mayer and wagner reagent, HNO3, Mg powder, and tissue.

2. Work Procedure

The work procedures in this study are:

2.1 Standardization of specific parameters

2.1.1 Extract identity parameters

The identity parameter of the extract is carried out by providing a description of the name which includes the name of the extract, the Latin name of the plant, the plant part used, and the Indonesian name of the plant (Ditjen POM, 2000).

2.1.2 Organoleptic tests

Organoleptic tests are carried out using the five senses to describe the shape, color, smell, and taste of plant extracts (Ditjen POM, 2000).

2.1.3 Chemical content test of extracts

a. Alkaloid

Alkaloid test can be done by detection of color or spot on the KLT plate that has been bottled extract and eluted. This test is done by spraying Dragendorff reagent on the KLT plate and by heating for ± 5 minutes at 100^0C. Positive results are indicated by the formation of orange color on the spot (Raihan et al., 2020).

b. Flavonoid

Flavonoid test can be done by detecting the color or spot on the KLT plate that has been bottled extract and eluted. This test is carried out by spraying cyroborate reagent on the KLT plate and by heating for ± 5 minutes at 1000C. Positive results are indicated by the formation of a yellow color on the spot visibly (Raihan et al., 2020).

c. Phenolic

Extracts that have been bottled on silica gel GF254 KLT plate using n-hexan:ethyl acetate (9:1) eluent until completely eluted, then observed spots at UV 254 nm and 366 nm. After that, it was sprayed with 1% FeCl3 reagent. Positive results contain phenol if the stain is black (Ramadhan et al., 2021).

d. Terpenoid

In the terpenoid test can be done by detecting the color or spot on the KLT plate that has been bottled extract and eluted. This test is done by spraying Lieberman-Burchard reagent on the KLT plate. Positive results are indicated by the formation of red-purple, dark blue or blackish green color on the spot visibly (Raihan et al., 2020).

2.2 Standardization of non-specific parameters

2.2.1 Specific gravity

Use a clean, dry and calibrated pycnometer by determining the weight of the pycnometer and the weight of freshly boiled water at 25°C. Set the temperature of the liquid extract to approximately 20°C and add it to the pycnometer. Set the temperature of the filled pycnometer to 25°C, remove

the excess liquid extract and weigh. Subtract the weight of the empty pycnometer from the weight of the filled pycnometer. The specific gravity of the liquid extract is the result obtained by dividing the weight of the extract by the weight of water, in a pycnometer at 25°C (Ditjen POM, 2000)

2.2.2 Microbial contamination

a. Total Plate Count (ALT)

The total plate number test was carried out using the serial dilution method. A total of 1 ml of patchouli oil that has been diluted with distilled water in a test tube is added to 9 ml of 0.9% NaCl to obtain a concentration of 10-1 dilution is continued until a concentration of 10-5 is obtained. The dilution results from each tube were pipetted 1 ml and then poured on a sterile petri dish to be given PCA media in a pour plate. Blank was made by pouring 1 ml of 0.9% NaCl into a sterile petri dish and then adding PCA by pour plate. Petri dish was incubated at 37ºC for 24 hours - 48 hours petri dish in an inverted state. Then observations were made related to the number of colonies which were then multiplied by the dilution factor (Rahmawati et al., 2022).

b. Mold Yeast Number (AKK)

The yeast mold number test was carried out using the serial dilution method with three levels of dilution, namely concentrations of 10-1 to 10-3. The results of the dilution were inoculated in a pour plate on a sterile Petri disk with PDA media. Blank was made by pouring 1 mL of 0.9% NaCl into a sterile petri dish and then adding PDA by pour plate. Petri dish was incubated at 25ºC for 5 - 7 days. Then observations were made related to the number of colonies which were then multiplied by the dilution factor (Rahmawati et al., 2022).

2.2.3 Heavy metal contamination

Weighed as much as 2 grams of extract was put into a porcelain cup and then fumigated using a furnace for ± 2 hours at 600 ° C. Then the ash was dissolved using HNO3 and filtered and then sufficient volume to 25 mL. Then the ash was dissolved using HNO3 and filtered and then the volume was sufficient to 25 mL. the sample was analyzed using an atomic absorption spectrophotometer with a wavelength of 217 nm for Pb (lead) (Handayani et al., 2019).

2.3 Analysis of *patchouli alcohol* content with GC-MS

Determination of the *patchouli alcohol* component of *patchouli oil* samples was carried out using a set of gas chromatography-mass spectrometer (GC-MS) Thermo scientific TRACE 1310. The analysis conditions used a TG-5MS capillary column, 30m long, 0.25mm diameter, injector temperature 250°C, helium carrier gas with a flow rate of 1ml/min. The column temperature was temperature programmed with an initial temperature of 50°C for 5 minutes, then increased slowly at a rate of 10°C/minute to a final temperature of 280°C which was maintained for 15 minutes (Teruna & Rahayu, 2021).

III. RESULT AND DISCUSSION

This *patchouli oil* (*Pogostemon cablin* Benth.) standardization research aims to determine the value of specific parameters and non-specific parameters of patchouli oil so that in the future it can provide information to the public in the form of scientific data that ensures product safety in accordance with general product standards, and its use can be accepted and used as raw material for traditional medicine. Specific parameters include extract identity, organoleptic, extract chemical content test. The non-specific parameters include specific gravity, microbial contamination and heavy metal contamination. In addition, GC-MS analysis was also carried out with the aim of knowing the PA levels contained in *patchouli oil*.

There are several stages in this research, namely preparation of tools and materials, sample processing, standardization and analysis by GC-MS. Patchouli oil was first filtered, then continued with the standardization process. Specific parameters are parameters that focus on compounds or classes of compounds in the sample. These parameters include extract identity, extract organoleptic examination, and extract chemical content test.

Meanwhile, non-specific parameters include specific gravity, microbial contamination and heavy metal contamination.

TABLE 1. The results of determining the identity parameters of patchouli oil (*Pogostemon cablin* benth.)

Extract identity parameters	Results
Name	Patchouli oil
Parts used	Leaves
Name of simplisia	*Pogostemon cablin* Benth
Latin name	*Pogostemon cablin* Benth
Indonesian name	Patchouli oil

The table above is the result of determining the identity parameters of patchouli oil. This identity parameter aims to provide an objective identity of the plant used (Ditjen POM, 2000).

TABLE 2. Results of organoleptic parameters determination of patchouli oil (*pogostemon cablin* Benth)

Organoleptic parameters	Results
Form	Liquid
Color	Brownish yellow
Odor	Typical
Taste	Bitter

Organoleptic parameters of extracts aim for simple and subjective initial recognition using the five senses (Ditjen POM, 2000). Based on (Table 2), the results of organoleptic determination of patchouli oil in the form of liquid concentration, brownish yellow color, distinctive odor and bitter taste.

Then test the chemical content of the extract which aims to provide an initial description of the composition of the chemical content of the extract (Ditjen POM, 2000). In the chemical content identification test includes alkaloid, flavonoid, terpenoid, and phenolic tests the results can be seen in (Table 3.). This test was carried out with the aim of providing an overview of the chemical content contained in patchouli oil.

TABLE 3. Chemical content identification results of patchouli oil (*Pogostemon cablin* benth.)

Compound	Reagent	Result	Description
Phenolic	$FeCl_3$ 1%	+	Formed black stains
Flavonoids	$AlCl_3$ 1%	-	No color change occurs
Alkaloids	Dragendroff	-	No color change occurs
Terpenoids	Lieberman-Burchard	+	Formation of red-purple color

The chemical content test of patchouli oil was carried out by Thin Layer Chromatography (KLT). For alkaloid, flavonoid, phenolic and terpenoid compounds using eluents such as N-Hexan: Ethyl acetate (9:1).

From the test results of chemical compounds that show positive are phenolic compounds and terpenoids, 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).

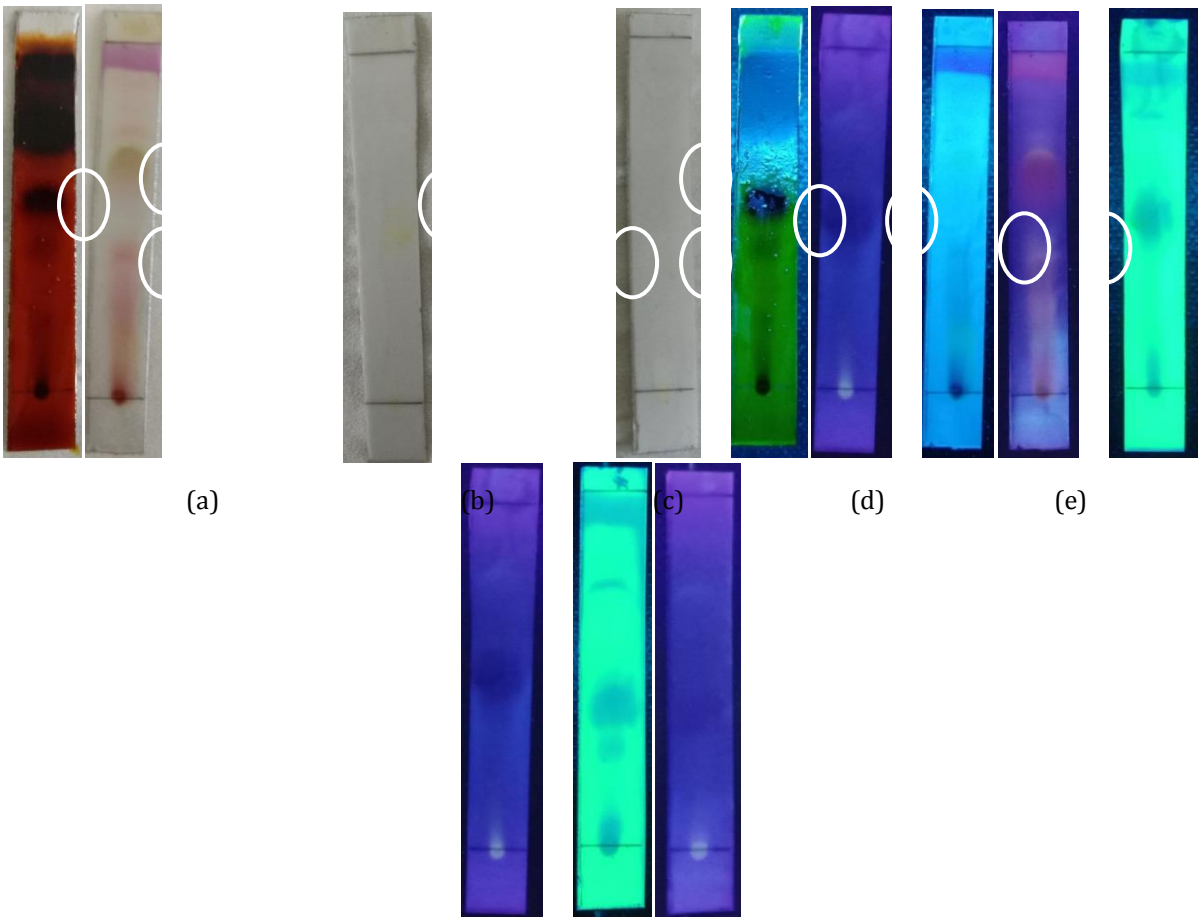

Figure 1. Qualitative test results of chemical compounds by thin layer chromatography (a) visible light; (b) phenolic compounds; (c) terpenoid compounds; (d) alkaloid compounds; (e) flavonoid compounds

TABLE 4. Results of determination of specific gravity of patchouli oil (*Pogostemon cablin* benth.)

Parameters	**Results (g/mL)**	**Condition**
Specific gravity determination	0.968 g/mL	0.950-0.975

According to SNI 06-2385-2006, the specific gravity value of patchouli oil ranges from 0.950-0.975 and the specific gravity result obtained is 0.968 g/mL which means it meets the requirements.

Then the microbial contamination parameter is carried out to identify the presence of pathogenic microbes which aims to provide assurance that the extract should not contain pathogenic microbes and non-pathogenic microbes exceeding the specified limits because it affects the stability of the extract and is harmful (toxic) to health (Ditjen POM, 2000 & Ahmad et al, 2023).

TABLE 5. Microbial contamination test results of patchouli oil (*Pogostemon cablin* benth.)

Parameters	**Results**	**Condition**

Bacterial total plate count	< 1.0 × 100 colonies/ml	-
Yeast mold number	1.0 × 100 colonies/ml	-

Based on the results of microbial contamination testing in the table, patchouli oil has a total bacterial plate number and yeast mold number of < 1.0 × 100 colonies / ml. There are no requirements for the total bacterial plate number and yeast mold number of patchouli oil and it is not in the scope of ISO 17025.

TABLE 6. Metal contamination test results of patchouli oil (*Pogostemon cablin* Benth.)

Parameters	**Results**	**Condition**
Lead (Pb)	0.244 mg/L	≤ 10 mg/Kg

Testing for Pb heavy metal contamination is carried out using an atomic absorption specrophotometer where the preparation of extract samples for metal contamination testing uses nitric acid (HNO3) which aims to dissolve metal analytes and dissolve other compounds from the metal to be analyzed.

According to the Decree of the Food and Drug Administration Number 12 concerning the maximum limit of metal contamination states that the maximum limit of lead metal contamination (Pb) is ≤ 10 mg/Kg (BPOM RI, 2014). In the examination of patchouli oil metal contamination, the result for lead is 0.244 mg/L, which means it meets the requirements.

TABLE 7. Analysis of Patchouli Alcohol content with GC-MS

Parameters	**Results**	**Condition**
Patchouli Alcohol	31.80%	≤30%

According to SNI 06-2385-2006, PA content is at least 30%, the greater the PA content, the better the quality of patchouli oil. The results of the analysis of patchouli oil PA content amounted to 31.80% which shows the quality and quality of patchouli oil is getting better.

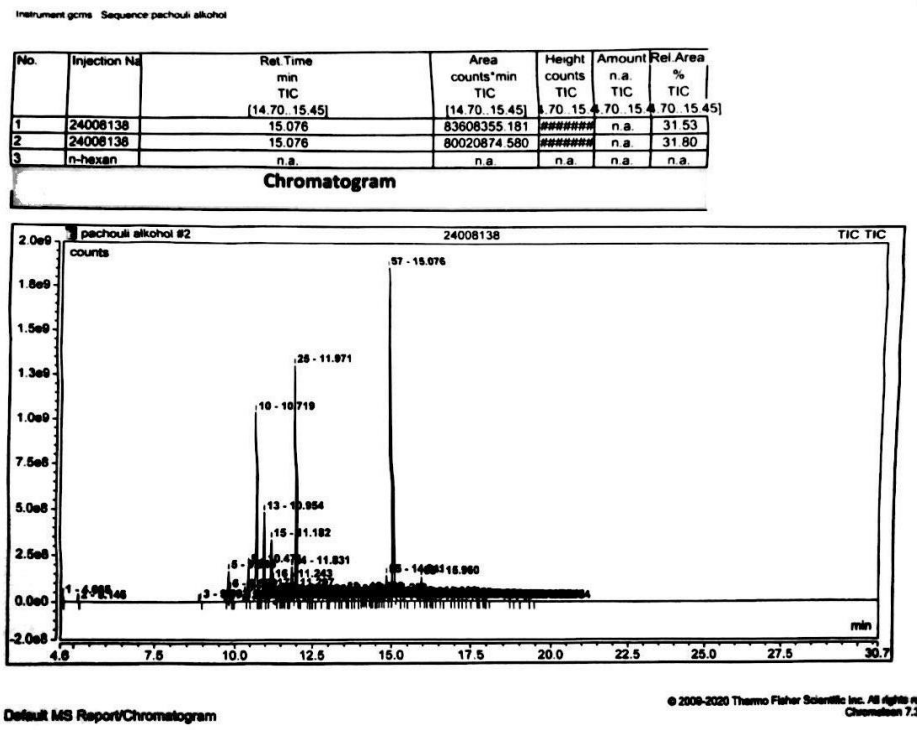

Figure 2. The GC-MS curve graph shows that the patchouli alcohol content is at the highest peak with a level of 31.80%.

IV. CONCLUSION

Based on the results of the research that has been done, it can be concluded that patchouli oil (Pogostemon cablin Benth.) gives results according to the general standard parameters of medicinal plant extracts with specific parameter values as follows organoleptic test obtained oil in liquid form, brownish yellow in color, distinctive odor and tasteless; positive contains phenolic and terpenoid senyala; and non-specific parameter values as follows: specific gravity 0.968 g/mL; total plate count < 1.0 × 100 colonies/ml; mold and yeast count < 1.0 × 100 colonies/ml; lead metal contamination (Pb) 0.244 mg/L. Patchouli alcohol content of 31.80% indicates that the quality and quality of patchouli oil has met the SNI requirements.

REFERENCES

[1] Bahri, S. (2021). 4179-10557-1-Sm. *Formulasi Sediaan Gel Minyak Atsiri Tanaman Nilam (Pogostemon Cablin Benth) Sebagai Antiseptik Tangan (Hand Sanitizer)*, *1*(Mei), 87–99.

[2] Muna, T., Zakaria, N., & Fonna, L. (2021). FORMULASI DAN EVALUASI SEDIAAN SABUN CAIR MINYAK ATSIRI DAUN NILAM (Pogostemon cablinBenth.). *Jurnal Sains Dan Kesehatan Darussalam*, *1*(1), 10.

[3] Jumardin, J., Ilham, J., & Salim, S. (2019). Studi Karakteristik Minyak Nilam Sebagai Alternatif Pengganti Minyak Transformator. *Jambura Journal of Electrical and Electronics Engineering*, *1*(2), 40–48.

[4] Base, N. H., & Syamraharji, R. (2018). Uji Efek Antiinflamasi Pemberian Minyak Nilam (Pogostemon cablin, Benth) Secara Topikal Terhadap Udem Pada Kulit Punggung Mencit Jantan (Mus musculus). *Jurnal Kesehatan Yamasi Makassar*, *1*(1), 10–27.

[5] Ditjen POM, D. R. (2000). Parameter standar umum ekstrak tumbuhan obat, Jakarta: Departement Kesehatan Republik Indonesia. *Edisi IV*, 9–11, 16.

[6] Raihan, M., Taqwa, N., Hanifah, A. R., Lallo, S., Ismail, I., & Amir, M. N. (2020). SKRINING FITOKIMIA EKSTRAK KULIT BUAH NANGKA (Artocarpus heterophyllus) DAN AKTIFITAS ANTIOKSIDANNYA TERHADAP [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] (ABTS). *Majalah Farmasi Dan Farmakologi*, *23*(3), 101–105.

[7] Ramadhan, H., Rezky, D. P., & Susiani, E. F. (2021). Penetapan Kandungan Total Fenolik-Flavonoid pada Fraksi Etil Asetat Kulit Batang Kasturi (Mangifera casturi Kosterman). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *8*(1), 58.

[8] Rahmawati, A. N., Saryanti, D., Nurita Sari, F., & Yovita Turnip, I. (2022). Uji Cemaran Mikroba dan Kapang Khamir Ekstrak Air Daun Muntingia calabura L. (Kersen). *Pharmacon: Jurnal Farmasi Indonesia*, *19*(1), 72–78.

[9] Handayani, V., Syarif, R. A., Najib, A., Ahmad, A. R., Mahmud, A., & St, N. J. (2019). Standardization and bacteria inhibitory test of purified extract of mahogany (Swietenia mahagoni (L.) Jacq) seeds and leaves. *International Journal of Research in Pharmaceutical Sciences*, *10*(3), 2132–2138.

[10] Teruna, H. Y., & Rahayu, W. N. (2021). Analisis komponen minyak atsiri daun nilam (Pogostemon cablin) lokal Pekanbaru menggunakan GC-MS. *JFIOnline | Print ISSN 1412-1107 | e-ISSN 2355-696X*, *13*(1), 19–24.

[11] Kinam, B. O. I., Prabowo, W. C., Supriatno, S., & Rusli, R. (2021). Skrining Fitokimia dan Profil KLT Ekstrak dan Fraksi dari Daun Berenuk (Cresentia cujete L.) serta Uji DPPH. *Proceeding of Mulawarman Pharmaceuticals Conferences*, *14*, 339–347.

[12] Badan Standarisasi Nasional. 2006. Minyak Nilam SNI 06 – 2385 – 2006. Jakarta.

[13] BPOM RI. (2014). Peraturan Kepala Badan Pengawas Obat Dan Makanan Republik Indonesia Nomor 12 Tahun 2014 Tentang Persyaratan Mutu Obat Tradisional. *Badan Pengawas Obat Dan Makanan*, 1–25.

[14] Ahmad, A. R., Usman, I. N. R., Syarif, R. A., & Handayani, V. (2023, May). The identify of antioxidants constituents of Cemba leaves (Acacia rugata (Lam.) Fawc. Rendle). In *AIP Conference Proceedings* (Vol. 2595, No. 1). AIP Publishing.