Isolation and crystallization of patchouly alcohol from patchouly oil

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Abstract. Patchouly alcohol is the main component in patchouly oil, and its quantity influences to the quality and price of patchouly oil in trade. According to ISO 3757:2002 standard, the patchouly oil have to contain 27-35% of patchouly alcohol and according to SNI 06-2385-2006 standard, they have to contain at least 31%. Cromatography gas is the standard method recomended by ISO 3757/2002 for testing the quality of patchouly oil. This method requires the original compound of pure patchouly alcohol as the reference material. However the authentic compound of pure patchouly alcohol is not yet available in the chemical trade. Therefore, research should be focused on determining a patchouly oil standar as laboratory reference testing in Indonesia. The experiment of isolation patchouly alcohol from patchouly oil was conducted using fractional distillation method. The isolated patchouly alcohol was identified by gas chromatography-mass spectrometry method. The isolation gave 6.23% yield of patchouly alcohol, and the purity of isolated patchouly alcohol was 95.68%.

Keywords : Patchouli oil, isolation, crystallization, patchouli alcohol

Introduction

Patchouli (*Pogostemon cablin* Benth) is one of be the vegetation species that produces essential oil known as patchouli oil. The patchouli oil generally is produced by a steam distillation, although other methods can be used also i.e. a solvent extraction method and supercritical CO_2. Patchouli oil is obtained from the leaves of *Pogostemon cablin* Benth (patchouli), a plant of the Lamiaceae family, originating from Indonesia, Malaysia and India (Hu et al.., 2006). It is an important essential oil in the perfume industry, used to give a base and lasting character to a fragrance (Singh et al.., 2002). The essential oil is very appreciated for its characteristic pleasant and long lasting woody, earthy, and camphoraceous odor, as well as for its fixative properties, being suitable for use in soaps and cosmetic products (Deguerry et al.., 2006). It is also on the FDA's (Food and Drug Administration) list of substances approved for human consumption, in section 172.510, as a natural additive for food flavoring (FDA, 2002). Moreover, the plant (*P. cablin*) is widely used in traditional Chinese medicine as it offers various types of pharmacological activity according to the composition of the oil (Hu et al.., 2006).

Patchouli oil industry is the largest foreign exchange earner among the resulting essential oil exports Indonesia. One of the constraints faced by industry in Indonesia is the quality of patchouli oil, because patchouli oil from Indonesia often mixed with vegetable oil, oil keruing or other oil. This resulted in patchouli oil from Indonesia valued cheaper than patchouli oil produced from other countries. One effort that can be done is to improve the ability of essential laboratory testing in Indonesia. One of the requirements for laboratory testing is the availability of reference material. However, the authentic compound of pure patchouly alcohol is not yet available in the chemical trade (Merck, 2005; Sigma, 2005; TCI, 2005). Therefore, research should be focused on determining a patchouly oil standar as laboratory reference testing in Indonesia. Therefore, research should be focused on determining a patchouly oil standar as laboratory reference testing in Indonesia.

This study is expected as a first step to produce standard reference materials patchouli oil in Indonesia to improve the ability of the laboratory testing of patchouli oil. Increasing number of laboratories capable of testing of patchouli oil are expected to reduce counterfeiting and can also be members of quality assurance in the trade of Indonesian patchouli oil.

The isolation of patchouli alcohol from patchouli oil was made by Hernani and Wijaya (2002) by extraction, using organic solvents, purity isolation results obtained for 82.1%. In addition, Yanyan et al.., 2004, has been doing research isolation of patchouli alcohol from patchouli oil through three stages of the fractio distillation method with low pressure,

followed by extraction with 1 M NaOH solution, then vacuum liquid chromatography method. Stages of the method can raise levels of patchouli alcohol from 35.77% to 75.1%. Ma'mun and Maryadhi (2008), also have done research isolation of patchouli alcohol from patchouli oil using vacuum distillation method and obtained patchouli alcohol with the highest concentration of 91.5%. In this study, the experiment of isolation patchouly alcohol from patchouly oil was conducted using fractionation distillation method and fractionation re-distillation. Separation of the components contained in oil based on differences in their boiling points.

Methodology

Materials

Main material for the research is patchouli oil from steam distillation that are obtained from South Aceh sub district, Tapaktuan regency, Nanggroe Aceh Darussalam. The chemical materials were used such as ethanol, alcohol 96%, petroleum ether, and dietyl ether purchased from Merck.

Identification of the quality and composition of patchouli oil

The Identification quality parameters of patchouli oil including specific weight, refractive index, optical rotation, solubility in alcohol, acid value, esther value, patchouli alcohol content and alpha copaene content by followed the SNI 06-2385-2006 method (SNI, 2006). While chemical composition is determined by gas chromatography–mass spectrometry (GC-MS) analysis. The components of patchouli oil were identified on the basis of comparison of their mass spectra and retention time with databased.

Isolation and crystallization of patchouli alcohol procedure

The isolation of patchouli alcohol from patchouli oil was carried out with fractional distillation method. The system was operated at low pressure (6 mmHg) and at temperature range of 95-155°C. While redistillation is was carried out at 4 mmHg and temperature range of 125-140°C. The composition of the fractions were determined by GC-MS. The crystallization of the last fraction is done by modified of the developed methods by Bulan (2000). The patchouli alcohol content of the crystal forms was determined by GC-MS.

Chromatographic analysis

Identification of essential oil compounds and patchouli alcohol content was based on comparison of the mass spectra obtained in the gas chromatographnt with those obtained from he GC-MS library (Electronic LibraryWiley). The chromatography was performed on a gas chromatograph (Shimadzu GCMS-QP 20105), using a fused-silica capillary column (Rtx-5MS), coupled to a selective mass detector. The injector temperature was 300°C. The oven temperature was 80°C. The carrier gas was helium at a flow rate of 0.68 mL/min and the sample volume injected was 1 µl with a split rate 99.7

Results and Didcussion

The quality and composition of patchouli oil

Table 1. shows the quality parameters of patchouli essential oil including specific weight, refractive index, optical rotation, solubility in alcohol, acid value, esther value, patchouli alcohol content and alpha copaene content.

Table 1. The standard quality of patchouli essential oil

Parameters	Patchouli oil
Colour	yellow-brown
Specific weight (25°C/ 25°C)	0.965
Refractive index ($_nD^{20}$)	1.515
Solubility in alcohol 90%	Clear solution in volume ratio of 1:10
Acid value	0.50
Esther value	12.65
Optical rotation	(-) 52°
Patchouli Alcohol ($C_{15}H_{26}O$)	32.60%
Alpha copaene ($C_{15}H_{24}$) (%)	Not detected

Table 1. indicated that patchouli oil used had specific weight, refractive index, optical rotation, solubility in alcohol, acid value, esther value, solubility in alcohol, the content of patchouli alcohol, and alpha copaene that met the standard of the SNI standard quality. Table 2 shows the composition of patchouli essential oil. In Table 2, it can be observed that the patchouli oil used in this experiment consist of 15 components with the five components were patchouli alcohol (32.60 %), δ-guaiene (23.07 %), α-guaiene (15.91 %), seychellene (6.95 %), and α-patchoulene (5.47 %). It is similar with Corine and Selliers (2004)], finding on the most components are patchouli alcohol (32.2%), δ-guaiene (16.7%), α-guaiene (15.6%), seychellene (5.3%), and α-patchoulene (5.5%).

Table 2. Composition of patchouli essential oil

Peak Number	Retention Time (min)	Character Relatively Intensity MS m/z	Compound	%
1	14.69	41(45,0),55(28,4),69(19,2),79(25,4),93(50,6),105(54,5),119(24,7),133(35,3),147(22,3),161(100),175(8,4),189(65,0),204(29,0)	β-patchoulene	2.34
2	14.73	41(85,2),53(63,5),68(65,3),81(100),93(79,1),107(41,6),121(29,7),133(15,2),147(24,3),161(15,4),175(2,0),189(10,6)	β –elemene	1.17
3	15.18	41(31,2),43(69,8),67(18,7),81(56,1),85(45,5),109(31,4),123(100),135(18,3),150(57,4),175(2,0),190(2,0)	4-(2^1,6^1,6^1-trimetil-siklohek-2^1-en-1^1-yl)-pentan-2-one	0.55
4	15.26	41(100),55(44,2),69(81,5),79(59,4),93(77,2),107(38,9),120(30),133(62,1),148(15,2),161(16,0),175(4,5),189(5,5),204(7,4)	Trans-caryophyllene	3.49
5	15.45	41(42,5),55(38,2),67(31,0),79(57,4),93(75,2),105(100),119(39),133(46,5),147(70,8),161(21,6),175(4,0),189(30,2),204(39,3)	α-guaiene	15.91
6	15.69	51(8,2),55(16,5),57(14,4),79(22,7),93(28,1),107(24,6),122(100),133(141,4),147(13,2),161(38,8),175(7,5),189(13,0),204(25,0)	Seychellene	6.95

Table 2. continued

7	16.76	41(28,2),43(12,6),67(11,4),80(28,4),93(100),107(10,2),121(25,8),136(4,5),147(5,5),161(2,0),189(1,5),204(5,3)	α-humulene	0.69
8	15.87	41(34,2),55(26,5),69(14,0),79(25,4),93(76,8),107(61,4),119(25,5),135(100),147(9,2),161(23,4),175(2,3),189(14,4),204(25,0)	α-patchoulene	5.47
9	15.92	41(34,2),55(26,5),69(14,5),79(25,6),93(76,1),107(61,0),119(25,5),135(100),147(9,8),161(23,5),175(2,5),189(14,2),204(25,8)	α-gurjunene	1.47
10	15.98	41(100),55(43,5),69(81,2),79(59,0),93(78,5),107(37,9),120(29,5),133(62,0),148(46,5),161(18,2),174(4,5),189(10,4),204(7,5)	cis-kariofilene	1.45
11	16.05	41(42),55(35),67(31),79(54),93(75),105(100),119(36),133(48),147(70),161(20),175(5),189(25),204(24,8)	β-chamigrene	0.49
12	16.24	41(98,6),55(49,2),67(55,5),79(68,8),91(100),105(86,9),119(55,2),133(59,4),147(42,3),161(64,5),175(8,0),189(21,5),204(44,5)	Alloaromadendrene	0.62
13	16.35	41(68,1),55(46,5),67(38,8),79(61,0),93(78,4),105(100),121(44,2),133(50,0),147(79,7),161(24,5),175(6,2),189(40,5),204(50,6)	Cis-cariyophyllene	3.73
14	16.45	41(82,2),55(54,5),67(39,8),79(64,2),93(98,5),107(100),119(31,4	δ-guaiene	23.07
15	18.71	),135(35,0),147(28,6),161(16,2),189(30,5),204(11,5) 41(92,5),69(49,5),83(100),95(46,8),98(93,2),125(50,0),138(75,5),161(38,8),179(18,5),189(16,4),207(26,5),222(92,2)	Patchoulialcohol	32.60

Isolation and crystallization of patchouli alcohol procedure

The results isolation of patchouli alcohol and analysis of gas chromatography mass spectra are expressed in two parameters, namely the retention time (minutes) and concentration (%). Retention time is a specific number of the interactions between the molecules of compounds in the column chromatography. The concentration shows levels of purity of the analyzed samples. The results of analysis of patchouli alcohol fractions are shown in Table 3.

Table 3. Fractions of patchouli alcohol

Fractions	Patchouli alcohol. %
Fraction 1	9.88
Fraction 2	23.93
Fraction 3	36.79
Fraction 4	47.60
Fraction 5	76.73
Fraction 6	81.78
Fraction 6.1	85.2
Fraction 6.2	87.8
Fraction 6.3	89.9

The 6 fractions resulted from fractional distillation of patchouli oil at the condition as scaled before found to be correlated with the patchouli alcohol content, with the highest patchouli alcohol content in the 6^{ts} fractions containing 81.78% of patchouli alcohol. In this fractions, other components of patchouli oil were considered have already been vaporized in the previous fractions. The increasing level of patchouli oil is considered due to the reduction of other components. Re-distillation of 6^{eh} fraction gave three sub fractions having boiling point of 110-118°C. The highest level of patchouli alcohol was found to be in the last fraction (fraction 6.3), which was 89.91 %.

The first crystallization of sub fraction 6.3 gave the yield of 6.86% and the colour the crystal was yellowish white. The yield of the second crystallization, found to be 6.23% with the colour of the crystal was white. Patchouli alcohol has melting point about 55°C-56°C in the first crystallization process, and about 55.5°C-56°C in the second crystallization. According to Dummond [15], patchouli oil has melting point of 56°C. The final crystal found to be almost pure patchouli alcohol, with purities up to 95.68%, the data were organized shown in Fig 3, Table 4 and Fig. 4.

The peak patchouli alcohol in patchouli oil chromatogram is located at the end of the chromatogram. This shows that patchouli alcohol is a component that has a high boiling point in patchouli oil compound classes other than terpenes. Relatively high boiling point may explain why patchouli oil has fixative properties. Based on the results chromatogram, patchouli alcohol content of 95.68% at retention time 16.88, then we could see the peak that occurs is not broken. Peaks that do not split in the GC-MS test conditions shows the components in the patchouli oil relatively stable.

On the basis of these assumptions patchouli alcohol has a relatively good stability. Homogeneity in reference materials produced were tested by separating the reference material into ten different bottles and tested by means of GC on the same operating conditions. Test results on three bottles of random sampling shows levels of patchouli alcohol have the same levels of patchouli alcohol, meaning that the resulting reference materials are relatively stable. To be more convincing patchouli oil with patchouli alcohol content of 95.68% was also tested by GC-MS. Patchouli alcohol is then tested by GC-MS and compared with a database of test results contained in the GC-MS. The test results showed that the largest component in the tested samples containing alcohol compounds and has a chemical formula in accordance with patchouli alcohol.

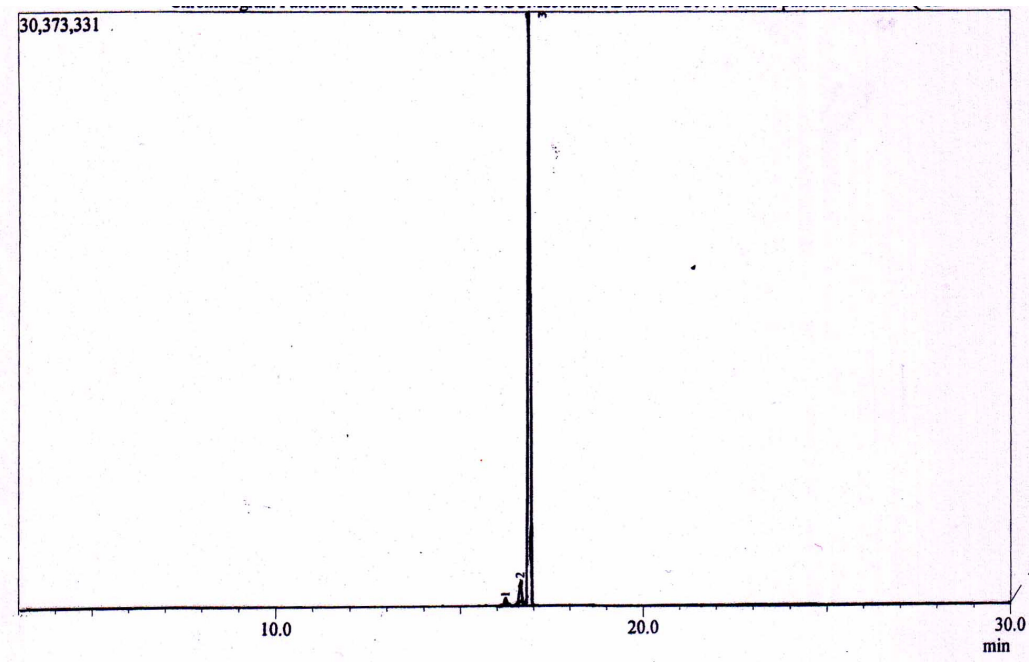

Figure 3. Chromatogram the crystal of patchouli alcohol

Table 4. Composition crystal of patchouli alcohol

Compound	Retention time	%
4-(2.6.6-Trimethyl-cyclohex-1-en-1-yl)butan-2-ol	16.23	0.93
1H-Cycloprop azulen-4-ol,decahydro-1,1,4.7-tetramethyl	16.64	3.40
Patchouli alcohol	16.88	95.68

Figure 4. Crystal of patchouli alcohol

Conclusions

Isolation and crystallization of patchouli alcohol gives results with the highest concentration of 95.68%. Based on test results reproducibilitas GC-MS and retention time, the crystal patchouli alcohol obtained is eligible as reference material for the analysis of patchouli alcohol in patchouli oil.

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