DETERMINATION OF EUGENOL IN VOLATILE OIL FROM CLOVE LEAF AND FLOWER (*Eugenia aromatica* OK) BY TLC-DENSITOMETRY

Penentuan eugenol dalam minyak atsiri daun dan bunga Cengkeh (Eugenia aromatica OK*) menggunakan KLT densitometri*

Zuhelmi Aziz, Ratna Djamil, Desi Rahmi Suciati
Faculty of Pharmacy, University of Pancasila
Srengseng Sawah, Jagakarsa, Jakarta 12640
e-mail: zuhelmi_aziz@yahoo.com

ABSTRAK

Cengkeh (*Eugenia aromatic* OK) adalah tanaman tropis yang berasal dari Maluku. Minyak atsiri cengkeh biasanya didapatkan dari bunga menggunakan metode destilasi, tetapi beberapa literature menyebutkan bahwa daun cengkeh juga mengandung minyak atsiri. Untuk itu dilakukan penelitian penentuan eugenol dalam minyak atsiri dan dan bunga cengkeh. Dengan menggunakan TLC Densitometer diperoleh minyak atsiri dari bunga 82,49% dan daun 79,03%.

Kata kunci: Cengkeh (*Eugenia aromatic* OK), minyak atsiri, eugenol, TLC Densitometer

ABSTRACT

Clove (Eugenia aromatic OK*) is a tropical plant that come from Maluku. Clove oil contain volatile oil that usually obtained from flowers by the distillation method, but there are some literatures mentioned clove leaves also contain volatile oil. Based on this, research was conducted to determine the content of eugenol in volatile oil of clove leaf and flower. Determination of eugenol by TLC-Densitometry showed that, eugenol content in clove volatile oil from flower 82.49% and from leaf 79.03%.*

Key words: Cengkeh (*Eugenia aromatic* OK), *volatile oil, eugenol, TLC-Densitometry.*

INTRODUCTION

Indonesian have been using a plant as medicine for a long ago. One of the plants is a clove (*Eugenia aromatica* OK). Clove is a tropical plant that come from Maluku, which was widely cultivated for flowers and clove oil. Clove oil usually used as a drug because it contains volatile oil. The volatile oil have a special odor, that comes from plants which easy to evaporate at room temperature without decomposition. Clove oil is a volatile oil also contains eugenol, which is a chemical component that provides a sense of bitter and spicy smell of cloves.

Until now the use of cloves in Indonesia among other diverse as a traditional medicines (cough medicines, cold medicines and also to eliminate bad breath, toothache cure, relieve nausea and vomiting, accelerate heart rate), spices, raw tobacco mixture and material for making clove oil.

There are some literatures mentioned clove leaves also contain volatile oil with eugenol as a major component of terpenes. Eugenol is an important component in industry as in pharmaceu-

ticals, paints, perfumes and others.

Based on this, research was conducted to determine the content of eugenol in volatile oil of clove leaf and flower by TLC-Densitometry.

MATERIALS AND METHODS

Materials:

Clove oil from leaf and flower of clove (*Eugenia aromatica* OK.) and reference standard eugenol, anisaldehide, glacial acetic acid, methanol, sulfuric acid, toluene, ethyl acetate. Instrumentation: Densitometer- Camag TLC Scanner-3.

Methods

Preparation

1) Test Solution of clove oil from clove leaves and flowers (*Eugenia aromatica* OK) obtained from previous studies.
2) Standard solution (Eugenol Rs Solution) dissolved 85.3 mg accurately weighed of eugenol Rs in toluene at volumetric flask 25-ml (solution A).

Identification by thin layer chromatography.

Apply separately 10 µL portion of the test solution and eugenol Rs solution to a suitable thin layer chromatography (TLC) plate. Place the plate in the *chromatographic chamber* with toluene-ethyl acetate (93:7) a mobile phase, and develop the chromatogram until the solvent front has moved 15 cm. Plate dried at room temperature, consecutive observed at UV 254 nm and 366 nm. Then spray the plate with sulfuric acid and anisaldehide spray reagent solution and heat at 110^0C for 10 minutes. Then spots were observed in normal light, UV 254 nm and 366 nm UV light and the principal spot obtained from the test solution corresponds in hRf value and color to that of the standard solution.

Determination of the maximum absorption wavelength of eugenol

Apply separately 10 µL of solution A to TLC plate, then develop in toluene-acetate (93:7) like on (b), made the absorption spectrum of substances with a densitometre at a particular wavelength, and obtained the maximum absorption wavelength.

Linearity test

Linearity is usually demonstrated by dilution of standard stock solution. *Preparing*

Series of solutions with concentration 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5 ml of solution A in 5 ml toluene accurately. Apply separately 10 µl each solution to TLC plate then develop in toluene-ethyl-cetate (93:7.) Furthermore, the absorption spectrum for the substance by means of densitometry at a wavelength of 283 nm. Then made the regression line with equation Y = a + bx and calculated the correlation

Limit of detection

Create a series of solutions with concentration 0.5 ml, 0.4 ml, 0.3 ml, 0.2 ml and ml of solution A in 5 ml toluene accurately. Apply separately 10 µl each solution to TLC plate then develop in toluene-ethyl-acetate (93:7). Furthermore, substance absorption spectrum is made by densitometer at a wavelength of 283 nm.

Assay

Reference standard solution of eugenol

Dissolved 33.8 mg accurately weighed of eugenol Rs in toluene at volumetric flask 5-ml.

Test solution

Dissolved 40 mg accurately weighed of

clove oil of flowers and leaves in toluene at volumetric flask 5-ml.

Apply separately 10 µl each solution to TLC plate then develop in toluene-ethyl-cetate (93:7.) means of. Then measured the area spots with densitometer at a wavelength of 283 nm. The content of eugenol calculated using the equation:

$$\% \text{ eugenol} = As/ A_{Rs} \times Bs/ B_{Rs} \times 100\%$$

Description :

As : Area spot of sample Bs : sample weight

A_{Rs} : Area spots eugenol Rs

B_{Rs} : eugenol Rs weight

Recovery Test

Standard solution

Diluted 2.0 ml accurately of eugenol Rs in toluene at volumetric flask 5-ml.

Sample solution

Carefully weighed amount of approximately 47.1 mg of a solution volatile oil of flower, then put into the volumetric flask 10 ml. Dissolved and diluted to the mark with toluene. Pipette 2 ml of test solution is then inserted into the volume flask 5 ml, then diluted to the mark with toluene.

Sample solution plus eugenol Rs

Pipette 2 ml of s solution is inserted into the volume flask 5 ml and then added 2.0 ml of eugenol Rs solution, then diluted to the mark with toluene.

Determination

Apply separately 10 µl standard solution, sample plus eugenol Rs solution and sample solution to TLC plate then develop in toluene-ethyl-cetate (93:7), then measured each spots with densitometer at 283 nm

RESULTS AND DISCUSSION

Identification By TLC

From the results of ordinary-light observations on the chromatogram obtained the following results: on chromatograms sprayed with spray reagent that was not seen spots in eugenol Rs and eugenol in clove samples of leaves and flowers, while in the chromatogram was sprayed with the reagent shown the existence of two spots on eugenol Rs and three spots on the flower and leaf samples of clove, the values of hRf value can be seen in Table 1.

And from observations of UV chromatograms at 366 nm obtained results as follows: the chromatograms that was not sprayed with the reagent visible presence of the purple spots on eugenol Rs, cloves sample of flower and leaves, while the chromatogram is sprayed with a reagent visible two spots on eugenol Rs and three spots on the leaf and flower samples cloves. The values of hRf can be seen in Figure 1 and hRf can be seen in Table 2.

Tabel 1. Chromatogram profile in ordinary light

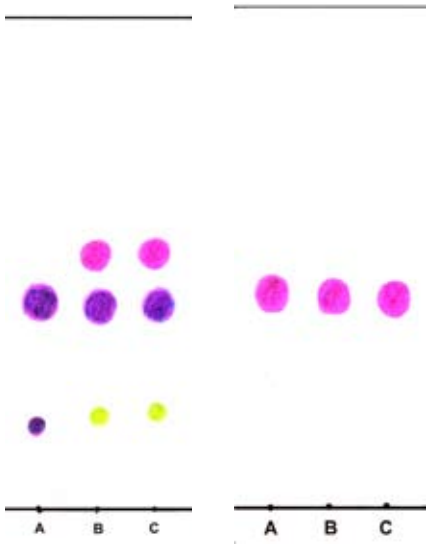
Number of spots	Before spraying					
	A		B		C	
	hRf	color	hRf	Color	hRf	Color
-	-	-	-	-	-	-
Number of spots	**After spraying**					
	A		B		C	
	hRf	color	hRf	Color	hRf	Color
1	16,9	purple –light blue	19,2	purple –light blue	20,8	purple –light blue
2	42,3	purple –light blue	42,3	purple –light blue	41,5	purple –light blue
3	-	-	52,3	Pink	52,4	pink

Tabel 2. Chromatogram Profile in UV 366 nm

Number of spots	Before spraying					
	A		B		C	
	hRf	color	hRf	color	hRf	color
1	42,3	purple	42,3	purple	41,5	Purple
Number of spots	**After spraying**					
	A		B		C	
	hRf	Color	hRf	color	hRf	color
1	16,1	purple –light blue	18,4	Green	19,2	green
2	42,3	purple –light blue	42,3	purple –light blue	41,5	purple –light blue
3	-	-	52,3	Pink	52,4	pink

From the observation of UV chromatograms at 254 nm before spraying showed there was seen a single brown spot on raw eugenol Rs, clove volatile oil from leaf and clove volatile oil from flower with hRf values: 42.3, 42.3 and 41.5, while in the chromatogram after spraying with spray reagent there was not spots of eugenol Rs, clove oil of leaf and flower. The profile chromatogram be seen in Figure 2.

Based on the results of identification by TLC showed that the volatile oil of clove leaf and flower contains eugenol.



Before sprayed After sprayed

Figure1. Chromatogram-TLC of volatile oil of clove leaf and flower at UV 366 nm with migration distance 15 cm

Description:

A : Raw comparator eugenol

- Plate: Silica Gel GF254

B : volatile oil of flower

- mobile phase: toluene-ethyl acetate (93:7)

C : volatile oil of leaves

- spray reagent: Anisaldehide - H2SO4 conc

Before sprayed

Figure 2. Chromatogram-TLC of volatile oil of clove leaf and flower at UV 254 nm with migration distance 15 cm

Descriptions:

A : eugenol Rs

B : volatile oil of flower

C : volatile oil of leaf

plate : Silika Gel GF_{254}

mobile phase : Toluene – Ethyll acetate (93:7)

spray reagent : Anisaldehide – H_2SO_{4C}

migration distance : 15 cm

Determination of the maximum absorption wavelength of eugenol

From of absorption spectrum obtained by densitometer there had been a maximum absorption wavelength is 283 nm. (Figure 3).

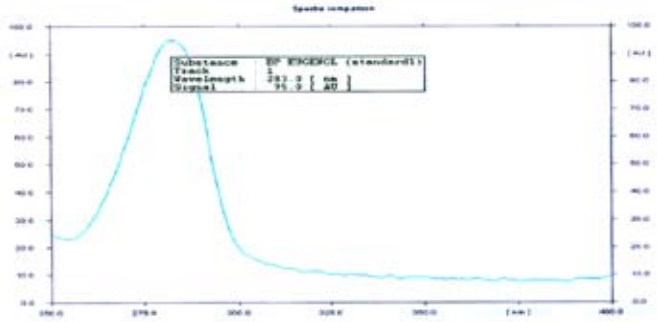


Figure 3. The curve determination the maximum absorption wavelength

Linearity Test

In the linearity test results obtained by a linear relationship between spot area with concentration, where *r values* obtained for reference standard solution of 0.9961 eugenol. The *r value* shows the ideal value for close to 1, so that the densitometry equipment to be used in future research. Linearity test results can be seen in Table 4.

Table 4. Test linearity standard solution eugenol

No	consentration (bpj)	Spots area	Regretion line	r
1	341,2 bpj	12997,99	Y= 11311,72 + 5,2080 x	0,9961
2	628,4 bpj	14795,74		
3	1023,6 bpj	16749,04		
4	1364,8 bpj	18779,76		
5	1706 bpj	19890,92		

Limit of Detection

There was showed that the detection limit densitometer tool used in research to detect the reference standard solution with a concentration of eugenol to 136.4 ppm.

Determination of Eugenol Content

There was obtained Eugenol content in clove volatile oil from flower has an average concentration greater than eugenol content in volatile oil from leaves. Determination of eugenol by TLC-Densitometry showed that, eugenol content in clove volatile oil from flower 82.49% and from leaf 79.03%.

Recovery Test

Recovery test performed on the volatile oil of clove flower with the addition of eugenol Rs. Recovery test by TLC-Densitometry was obtained 102.03%, which meets the requirements of the range between 80-120%.

CONCLUSIONS

1. Based on the TLC chromatograms of the pattern can be concluded that the volatile oil of clove leaf and flower have the same chemical components of Eugenol.
2. The content of eugenol in clove volatile oil from flower has an average concentration greater than the content of eugenol in clove volatile oil from leaf Eugenol content in clove volatile oil from flower 82.49% and from leaf 79.03.

REFERENCES

Anonim. 2006. Tanaman obat. http://www.iptek.net.id/ind/pd_tanobat/view.php?id=9. Diakses tanggal 20 Desember 2006.

Departemen Kesehatan Republik Indonesia. 1989. *Material Medika Indonesia*. Edisi V. Jakarta. hal 120-3

Grinberg N. 1990. *Modern thin layer chromatography.* Marcel Dekker, Inc. New York. hal. 89-249

Guenther E. 1987. *Minyak Atsiri* Jilid 1, Terjemahan Ketaren S, Universitas Indonesia. Jakarta. hal. 19-38

Johnson E.L., Stevenson R. 1991. *Dasar kromatografi cair,* Alih Bahasa Padmawinata Penerbit Institut Teknologi Bandung. Bandung. hal. 2-4

Ketaren, S. 1985. *Pengantar teknologi minyak atsiri.* PN Balai Pustaka. Jakarta. hal. 21-73

Macek, K. edited. 1972. *Pharmaceutical application of thin layer and paper chromatography*. Third Edition, Medical Departement Elsevier Publishing Company. Amsterdam. hal 189-205

Munson, J.W. 1991. *Analisis farmasi modern.* Bagian B. Diterjemahkan oleh Harjanah. Penerbit Airlangga University Press. Surabaya. hal. 125-137

Nurwahidah E. 2007. Perbandingan pola kromatogram minyak atsiri dari daun cengkeh dan bunga cengkeh (*Eugenia aromatica* O.K). Universitas Pancasila. Jakarta. hal 1-50

Sutrisno R.B. 1998. *Taksonomi spermatophyta untuk farmasi*. Edisi I. Fakultas Farmasi Universitas Pancasila. Jakarta. hal 152-6

Wagner H., Bladt S., Zgainski E.M. 1984. *Plant drug analysis " A Thin Layer Chromatography Atlas*". Berlin Heidelberg New York Tokyo. hal. 9-28