

Antioxidant Activity, Skin Irritation Potential and Chemical Composition of Clove Leaf Oil from West Java Indonesia

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ABSTRACT – Essential oils with specific chemical compositions have the potential as a source of antioxidants in cosmetics, however, in general, essential oils are not safe because of the potential for skin irritation. This study aims to perform fractionation to obtain clove oil with a chemical composition that is safe on the skin and does not reduce its antioxidant activity. Fractionation was performed at a temperature of 100 – 285 °C. Chemical composition was determined by Mass Chromatography Spectrometry (GC-MS), antioxidant activity by DPPH method, and irritation potential by in-vivo patch test. The results showed chemical components of clove oil were eugenol, copaene, beta caryophyllene, iso-eugenol, alpha caryophyllene, cadinene, caryophyllene oxide, caryophylla and beta caryophylla. Fractions with chemical compositions of eugenol and beta caryophyllene as the main components and copaene, iso-eugenol, alpha caryophyllene, cadinene as secondary components have very light erythema and no potential irritation to the skin. The fraction produced from the fractionation of crude clove oil at a temperature of 235 – 260 °C has the highest antioxidant activity of 10.17 mg/L.

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INTRODUCTION

Essential oils are plant extracts consisting of complex organic substances with a distinctive aroma [1]. The composition of compounds and functional groups in essential oils determines the organoleptic properties and biological activities including antioxidant activity, antibacterial activity, and anti-inflammatory activity [2]. Essential oils have been used regularly in cosmetics because they have a pleasant aroma [3]. Over time essential oils are not only known as aromatherapy but also have other activities including antioxidant activity [4]. All essential oils have antioxidant activity with different oxidation-inhibiting abilities for each type. Essential oils that have phenolic elements such as those in leaves and twigs of *Vireya Rhododendron* from Indonesia have antioxidant capacity until 1793.62 $\mu\text{mol trolox/g}$ dried powder [5]. Beside the elements contained, chemical composition of essential oil also determines its antioxidant activity [6].

Clove oil consists of the main components eugenol (50 – 80 %), caryophyllene (4 – 20 %), and more than 20 types of other components [7]. Clove oil is produced from the distillation of the leaves of cloves. The distillation condition process determines the percentage of chemical composition. The main composition clove bud oil from West Java Indonesian obtained by PT. Indesso is eugenol 77.53%, β -caryophyllene 5.90% and eugenyl acetate 12.07% [8]. Eugenol is a phenolic which have high antioxidant activity that IC_{50} values below 50 $\mu\text{g/mL}$ [2]. The antioxidant activity of clove oil is higher than the antioxidant activity of synthetic butylated hydroxytoluene (BHT) [6]. The inhibition of oxidation of clove oil was 94.18 % higher than that of synthetic antioxidants BHT by 92.15 % [9]. Meanwhile, in another study, it was found that the inhibition of BHT oxidation was greater than that of trolox which was indicated by the lower antiradical activity of BHT 16.14 μM (IC_{50}) compared to trolox 23.84 μM (IC_{50}) [10]. According to the Boulebd, H., 2020 the antiradical potential $\text{BHT} > \text{BHA} > \text{Trolox} > \text{ascorbic acid}$. BHT, BHA, and ascorbic acid (vitamin C) is used in similar food applications, while Trolox (vitamin E derivatives) is medicinal and industrial applications [10]. The need for cosmetic antioxidants increases especially for the elder because our skin becomes old due to physiological changes [11]. Currently, the urge to use natural ingredients as a source of antioxidants such as plant extracts is increasing to meet the antioxidant needs of the cosmetic industry [12, 13].

Essential oils have a great opportunity as a source of antioxidants, however, the complex compounds and chemical components in essential oils have caused the potential sensitivity allergic reactions be present in cosmetics, topical pharmaceuticals and household products [14]. It is known that essential oils with alcohol, phenol, and aldehyde functional groups [13] have a very high potential for irritation. Phenol compounds such as eugenol have an allergic effect on the skin [3], therefore it is necessary to research the use of clove oil as a safe antioxidant in cosmetics. The aims of this study were to determine the antioxidant activity, the allergy potential and chemical composition of clove oil and its fractions. This paper is expected used as a reference in selecting the chemical composition of clove oil to be used as a source of cosmetic antioxidants.

EXPERIMENTAL METHOD

The research was conducted at the research laboratory of the Center of Chemical and Packaging, Ministry of Industry, Jakarta, Indonesia.

Materials and Instruments

Crude clove leaf oil was obtained from PT Sinkona International Lestari which was placed on Tasikmalaya, West Java, latitude -7.319563 and longitude 108.202972. While DPPH (2,2-diphenyl-1-pikrilhidrazil), L(+)-Ascorbic Acid, and ethanol for Spectrophotometric Grade was obtained from Sigma-Aldrich.

Spinning Band Column Distillation 9600 Series with M690 fractionation tool program was used as fractionation equipment. Moreover, this study also used analytical balances and other glassware.

Method and Procedure

Fractionation

Fractionation was carried out to obtain clove oil fraction with several chemical compositions. In this study, the fractionation was carried out using a Spinning Band Equipment. The chiller temperature was set at 20 °C. Samples of clove oil was placed on the flask of raw material and boiling chips were put into the flask. Furthermore, the heating mantle and flask containing the sample were mounted on the tool column. The vacuum pump was turned on and the fractionation temperature was set at 100 - 125 °C, 125 - 236 °C, 236 - 260 °C, 260 - 285 °C. The pressure was at 17.2 mmHg and the process heat rate was 50 %. Fractionation was carried out until the final temperature was reached and the fraction product was accommodated in the product bottle.

Chemical Composition Test

Chemical composition was tested using Gas Chromatography Mass Spectrometry (GC-MS) with Agilent brand, type GC 6890/MS 5975 MSD, the column was HP5-MS capillary column (30 m x 0.25 mm x 0.25 m). The operating conditions were carried out with the initial temperature: 60 °C for 5 minutes. Then it increased at a speed of 10 °C/min to a temperature of 250 °C and maintained it for 5 minutes. The split ratio was 1:20. Helium was used as a carrier gas at a constant pressure of 7.65 psi.

Antioxidant Test

The antioxidant testing method was taken from the method of Rafi et al, 2018 with modifications. First of all, 1% (w/v) essential oils sample was dissolved in ethanol p.a. and then this solution was divided in half as a negative correction. Elsewhere, a 0.1 nM DPPH solution in ethanol p.a. was prepared. After that, DPPH solution and the volatile oil sample solution were mixed in a ratio of 1:1. In another place, 15 mg/L ascorbic acid solution was prepared in ethanol p.a. And then, a series of ascorbic acid solutions were made with successive concentrations of 0, 1.5, 3, 4.5, 6, 7.5 mg/L. Then, the absorbance was measured at a wavelength of 517 nm using a UV-VIS Spectrophotometer. Antioxidant activity was determined by determining the concentration in mg/L.

Skin Irritation Potential Test

The test method is In-Vivo Semi Occlusive Single Patch Testing and clinical evaluation by Spincontrol Idea Indonesia. The test was conducted by involving 33 people as test subjects. Subjects were selected based on the criteria of healthy Asian men and women aged 18-65 years, having various skin types such as dry skin, oily skin and combination skin. First of all, each subject filled out a health and cosmetic use questionnaire. Subjects did not use cosmetics on the back for 12 hours before starting the test. Then, apply the sample to the subject's back. After 48 hours the back skin is cleaned. Subsequently, a clinical evaluation was carried out on the back skin that was attached to the sample. The assessment criteria are divided into 4 types; first, no effect if there is no change in skin color; second, mild irritation if the skin becomes pink; third, moderate irritation if the skin turns to be pinkish-red; and finally severe irritation if the skin becomes red.

RESULT AND DISCUSSION

Chemical Composition

Clove oil has about thirty-two components [8]. Based on the GC-MS chromatogram (Table 1), it is known that West Java Indonesian clove oil consists of ten types of components detected with concentrations above 0.1 %. The crude oil in this study had a relative density of 1.02 g/mL, a refractive index of 1.553, and an optical rotation of -0.37 and was fractionated at a temperature of 100 °C to 285 °C. Fractionation was divided into four process temperature ranges such as fraction-1 at temperature of 100 – 125 °C, fraction-2 at a temperature of 125 – 235 °C, fraction-3 at a temperature of 235 – 260 °C, and fraction-4 at a temperature of 260 – 285 °C which were accommodated in pot-1, pot-2, pot-3 and pot-4, respectively. This temperature division was carried out to separate eugenol as the main component from other components. Fraction-1 which accommodated in pot-1 was a very light and volatile component that is lost during the process and could not be tested. Fraction-1 was thought to be terpene or alcohol compounds such as pinene, eucalyptol, chavicol, linalool, dodecane as also detected in Chinese clove oil [7]. In this study, 2.5 L of crude clove oil produced 0.1

% (v/v) fraction-2, 92 % (v/v) fraction-3, 0.05 % (v/v) fraction-4 and left it about 0.4 % as a residue. Based on the volume of the resulting fraction, there is 7.45 % of the product lost during the process. It might be a very light component that evaporates during processing or remains in the pot when measured.

The chemical composition of crude clove oil, fraction-2 product, fraction-3 product, fraction-4 product and residues are summarized in Table 2. In this study, the chemical components indicated by the library of GC-MS. The main components with a concentration of 20 – 90 % in clove oil, fraction and residue are eugenol and beta caryophyllene, while the secondary components with a concentration of 1 – 20 % and minor components range of 0-1 % are found to be different in each fraction. In fraction-2 the secondary component is copaene, while the minor components are iso-eugenol and alpha caryophyllene. In fraction-3 which is a secondary component is copaene, iso-eugenol and alpha caryophyllene. Meanwhile, the main component in fraction-4 is only eugenol, while the secondary components are beta caryophyllene, alpha caryophyllene, cadinene while the minor components are caryophyllene oxide and iso-eugenol. The fractionated components showed that the eugenol increased with the increase of process temperature. The copaene component with high concentration is found in fraction-2, while beta caryophyllene and alpha caryophelene are found to be high in fraction-3.

The percentage of components detected in 100 % on crude clove oil, while on fraction-2, fraction-3, fraction-4 and residue is 99.8 %, 95,9 %, 87.7 %, and 94.9 % sequentially. Eugenol is a phenylpropanoid compound with a phenol functional group [15]. In this study, eugenol contained in crude clove oil as much as 68.1 % is also contained in all fractions with values 27.9 %, 54.7 %, 75.9 % and 84.1 % for fraction-2, fraction-3, fraction-4 and residue, respectively. The highest concentration of eugenol was found in the residue which is the final product of the fractionation process. Generally, clove oil is produced by hydrodistillation, steam distillation or dry distillation of a plant. Eugenol produced by the hydrodistillation process is 79% and more than 30 other components [8].

Table 1. Temperature of fractionation process and gc-ms chromatography of crude clove oil and its fraction

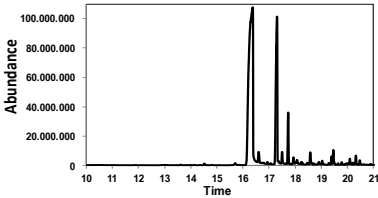
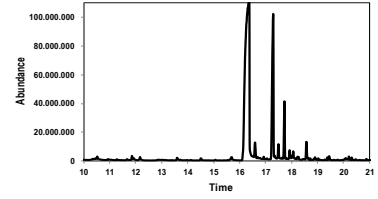
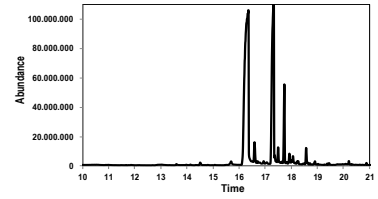
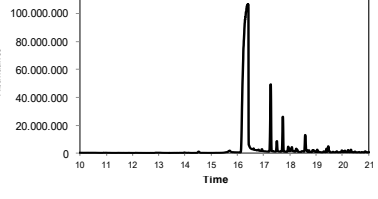
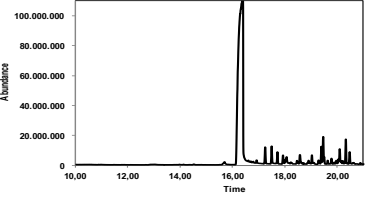
Name of Sample	Temperature Set (°C)	GC-MS Chromatogram
Crude Clove Oil	-	
Fraction - 1	100 – 125	-
Fraction - 2	125 – 235	
Fraction - 3	235 –260	
Fraction - 4	260 – 261	
Residue	-	

Table 2. Chemical Composition

RT	Composition	Percentage (%)				
		Crude Clove Oil	Fraction - 2	Fraction - 3	Fraction - 4	Residue
16.4	<i>Eugenol</i>	68.1	27.9	54.7	75.9	84.1
16.6	<i>Copaene</i>	1.3	6.1	2.0	-	-
17.3	<i>Beta Caryophyllene</i>	21.1	23.8	30.5	5.8	1.4
17.5	<i>Iso-Eugenol</i>	1.6	0.9	1.4	0.9	1.4
17.7	<i>Alpha Caryophyllene</i>	4.0	0.7	5.9	2.8	0.9
18.6	<i>Cadinene</i>	0.9	-	1.4	1.7	0.8
19.5	<i>Caryophyllene Oxide</i>	1.9	-	-	0.6	2.9
20.1	<i>Caryophylla</i>	0.4	-	-	-	1.3
20.3	<i>Beta Caryophylla</i>	0.7	-	-	-	2.1

Note : RT = Retention time

This study, although the boiling point of eugenol is known to be around 254 oC, the results of the fractionation showed that not all eugenol can be separated and collected in one pot with a fractionation temperature of 235 – 260 °C. This is because the temperature setting range in the fractionation column was too large. In the fractionation process to obtain components with the high level of purity, the fractionation process can be carried out gradually with a small temperature range. In this study, the desired fraction is a complex compound that is expected to not cause allergies. There are about 79 types of essential oils that cause skin allergies [3]. Copaene and caryophyllene are terpene compounds commonly contained in essential oils [7, 4, 16]. In this study, copaene is contained in crude clove oil, as well as in fraction-2 and fraction-3 with significantly different concentrations. It happens due to the process conditions for fraction-2 correspond to the boiling point of copaene around 124 oC. While beta and alpha caryophyllene was detected in all samples with significantly different concentrations for each fraction. The highest concentration of caryophyllene component which has a boiling point between 262 – 264 °C was in fraction-3 which operated at a fractionation temperature setting at 235–260 °C.

Antioxidant Activity

Essential oils are natural antioxidants that are expected to replace synthetic antioxidants. Essential oils are a mixture of complex organic compounds with different functional groups. The composition of functional group determines the organoleptic properties and biological activities including antioxidants [17]. Eugenol with a phenol group is known to have high antioxidant activity [2]. Copaene, caryophyllene and cadinene are common terpene groups in essential oils. Functional groups of terpenes with alpha or gamma isomers have high antioxidant activity compared to other terpene isomers [3, 17].

In this study, the antioxidant concentration of clove oil is calculated from the calibration curve of ascorbic acid as a standard, the test solution is shown in Figure 1. Ascorbate is one of the most important vitamins in the plant material. The color of the solution is an indicator of the concentration of antioxidants. The color of a solution becomes increasingly faded with the a higher concentration of antioxidants. It can be seen that almost all sample solutions have higher concentration than the standard. High concentration indicates high activity.

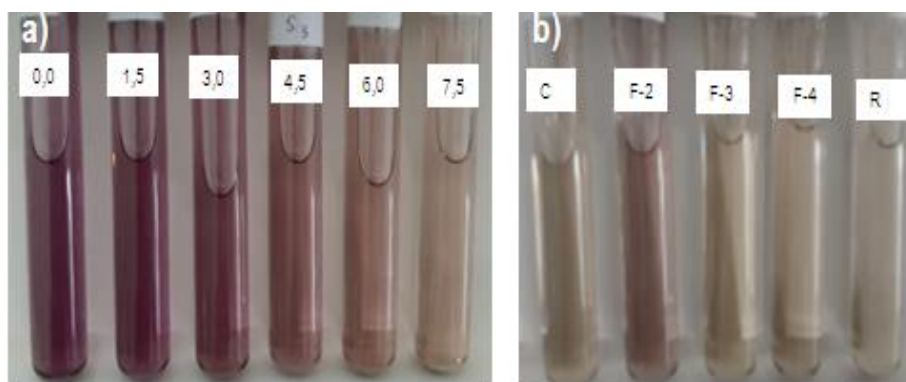


Figure 1. Antioxidant test solution a) ascorbic acid as standard (0.0 mg/L, 1.5 mg/L, 3.0 mg/L, 4.5 mg/L, 6.0 mg/L, 7.5 mg/L, b) clove oil solution (crude, fraction-2, fraction-3, fraction-4 and residue).

The antioxidant concentration of crude clove oil and then fractionation product namely fraction-2, fraction-3, fraction-4 and residue were 10.1 mg/L, 7.9 mg/L, 10.2 mg/L, 9.8 mg/L and 10.2 mg/L, respectively as shown in Figure 2. It showed that the concentration of antioxidants in the sample was not significantly different, except for the sample fraction-2 which was significantly lower than the other samples. Even though the concentration of eugenol components is low, the antioxidant activity in clove oil can be minimize lipid oxidation, retarding the formation of toxic oxidation products

and prolonging the shelf life of pharmaceutical products [7]. Furthermore, the antioxidant properties of oils with high substituted phenol such as eugenol content are able to significantly inhibit the oxidation process [9][18].

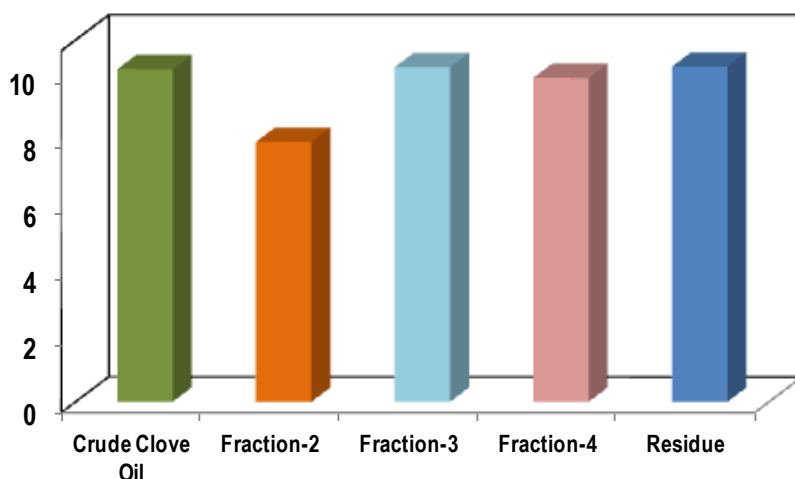


Figure 2. Antioxidant concentration of crude clove oil, fraction-2, fraction-3, fraction-4 and residue

Skin Irritation Index

Essential oils and their component are widely used topically on mucous membranes and skin, so testing for potential allergy on the skin is necessary. The potential for essential oil allergy on the skin can be known by knowing the skin irritation index. In general, the method used to obtain the skin irritation index is the patch test method [3]. Allergy potential testing is necessary to avoid the use of essential oil products that cause skin irritation (erythema clinical evaluation). Table 3 showed the results of the evaluation of irritation level from 33 people as subjects with various ages. The irritation index (M.I.I.) with numbers of 0.00-0.20 indicated no irritation or can be ignored. M.I.I. from 0.20 to 0.50 indicated mild irritation, while M.I.I. from 0.50 to 2.00 indicated moderate irritation, and M.I.I. from 2.00 to 3.00 indicated severe irritation. Based on test results using crude clove oil sample showed that the use of 10 % crude clove oil could irritate to the skin, this is indicated by M.I.I. of 0.39. On the other hand, the use of fraction-3 which has an M.I.I. 0.06 is non-irritating. The irritant properties of essential oils are determined by the composition of the components that compose them. The groups with high irritant potential are phenol, alcohol, aldehyde, ketone and ether respectively. Phenol, monoterpene alcohols and aromatic aldehydes have the highest irritant properties compared to monoterpene aldehydes, ketones and ethers. Eugenol and isoeugenol are phenylpropanoid phenolic compounds with lower irritant potential than monoterpene phenols such as thymol and carvacrol [3].

Table 2. Erythema Clinical Evaluation Results

No	Age (year)	Gender	Score	
			Crude Clove Oil	Fraction - 3
1	23	Female	0,5	0
2	43	Female	0	0
3	31	Male	0	0
4	38	Female	0	0
5	30	Female	0,5	0,5
6	44	Female	2	0
7	36	Female	2	0
8	40	Female	0	0
9	48	Female	2	0
10	28	Female	0	0
11	49	Female	1	0
12	39	Male	0	0
13	39	Male	0	0
14	41	Female	Not Exploited	Not Exploited
15	49	Female	Not Exploited	Not Exploited
16	39	Male	0	0
17	53	Female	0	0
18	44	Female	0,5	0
19	41	Female	0,5	0,5
20	49	Female	0	0
21	45	Female	0,5	0

22	45	Female	0	0
23	23	Male	0,5	0
24	24	Female	0	0
25	47	Male	0	0
26	48	Female	0	0
27	24	Female	0	0
28	35	Female	1	0
29	19	Female	0	0
30	18	Female	1	1
31	32	Female	0	0
32	58	Female	0	0
33	35	Female	0	0
Mean Irritation Index (M.I.I.)			0,39	0,06
Maximum			2	1

Note : 0 = no evidence of any effect, 0,5 = very mild erythema, 1 = mild, 2 = moderate, 3 = marked

In this study, the components of caryophylla and beta caryophylla (see Tabel 2) which are monoterpene aldehyde compounds play a role in increasing the potential for skin irritation when mixed with eugenol components. These components increase the potential for skin irritation in raw clove oil.

CONCLUSION

Commercial tempeh inoculums and cassava waste pulp as LA producers makes the effective fermentation process for bioplastic technology. The optimum condition at a cassava waste pulp concentration of 20 g/L and 0.30% (w/v) tempeh inoculum with 72 hours of incubation time yields a maximum LA production of 6.65 g/L. However, a well-established study on microorganisms producing LA with renewable raw material using the RSM technique still requires more research to be generalized and applied to a broader LA industry scope.

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