

## OPTIMIZATION OF ETHANOL CONCENTRATION IN EXTRACTION OF EUGENOL FROM GALANGAL RHIZOME

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### ABSTRACT

**Objective:** The main objective of this study was to determine the optimal ethanol concentration as solvent for extracting eugenol from galangal.

**Methods:** Galangal rhizome extraction was conducted with kinetic maceration at 50°C with various ethanol concentration as solvent (0%, 30%, 50%, 70%, and 96%). A 1:10 ratio of rhizome and ethanol was applied. The extract was obtained, then its eugenol concentration was analyzed qualitatively and quantitatively by thin-layer chromatography-densitometer with n-hexane:ethyl acetate (4:1) by using mobile phase and silica gel 60 F<sub>254</sub> as stationary phase and applying a wavelength of 283 nm.

**Results:** The result of qualitative analysis showed that extract of 70% and 96% ethanol had a spot with R<sub>f</sub> value 0.63, which was equal to the R<sub>f</sub> value of standard eugenol. Standard curve equation for the 70% ethanol extract was  $y=89318x+656.07$  ( $r=0.9993$ ) and for the 96% ethanol extract was  $y=8658x+1743$  ( $r=0.9999$ ). The result of the quantitative analysis showed that the 70% and 96% ethanol extract contained 4.85% and 4.79% eugenol, respectively.

**Conclusion:** Extraction of galangal rhizome in 70% and 96% ethanol was positively containing eugenol. Highest eugenol concentration (4.85%) was obtained from galangal rhizome extraction in 70% ethanol.

**Keywords:** Eugenol, Galangal, Extraction, Densitometer.

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### INTRODUCTION

One of the medicinal plants that contains essential oil is galangal (*Alpinia galanga*). Galangal is included into Zingiberaceae familia, rhizome class, that is often used as food seasoning or spices since it has a fragrant aroma and spicy flavor. Galangal also used as traditional medicine. It could treat gastrointestinal disorders, eliminate bloating, infection, and potential for curing cancer because of its antioxidant activity (chemoprevention) [1-3].

Eugenol is one of essential oil contained in galangal. It is beneficial for human health. Eugenol has anti-inflammatory activity in acute lung injury [4]. Eugenol also has antioxidant properties [5,6]. Another study showed that eugenol has anticancer activity [7,8]. Eugenol also has antidepressant and anthelmintic activities [9,10]. Various benefits of eugenol was a reason to optimize the solvent used to extract eugenol from galangal rhizome, to obtain the highest concentration of eugenol.

### METHODS

#### Plant

The galangal rhizome was collected in the Mojosongo market and authenticated by Suratman, Department of Biology, Sebelas Maret University.

#### Preparation of plant extract

The galangal rhizome was washed, dried at 25°C, and then powdered. Galangal powder then extracted using 5 series of ethanol concentration (0%, 30%, 50%, 70%, and 96%). The ratio of galangal powder and ethanol was 1:10. Simplisia powder and extraction solvent heated on water bath at 50°C for 1 hr with stirring. Liquid extract obtained was separated, then thickened with rotary evaporator at 40°C. Viscous extract obtained was stored in the refrigerator.

#### Optimization of analysis eugenol

Extract and eugenol standard spotted on a thin-layer chromatography (TLC) plate (silica gel GF<sub>254</sub>) and eluted with various proportion of mobile phase n-hexane:ethyl acetate, which were 1:4; 2:3; 3:2; and 4:1. Spots were observed at ultraviolet 254 nm and 366 nm. The separation then watched and calculated R<sub>f</sub> value continued by densitometry TLC scanning. Comparison of mobile phase n-hexane:ethyl acetate which optimal was 4:1, which gained good separation and R<sub>f</sub> value of eugenol 0.63.

#### Analysis quantitative of eugenol

Eugenol was created into a series of standard curve concentration, i.e., 950, 1000, 1050, 1150, 1200, and 1250 ppm, then by 2 µl spotted on TLC plate and eluted with mobile phase n-hexane:ethyl acetate (4:1). TLC plate then measured its area under curve and peak height using densitometer TLC scanner at wavelength of 283 nm.

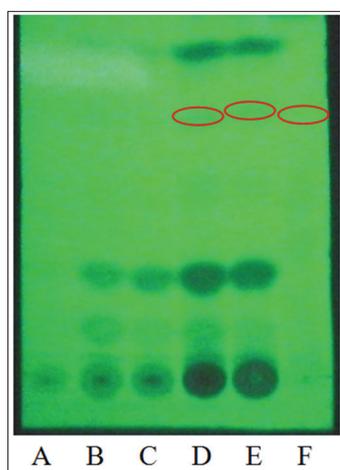
### RESULTS

The TLC result showed that eugenol were found in sample D and E (Figure 1). Densitometer spectra showed that sample D, E, and eugenol standard were having the same profile (Figure 2). The highest eugenol content was found in sample D (4.85±0.03) (Table 1).

### DISCUSSION

Extraction solvent used was ethanol because eugenol having a good solubility in ethanol [11]. Qualitative identification of eugenol was done by TLC. Advantages of TLC were easy to use, simple techniques, and quick time needed. TLC was a method of chemical mixture separation in a sample based on differences in migration of each component in the mixture to the stationary phase under the influence of mobile phase. Selection of mobile phase is important because it can affect

the migration of spots during elution progress or may affect the Rf value. Results of the selection of mobile phase showed that mobile phase n-hexane:ethyl acetate (4:1) gave separated eugenol from other compound in the extract with the value of Rf 0.63. Mobile phase of n-hexane:ethyl acetate (3:2); (2:3); and (1:4) did not show a good spot separation. It was because the mixture of mobile phase too polar, so the solute eluted until the upper limit. That was because the solute did not hold firmly on the stationary phase. The principle of TLC method is "like dissolve like", means that solubility is based on similarity of polarity properties. Eluent used was relatively non-polar because the



**Fig. 1: Qualitative identification of eugenol in ethanol extract of galangal rhizome (A - 10% ethanol, B - 30% ethanol, C - 50% ethanol, D - 70% ethanol, E - 96% ethanol, F - eugenol standard)**

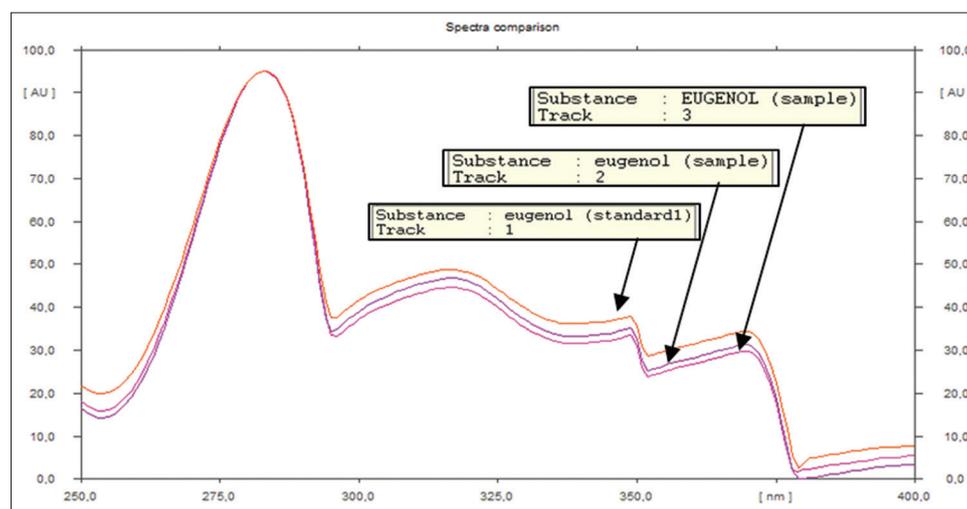
composition of n-hexane was more than ethyl acetate so that can elute eugenol which is relatively non-polar.

Based on TLC result, it can be seen that only sample D (70% ethanol) and E (96% ethanol) indicating a spot that were parallel to the eugenol standard (Spot F) with Rf value 0.63 for sample D and 0.64 for sample E. Rf value of eugenol standard and sample was similar, with difference value  $\leq 0.2$ . Similar value of Rf strengthen the suspicion that galangal rhizome extract in 70% and 96% ethanol were containing eugenol. To prove the suspicion, we were conducted further qualitative analysis using TLC scanner. The result showed that there were similarity between spectra profile densitometer of standard eugenol and galangal rhizome extract in 70% and 96% ethanol. It could be concluded that samples were positively containing eugenol.

Spectrum result by TLC scanner showed that maximum absorption of eugenol obtained at  $\lambda$  283 nm, whereas according to other study, maximum  $\lambda$  of eugenol was 282 nm [12]. Different wavelength of  $\leq 3$  nm was still within the tolerance limit allowed [13]. The linear regression equation obtained from standard curve I was  $y=89318x+656.07$  with a correlation coefficient (r) of 0.9993 and standard curve II was  $y=8658x+1743$  with a correlation coefficient (r) of 0.9999. The standard curve obtained have been fulfilled the linearity requirement, which is value of correlation coefficient  $>0.998$  [14]. The result of eugenol concentration from galangal rhizome extract was 4.85% by 70% ethanol extract and 4.79% by 96% ethanol extract.

#### CONCLUSION

The study showed that extract of galangal rhizome in 70% and 96% ethanol contains eugenol. The highest eugenol concentration is an extract of galangal rhizome in 70% ethanol, which was 4.85%.



**Fig. 2: Spectra profile of eugenol by densitometer**

**Table 1: Concentration of eugenol in ethanol extract of galangal rhizome**

Sample	Spotting replication	AUC	Concentration ( $\mu\text{g}$ )	Concentration $\pm$ SD (%)	% RSD
Ethanol 70%	1	22514.44	2.45	4.85 $\pm$ 0.03	1.51
	2	21927.52	2.39		
	3	22487.55	2.44		
Ethanol 96%	1	22783.07	2.43	4.79 $\pm$ 0.05	2.055
	2	21997.00	2.34		
	3	22668.82	2.42		

SD: Standard deviation

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