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Antioxidant Activity of Non polar and Semipolar Fractions of Ethanol Extract of *Zingiber zerumbet* Smith Leaves by Spectrophotometer and ELISA Reader

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Abstract

Zingiber zerumbet, known as the Shampoo Ginger, is one member of the family Zingiberaceae which widely used for traditional medicine. This research aims were compared antioxidant activity measeured by spectrophotomtry vis and ELISA reader and determination of chemical compositions. The antioxidant activity of the semipolar and non polar fractions of ethanolic extract of leaves of shampoo ginger was determine by DPPH method spectrophotometrically and ELISA Reader. Fractionation was performed using vacuum liquid chromatography, mobile phase hexane and chloroform (5:5; 4:6; 2:8; 1:9), ethyl acetate, and methanol. Chemical composition tested with spray reagent such as sitroborat, $FeCl_3$ and dragendrof. Results showed that semipolar and non polar fractions have antioxidant activity with IC_{50} value of 295.22±8.28 and 228.49±18.97 µg/mL, respectively by spectrophotometry Visible. Values of IC_{50} results by using ELISA Reader showed that semipolar and non polar fractions were 255.35±7.30 and 291.27±11.33 µg/mL, respectively. High precission measurement obtaned by spectrophotometric visible and ELISA Reader with RSD values of were 2.80% and 3.89%, respectively. Based on spray ragent reaction, semipolar and non polar fractions not detected phenolic and flavonoid compounds, but qualitative spray by DPPH showed yellow spot which indicate that compounds have antioxidant activity.

Keywords: Zingiber zerumbet, leaves, DPPH, ELISA Reader.

INTRODUCTION

Free radicals are molecules that have an odd electron, unstable and highly reactive [1]. The free radicals will take electrons from the cell, DNA, enzymes, and cell membranes, thus resulting cell damage. Either of enzyme, when the enzyme is exposed to free radical, the enzyme will not work properly and will be disturbed metabolic processes [2]. Increasing the amount of free radicals in the body can cause various diseases such as heart disease, cancer, aging, and autoimmune disorders [3]. Incidences of cancer were increased in line with increasing sources of free radicals such as cigarette smoke, pollution, and others. However, these free radicals can be prevented by antioxidants [4], that can stabilize free radicals by supplementing an electron to radicals which can inhibit free radical reactions [2].

Human body actualy have antioxidant called endogen antioxidant, but along with increasing free radical exposure endogen antioxidant can not stabilize the free radicals. This condition need antioxidan supplement, called exogen antioxidant [5]. Foods, vegetables, herbal medicine and fruits evidently have antioxidant activity. One of the herbal medicines that have antioxidant activity is Zingiber zerumbet from the rhizome. Previous study showed zingiber zerumbet have various activuty such as anti inflamation and antibacterial [6], [7], anti ulcer [8], [9], antioksidan [7], [9], [10]. Plant that usualy used is rhizome. Eventhough, the chemicals content not only in the rhizome but also in leaves. Bhuiyan et al [11] stated that rhizome and leaves have almost similar chemical content, such as eucaliptol, α-terpineol, 4-terpineol, borneol, agerospirol, and others. The difference between rhizome and leaves was on concentration levels. Accordingly, the research aimed to determine antioxidant activity of leaves extract of Zingiber zerumbet used DPPH method and compare result from spectrophotometer and ELISA Reader.

MATERIALS AND METHODS

Materials: glassware (pyrex), *rotary evaporator* (*Heiddolph*), *water bath (Memert)*, analytical balance (AND), TLC plate GF_{254} (merck), spectrophotometer UV-Vis (UV Mini SHIMADZU), *ELISA Reader (*Biotex elx 800), leaf of Z. zerumbet (Sleman, Yogyakarta, Indonesia), DPPH (E.Merck), chloroform, ethylacetate, n-hexene, metanol (E.Merck), $FeCl_3$(E.Merck), dragendorf, sitroborat.

Methods:

Extraction and fractination

Extration conducted by maceration method using metanol as solvent for 3 days. Macerate then evaporated by rotary evaporator to reduce ¾ of volume. Fractination was performed by vaccum liquid chromatography. Stationary phase was silica G60 with diameter 14 cm and heihgt 5 cm. Mobile phase used were mixture of n-hexene-chloroform (5:5; 4:6; 2:8; 1:9), each MP system performed twice elution with volume @ 150 mL, then elution by poured ethylacetateb 150 mL, followed by methanol twice elutiaon with each 200 mL. Fraction obtained collected and then evaporated to reduce volume by half volume. Fraction was evaluated by TLC method using mobile phase n-hexene-chlorofm (9:1) and stationary phase silica gel GF_{254}. Based on separation profile, similar fraction combined and grouped in semipolar and non polar fraction.

Identification of chemical compounds by Spray reagent on TLC

Semipolar and non polar fraction spotted on TLC plate and eluted by previous system. Spot eluted then calculated Rf values. Plate sprayed by spray reagent FeCl3 to evaluate phenolic content, dragendrof to know alkaloid content and sitroboric to know flavonoid content. Evaluation based on formed color at visual detection and change fluorescence on 355 nm detection.

Determination antioxidant activity by DPPH Method

Range concentration of non polar fraction made from 180, 220, 260, 300, and 340µg/mL, and semipolar fractions range 140,180, 220, 260 and 300 µg/mL. Solution incubated for 20 minute at dark places. Solution then measured of absorbantion at wave length 516.5 nm on spectrophotometer.

Range concentration of non polar fraction made from 180, 220, 260, 300, and 340µg/mL, and semipolar fractions range 140,180, 220, 260 and 300µg/mL. Solution incubated for 20 minute at dark places. Solution then measured of absorbantion at wave length 550 nm on ELISA Reader.

Table 1. Rf values of sample

No.	Rf						Note of spot
	Visualization on UV		Spray reagent				
	254 nm	366 nm	Sitroboric	DPPH	Dragendrof	$FeCl_3$	
1a	0.80		-	-	-	-	Burnout
1b	-	0.04	-	-	-	-	Blue
2b	-	0.30	-	-	-	-	Green-blue
3b	-	0.68	-	-	-	-	Blue
4b	-	0.80	-	-	-	-	Blue
1c	-	-	0.04	-	-	-	Blue
2c	-	-	0.10	-	-	-	Blue
3c	-	-	0.30	-	-	-	Green-blue
4c	-	-	0.68	-	-	-	Blue
5c	-	-	0.80	-	-	-	Blue
1d	-	-	-	0.30	-	-	Yellow
2d	-	-	-	0.48	-	-	Yellow
3d	-	-	-	0.68	-	-	Yellow
4d	-	-	-	0.80	-	-	Yellow

Table 2. IC_{50} Values of Samples using spectrophotometer UV-Vis (A) and ELISA Reader (B) (n=3)

Samples	A		B	
	IC_{50} (µg/mL)	SD	IC_{50} (µg/mL)	SD
Non polar fraction	295.22	8.28	291.27	11.33
Semipolar fraction	228.49	18.97	255.35	7.30

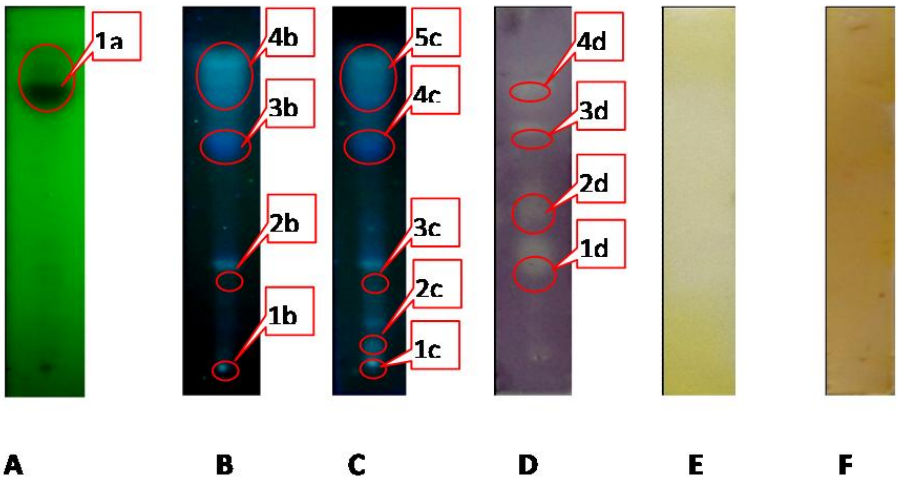

Figure 1. TLC profiles of non polar fraction sample on sicilca gel GF_{254} and mobile phase n-hexene:chloroform (9:1), visualization on UV_{254} (A), UV_{366}(B), after sprayed with sitroboric (C), DPPH (D), Dragendrof (E), and $FeCl_3$ (F)

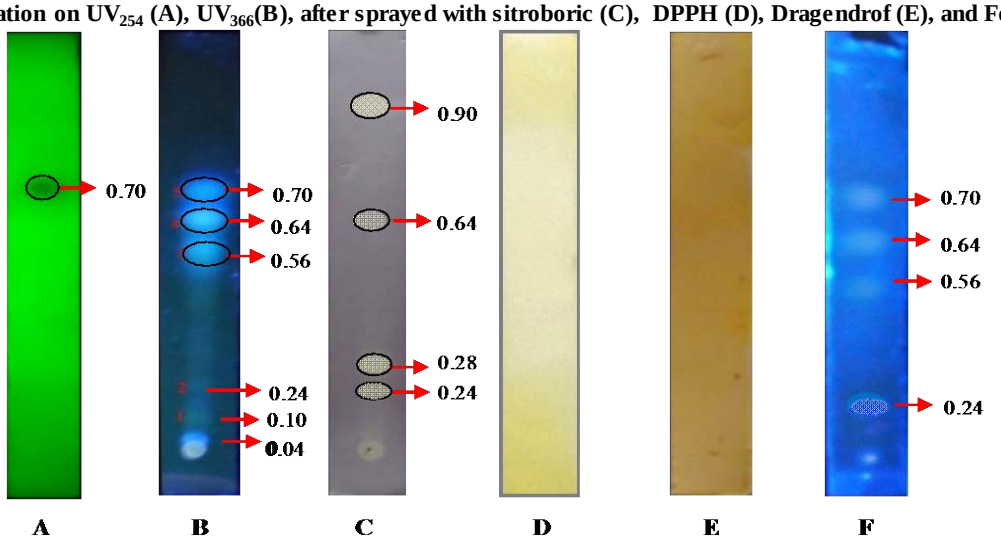

Figure 2. TLC profiles of non polar fraction sample on sicilca gel GF254 and mobile phase n-hexene:chloroform (9:1), visualization on UV_{254} (A), UV_{366}(B), after sprayed with DPPH (C), Dragendrof (D), $FeCl_3$ (E), and Sitroborat (F)

RESULT AND DISCUSSION

The sample was used proved as leaf of Zingiber zerumbet by Biology Departement of Universitas Muhammdaiyah Surakarta. Yielkd of extraction by maceration method was 9.82%. Compared with rhizome extraction yield, it was lower. Fractination and TLC profiles results showed 2 goups of fraction, non polar and semipolar fractions with yield, 107.56 and 100.35 mg, respectively. Polar fraction was not obtained, due to high polar of stationary phase.

Identification of chemical compounds of non polar and semipolar fractions

Spot of non polar and semipolar fraction sprayed with $FeCl_3$, positive result if color of spot formed green, blue, red, and black (violet to black). This color formed caused by ortho and meta-hydroxyl groups formed complex bond with iron. Dragendrof spray showed positive alkaloid if the spot formed color brown, orange to red brick. The formed of color due to charge transfer of lone pair electron from nitrogen of alkaloid. Stiroborat reagent spray used to evaluate flavonoid content of herbal medicine. The reaction mechanism is unclear, it is probably form complexes from boron and ortho hydroxyl of flavonoid formed color yellow (orange), blue and green. The result of spray reagent (fig 1 and fig. 2 and table 1) showed that alkaloid and phenolic compounds not contained in the fractions. Therefore to identification whereas has antioxidant activity, the spot sprayed by DPPH. The yellow color formed, which indicate the spots (R_f 0.3, 0.48, 0.68 and 0.8) have antioxidant activity. DPPH sprays have positive activity if the color of background violet and the spot is yellow [12].

Antioxidant Activity by Spectrophotometer dan *ELISA Reader*

DPPH method acceptance due to easy, simple, fast and require small samples [13]. The principle of measurement using a UV-Vis spectrophotometer is reading the absorbance of decreasing color intensity of DPPH. Measurement conducted at wavelength of 516.5 nm. The parameter in this method is IC_{50} (Inhibition Concentration 50%), which is smaller IC_{50} value better antioxidant activity or radical catcher power [14]. The antioxidant activity, IC_{50} values of non polar and semi polar fraction of ethanol extract of Z. zerumbet leaves using UV-Vis spectrophotometer were 295.22 and 228.49 µg/mL, respectively (table 2).

Antioxidant activity determine not only by spectrophotometer but also by ELISA reader [7]. The advantage of ELISA Reader is speed to measure samples faster compared than ones. In addition, the test with ELISA Reader requires only a small samples and reagent, in consequent it is cheaper compared than spectrophotometer. In contrast, the weakness of the ELISA Reader is that the wavelength is not as wide as in the UV-Vis spectrophotometer. It is depend on the availability of the filter. Due to availability of filter ELISA, the measurement of samples at 550 nm, but it is still in the range of the color. The IC_{50} value obtained by this instrument of non polar and semipolar fraction, in sequence were 291.27 and 255.35 µg/mL.

These results were quiet weak compared to vitamin E, as reference compound. The IC_{50} of vitamin E by spectrophotometer and ELISA Reader were 52 and 7 µg/mL, respectively. Based on IC_{50}, non polar and semipolar fractions classified as weak antioxidant [5]. The weak of antioxidant activity were in line with qualitative result which only few yellow spots on DPPH spray. However, antioxidant activity of non polar and semipolar fraction of ethanol extract of Z. zerumbet were better than ethanol extract of Z. zerumbet rhizome which has IC_{50} equal to 2,123 µg/mL.

Based on repeatability of measurements of spectrophotometer and ELISA reader which were RSD values 2.80% and 3.89%, respectively, it could be concluded that ELISA reader has potential as alternative instrument in measurement of antioxidant activity with DPPH method. Statistical test using t-test, antioxidant activity with spectrophotometer and ELISA reader showed there were insignificant. The t test result shows that t-count is 3.539 and t-crit value is 4.30.

CONCLUSIONS

1. IC_{50} values of non polar and semipolar fraction of leaves extract of Z. zerumbet were
2. ELISA Reader could be as alternative instrument to determine antioxidant activity by DPPH method

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