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ANTIOXIDANT ACTIVITY FRACTION OF KAFFIR LIME LEAVES (Citrus hystrix DC.) BY 1,1-DIPHENYL-2-PICRYLHYDRAZIL (DPPH) RADICAL SCAVENGING METHOD

[1]NUR CHAERUN NISA, [2]AKTSAR ROSKIANA AHMAD, [3]REZKI AMRIATI SYARIF, [4]ANDI AMALIAH DAHLIA

[1,2,3,4]Faculty of Pharmacy, Universitas Muslim Indonesia, Jl. Urip Sumoharjo KM 5 Makassar Indonesia
E-mail: [1]nur.chaerun.nisanew@gmail.com, [2]aktsar.roskiana@umi.ac.id, [3]rezkiamriati.syarif@umi.ac.id, [4]andiamaliah.dahlia@umi.ac.id

Abstract - The sample used in this study is the leaves of Citrus hystrix DC. belong to the family Rutaceae. This research aimed to determine the antioxidant activity of Citrus hystrix DC. leave fraction using 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging method. Citrus hystrix DC. leaf extract was extracted by maceration using ethanol 96% and obtained ethanol extract yield value of 5,044% and subsequently fractionated by Vacuum Liquid Chromatography (VLC) with the mobile phase is n-hexane:ethyl acetate and ethyl acetate:methanol. The stationary phase used silica gel F_{254}. The number of fractions obtained are 4 fractions. The results showed that the Citrus hystrix DC. leave fraction has antioxidant activity. The value of IC_{50} fraction 1 is 275,814 µg/mL, fraction 2 is 200,297 µg/mL, fraction 3 is 242,247 µg/mL and fraction 4 is 121,831 µg/mL. The 4thfraction of the Citrus hystrix DC. leave has higher antioxidant than the other fractions.

Index terms - Antioxidant, Citrus hystrix DC., kaffir lime leaves, 1,1-diphenyl-2-picrylhydrazil (DPPH), Vacuum Liquid Chromatography (VLC).

I. INTRODUCTION

One of the utilization of antioxidant compounds can be found in the family Rutaceae. Rutaceae is a family of 130 general in seven subfamilies, one of which is Citrus. Citrus (orange) is a growing plant in South Asia, Japan, and Indonesia. The plant is empirically efficacious to overcome fatigue and weak body after a severe illness [3].

Common citrus genus species are (Citrus hystrix DC.) containing phenolic, terpenoid [8], α-tokoferol [5], essential oils, flavonoids, myricetin, quercetin, luteolin, hesperetin, apigenin, isorhamnetin and phenol compounds such as carotenoid groups [1].

Results of research conducted by Tunjung et al in 2015 stated that IC_{50} value of chloroform extract of kaffir lime leaves (Citrus hystrix DC.) is 9.4 µg/mL and IC_{50} extract of ethyl acetate kaffir lime leaves is 25.2 µg/mL can reduce the viability of neuroblastoma (cancer). Besides, based on previous research of Fidrianny, Amaliah and Sukrasno (2016) mentioned that the antioxidant activity of ethanol extract of kaffir lime leaves (Citrus hystrix DC.) has a very strong antioxidant activity using DPPH test with IC_{50} value of 7.23 µg/mL.

II. MATERIAL AND METHODS

The kaffir lime leaves (Citrus hystrix DC.) were obtained from the Sidrap District, South Sulawesi Province, Indonesia.

A. Sample Extraction

Sample extraction by maceration using ethanol solvent. kaffir lime leaves powder (Citrus hystrix DC.) macerated with ethanol solvent. Maceration carried out for 3 days in a closed container and protected from light, stirring periodically. Maceration done 3 times. The filtrate obtained was collected further concentrated by using a rotary vacuum evaporator and the obtained extract ethanol.

B. Fractination by Vacuum Liquid Chromatography Methods

1. Preparation of Columns

The chromatographic column is cleaned and mounted perpendicularly, then the bottom of the column is corked so that the silica gel does not contaminate the fractional container. A total of 30 g of silica gel adsorbent 60 F254 (0.2-0.5 mm) versus 10 g with silica gel 60 F254 (0.063-0,200 mm) were mixed, then added to the column. The column filling is done under vacuum, in order to obtain maximum density.

2. Fractination Extract

Ethanolic extract of kaffir lime leaves (Citrus hystrix DC.) weighed 30 g and then put on top of adsorbent column previously read in column, above the extract was placed filter paper, then eluted gradually using mobile phase n-hexane: ethyl acetate in 50 mL with different degree of polarity (n-hexane, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and ethyl acetate), then ethyl acetate:methanol (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and methanol). The outflow is accommodated as a fraction.

C. Qualitative Test of AntioxidantActivity

Fraction of Citrus hystrix DC. obtained was bottled on a 7x1 cm silica gel TLC plate using a capillary pipe, then eluted with eluent n-hexane:ethyl acetate (8:2). Afterward, the plates were tested for antioxidant activity with sprayed DPPH solution and allowed to stand for 30 minutes. Stains that have

antioxidant activity will change from purple to yellow.

D. Quantitative Test of AntioxidantActivity

a. Preparation of DPPH Solution

DPPH powder of 3 mg was dissolved in 100 mL of methanol p.a in the forming pitch to obtain DPPH solution at 30 ppm concentration.

b. Preparation of Sample Solution

Made 1000 ppm stock solution by weighing the fraction of the kaffir lime leaves (Citrus hystrix DC.) as much as 10 mg and dissolved in methanol p.a while stirred and homogenized then enough volume up to 10 mL.

c. Preparation of Quercetin Solution as a Comparison

The 10 mg of quercetin powder was dissolved with 10 mL of methanol p.a in the stirred powders while stirred and homogenized and then adjusted the volume to the limit mark to obtain a stock solution of 1000 ppm. Then dilution with series of concentrations of 2, 4, 6, 8, and 10 ppm by means of 0.01 mL, 0.02 mL, 0.03 mL, 0.04 mL, and 0.05 mL respectively, each series of concentration and sufficient volume up to 5 mL. The concentration series that had been made was 0.5 mL each and then added 3.5 mL DPPH solution 30 ppm. The mixed solution was incubated for 30 minutes in dark space and the absorption was measured at 515 nm wavelength.

d. Measurement of Antioxidant Activity by UV-Vis Spectrophotometry

The stock solution that has been made is then diluted with concentration series of 20, 40, 60, 80, and 100 ppm. For a concentration of 20 ppm in a 0.1 mL pipette, a concentration of 40 ppm in a 0.2 mL pipette, a concentration of 60 ppm in a 0.3 mL pipette, a concentration of 80 ppm in a pipette of 0.4 mL, and a concentration of 100 ppm in a pipette of 0.5 mL, then each volume is sufficient with methanol p.a up to 5 mL. The concentration series that had been made in each pipette of 0.5 mL was then added 3.5 mL DPPH 30 ppm. The solution was incubated in the darkroom for 30 minutes, measured its absorbance at 515 nm wavelength [8].

E. Calculation of IC_{50}Value

The percentage of DPPH radical inhibition is calculated by the formula:

$$\text{percent inhibition} = \frac{[A_o - (A_s - A_e)]}{A_o} \text{ x } 100\%$$

Where A_o is the absorbance of the blank, A_s is the absorbance containing the sample and DPPH, A_e is the absorbance of the sample without DPPH [9].

The value of IC_{50} is a number indicating the concentration of test sample which gives 50% damping (can inhibit or damp the oxidation process by 50%). The value of IC_{50} is determined by means of a linear curve between the concentration of solution (x-axis) and percent inhibition (y-axis) of the equation y = a+bx can be calculated IC_{50} by using the formula:

$$IC_{50} = \frac{(50-a)}{b}$$

III. RESULTS AND DISCUSSION

In this study, the first step was performed to test the antioxidant activity fraction of kaffir lime leaves (Citrus hystrix DC.) by the determination of crops that aims to ensure that the samples of the plants used are true leaves of kaffir lime (Citrus hystrix DC.). The result of determination obtained by plant used in this research is Citrus hystrix DC species.

The next stage is making the extract of kaffir lime leaves (Citrus hystrix DC.) with maceration method. The results yield obtained from the extraction of the kaffir lime leaves (Citrus hystrix DC.) can be seen in the Table 1.

Table 1
Results yield of ethanol extract of the kaffir lime leaves (Citrus hystrix DC.)

Sample Weight (g)	Quantity of Solvent (mL)	Extract Weight (g)	Yield Extract (%)
600	4500	30,26	5,04

The stationary (adsorbent) rough silica gel (0.2-0.5 mm) of 30 g and fine silica gel (0.063-0,200 mm) of 10 g, the silica gel was mixed in order to prolong the contact time between the samples by the eluent used and prevent the elution process runs very slowly [14]. The mobile phase (eluent) used is n-hexane:ethyl acetate and ethyl acetate:methanol. Eluent has a different polarity of n-hexane is nonpolar, ethyl acetate is semipolar and polar methanol. The solvents are used because they have interesting properties of compounds that have the same or very like "like dissolve like" properties.

The outflow was accommodated as a fraction based on eluent used as much as 21 fractions. 21 fractions are then bottled on the TLC plate and seen the same chromatogram pattern. The fractions having the same chromatogram pattern were combined into one fraction so as to obtain 4 fractions, see in Table 2.

Table 2
Fractionation results of ethanol extract of kaffir lime leaves (Citrus hystrix DC.)

Fraction	Eluent	Weight of Fraction (g)
1	n-hexane:ethyl acetate (90:10) and (80:20)	5,94
2	n-hexane:ethyl acetate	5,57

	(70:30, 60:40, 50:50, 40:60, 30:70, and 20:80)	
3	n-hexane:ethyl acetate (10:90 and 0:100); ethyl acetate:methanol (90:10, 80:20, 70:30, 60:40, and 50:50)	9,34
4	ethyl acetate:methanol (40:60, 30:70, 20:80, 10:90 and 0:100)	9,33

The four fractions obtained were bottled on a 7x1 cm silica gel TLC plate with a capillary pipe, then eluted with eluent n-hexane:ethyl acetate (8:2). After that, the plate was tested by qualitative antioxidant activity with sprayed DPPH solution and allowed to stand for 30 minutes. Stains that have antioxidant activity will change from purple to yellow. It can be seen in Table 3.

Table 3
Qualitative test results of antioxidant activity of kaffir lime leaves fraction (Citrus hystrix DC.)

Fraction	Solution	Fraction of kaffir lime leaves (Citrus hystrix DC.)	Results
1	DPPH	Yellow stain, purple background	+
2	DPPH	Yellow stain, purple background	+
3	DPPH	Yellow stain, purple background	+
4	DPPH	Yellow stain, purple background	+

Description:
(+): has activity as an antioxidant
(-) : has no activity as an antioxidant

As according to Naik et al (2003), the capability of radical capture is related to the ability of the compound component to donate electrons or hydrogen. Any molecule that can donate electrons or hydrogen will react and will fade DPPH color, where the DPPH intensity will change from purple to yellow by electrons derived from antioxidant compounds.

Based on the results of qualitative research, 4 fractions of the kaffir lime leaves (Citrus hystrix DC.) have antioxidant activity.

The antioxidant activity test was done quantitatively using DPPH method. The DPPH method is used because the use of this method is fast enough, accurate and to evaluate antioxidant activity in foods and beverages, and is most widely used in screening antioxidant activity in medicinal plants [10]. The results of absorbance measurements, percent inhibition, and IC_{50} values can be seen in the Table 4.

Table 4.
Results of absorbance measurement, percent inhibition and IC_{50} values

Fraction	[ ] (ppm)	A sample and DPPH	A sample	Percent inhibition (%)	IC_{50} (µg/mL)
1	20	0,898	0,648	0,003	275,814
	40	0,898	0,637	0,005	
	60	0,898	0,626	0,009	
	80	0,898	0,611	0,013	
	100	0,898	0,601	0,017	
2	20	0,898	0,632	0,019	200,297
	40	0,898	0,617	0,023	
	60	0,898	0,602	0,027	
	80	0,898	0,590	0,032	
	100	0,898	0,579	0,039	
3	20	0,898	0,632	0,005	242,247
	40	0,898	0,621	0,012	
	60	0,898	0,610	0,015	
	80	0,898	0,602	0,021	
	100	0,898	0,589	0,028	
4	20	0,898	0,515	0,003	121,831
	40	0,898	0,510	0,007	
	60	0,898	0,501	0,012	
	80	0,898	0,489	0,014	
	100	0,898	0,480	0,018	
Quercetin	2	0,898	0,755	-	14,290
	4	0,898	0,691	-	
	6	0,898	0,648	-	
	8	0,898	0,601	-	
	10	0,898	0,557	-	

Description:
Absorbance Blank : 0,898

According to Phongphaichit et al (2007), a compound expressed as a very powerful antioxidant if IC_{50}<10 µg/mL, strong if IC_{50} value between 10-50 µg/mL, while if IC_{50} value ranges between 50-100 µg/mL, weak if the IC_{50} value is between 100-250 µg/mL and is inactive if IC_{50} is above 250 µg/mL.

The results obtained on the basis of measurement with UV-Vis Spectrophotometer at 515 nm wavelength are IC_{50} quercetin values obtained for 14.290 µg/mL, this indicates that antioxidant activity of quercetin is a powerful antioxidant (10-50 µg/mL). While for fraction 1 had inactive antioxidant activity (> 250 µg/mL) with IC_{50} 275,814 µg / mL, fraction 2, fraction 3 and fraction 4 had weak antioxidant activity (100-250 µg/mL) with IC_{50} value respectively 200,297; 242,247 and 121,831 µg/mL, indicating that the fraction of kaffir lime leaves (Citrus hystrix DC.) has antioxidant activity well below the comparison. It is influenced by the nature of polarity of active compounds distributed in the solvent used in the fractionation process [13]. Therefore, the use of solvent in the fractionation process will affect the value of IC_{50}.

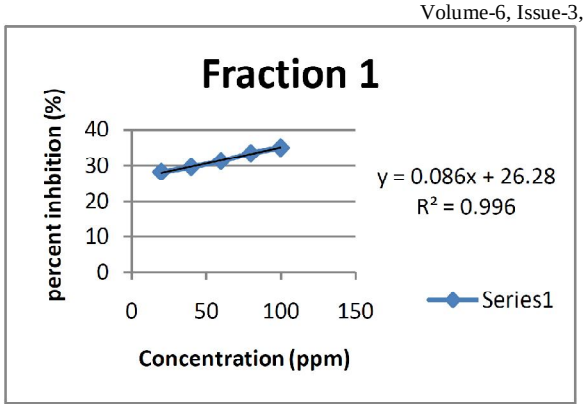

Figure 1: Graph of the relationship between the concentration of sample fraction 1 and percent of DPPH inhibition

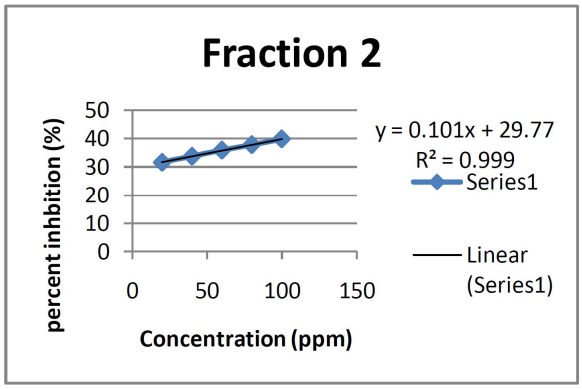

Figure 2: Graph of the relationship between the concentration of sample fraction 2 and percent of DPPH inhibition

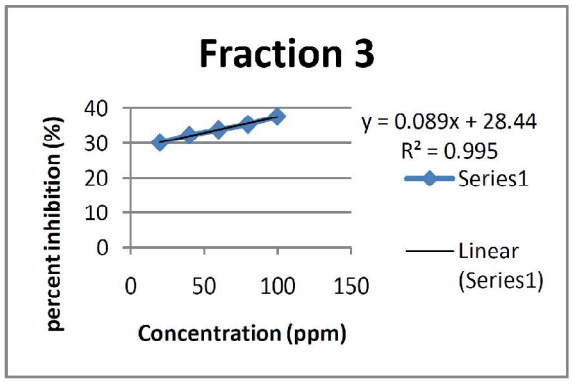

Figure 3: Graph of the relationship between the concentration of sample fraction 3 and percent of DPPH inhibition

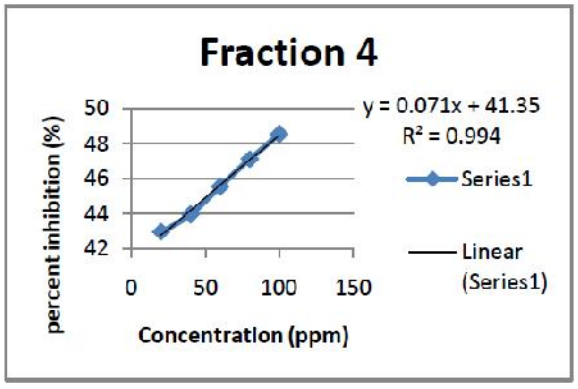

Figure 4: Graph of the relationship between the concentration of sample fraction 4 and percent of DPPH inhibition

From the above table, it is found that fraction 4 is the fraction which has the highest antioxidant activity compared with fractions 1, 2 and 3. This is thought to be caused of the fractions 1 and 2 consists of n-hexane-ethyl acetate and fraction 3 consisting of solvent n-hexane-ethyl acetate and ethyl acetate-methanol by comparison of the amount of ethyl acetate more so as to contain more non-polar and semipolar compounds. While fraction 4 consists of the ratio of ethyl acetate-methanol solvent with the ratio of the amount of methanol more so that the compound contained in fraction 4 is more polar. Barchan et al (2014) say methanol is a widely used and effective solvent for the extraction of antioxidants and phenolic compounds. Ali et al research results in 2015 also showed the highest total phenolic and flavonoid levels in kaffir lime leaves extract with some type of solvent is methanol extract.

CONCLUSION

In this research, the fraction of kaffir lime leaves (Citrus hystrix DC.) has antioxidant activity. The value of IC_{50} fraction 1 is 275,814 µg/mL, fraction 2 is 200,297 µg/mL, fraction 3 is 242,247 µg/mL and fraction 4 is 121,831 µg/mL. The fraction 4 of kaffir lime leaves (Citrus hystrix DC.) has higher antioxidant power than the other fraction.

REFERENCES

[1] Abeysinghe, DC, Li, X., Sun, C., Zhang, W., Zhou, C. and Chen, K., 2007. “Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species”, Food chem, vol. 104, no. 4, pp. 1338-1344.

[2] Ali, M., Rumana, A., Syeda, N., Mohammad, S. and Mohiuddin, A., 2014, “Studies of Preliminary Phytochemical Screening, Membrane Stabilizing Activity, Thrombolytic Activity and In-Vitro Antioxidant Activity of Leaf Extract of Citrus hystrix”, Int. J. of Pharm. Sci. and Resc., vol. 6, no. 6, pp. 2367-2374.

[3] Astarini, N., Perry, B. and Yulfi, Z., 2010, “Essential Oil of Citrus Citrus Citrus grandis, Citrus aurantium (L), and Citrus aurantifolia (Rutaceae) as Antibacterial and Insecticide Compounds”, Thesis, Faculty of Pharmacy, University of Muhammadiyah, Surakarta.

[4] Barchan, A., M. Bakkali, A. Arakrak, R. Pagan and A. Laglaoui, 2014, “The Effects of Solvents Polarity on the Phenolic Contents and Antioxidant Activity of Three Mentha Species Extracts”, Int. J. of Current Microbiol. and App. Sci., Vol. 3, no. 11, pp. 399-412.

[5] Ching, L.S. and Mohamed, S., 2001, “Alpha-tocopherol content in 62 edible tropical plants”, J. of Agric. and Food Chem., vol. 49, no. 6, pp. 3101-3105.

[6] Erawati, 2012, “Test of Antioxidant Activity of Garciniadaedalanthera pierre Leaf extract by using DPPH (1,1-diphenyl-2-picrilhidrazil) method and identification of the most active chemistry and fraction group”, S.Si Thesis, FMIPA Universitas Indonesia, Depok.

[7] Fidrianny I., Amaliah A. and Sukrasno, 2016, “Antioxidant Activities Evaluation of Kaffir lime leaves Extracts from West Java-Indonesia Using DPPH and FRAP Assays”, Int. J.of Pharmacogn. and Phytochem. Resc., Vol. 8, no. 4, pp. 611-618.

[8] Kooltheat, N., Kamuthachad, L., Anthapanya, M., Samakchan, N., Sranujit, RP, Potup, P., Ferrante, A. and Usuwanthim, K., 2016, “Kaffir lime leaves extract inhibits

biofilm formation by Streptococcus mutans", J. Nutr., vol. 32 no. 4, pp. 486-490.

[9] Maisuthisakul, P., Pasuk, S. and Ritthiruangdej, P., 2008, "Relationship between antioxidant properties and chemical composition of some Thai plants", J. of Food Comp. and Anal., vol. 21, no. 3, pp. 229-240.

[10] Marinova, G. and Batchvarov, V., 2011, "Evaluation of the Methods for Determination of the Radical Scavenging Activity by DPPH", Bulgarion J. Agric. Sci., Vol. 12, page 17.

[11] Maser, W.H, 2014, "Metabolomic application for identifying bioactive components: antibacterial component of Takokak fruit extract (Solanum torvum Swart)", Master Thesis, Bogor Agricultural University, Bogor.

[12] Naik, G.H., Priyadarsini, K.I., Satav, J.G., Banavalikar, M.M., Sohoni, D.P., Biyani, M.K. and Mohan, H., 2003, "Comparative Antioxidant Activity od Individual Herbal Components Used in Ayurvedic Medicine", Phytochem, vol. 63, no. 1, pp. 97-104.

[13] Phongphaichit, S., Nikom, J., Rungjindamai, N. and Jaruya, S., 2007, "Biological activities of extract from endophylic fungi isolated from Garcinia plants", FEMS Immunol. Med. Microbial, page 525.

[14] Septyaningsih, D., 2010, "Isolation and Identification of Main Component of Red Fruit Extract (Panandus conoideus Lamk.)", Thesis, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Surakarta.

[15] Tunjung, W.A.S., Jindrich, C., Martin, M. and Mark, S., 2015, "Anti-Cancer Effect of Kaffir Lime (Citrus hystrixDC) Leaf Extractin Cervical Cancer and Neuroblastoma Cell Lines", Procedia Chem., vol. 14, pp. 465 – 468.

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