Phytochemical screening, antioxidant activity and thin-layer chromatography test of methanol extract and simplicia leaves of loquat (*Eriobotrya japonica* Lindl)

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Abstract. Loquat (*Eriobotrya japonica* Lindl) is a medicinal plant that can be found in the Highlands in the region of Indnesia. Loquat has been widely used as a traditional medicine material by ethnic Chinese since ancient times. The research aims to analyse the phytochemical content and antioxidant activity of methanol extracts and simplisia loquat leaves. Phytochemical screening results show that methanol extract contains a secondary metabolite of alkaloids, flavonoids and steroid/terpenoid, while the Leaves Simplisia *E.japonica* contains a compound of secondary metabolites of alkaloids and flavonoids. Methanol extracts and Simplisia of *E.japonica* leaves were tested using DPPH with a high concentration of 20, 40, 60 and 80 ppm, incubated for 15 minutes and then measured by a spectrophotometer at a wavelength of 516 nm. Methanol leaves *E.japonica* extract has a value of IC_{50} 39.4 ppm very strong category, while the Leaves Simplisia *E.japonica* has a value of IC_{50} 102.4 ppm of medium category. The Rf value of methanol extract with a variation in solvent comparison (6:6, 7:3, 8:2, 9:1) is 0.78, 0.69, 0.51 and 0.40. The value of Simplisia leaves *E.japonica* with a variation in comparison of solvents (6:6, 7:3, 8:2, 9:1) are 0.80, 0.64, 0.49 and 0.18.

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical evergreen fruit tree originating in southeastern China. In Indonesia, Loquat is one of the Highland plants that has not been widely buable, but it can be found in some areas such as North Sumatera (Kab. Karo), North Tapanuli, Simalungun, Toba Samosir, and Dairi, in West Java (Cipanas, Kab. North Sulawesi (Tondano). Loquat plant is expected to flourish in Indonesia during the Dutch era [1][2].

In traditional Chinese medicine, loquat leaves have been used since ancient times to treat inflammatory diseases such as cough, acute bronchitis, and asthma. Research shows that loquat extracts contain many antioxidants, and different extracts exhibit bioactivity capable of counteracting inflammation, diabetes, cancer, bacterial infection, aging, pain, allergy and other health issues. Bioactive compounds such as phenolics and terpenoids have been isolated and characterized to provide a better understanding of the chemical mechanisms underlying the biological activities of loquat extracts [3][4].

The antioxidant activity contained in the loquat plant has not been extensively researched,

whereas antioxidant activity is closely related to its ability to treat various diseases. Therefore, research needs to be done to the antioxidant activity of leaves loquat using the method 1.1-Difenil-2-Pikrilhidrazil (DPPH).

2. Materials and methods

The materials used are methanol (Merck), DPPH (Sigma-Aldrich), *E.japonica* leaves. The tools used are Spectrophotometer (Shimadzu UV-1800), digital scales, Tentukur flask, micro pipette, volume pipette, glass beaker, Rotary evaporator, alluminium foil, stir bar.

2.1 Making of Extract

The leaves are washed with water and drained until the weight of the leaves becomes constant, then smoothed and sifted until the dry powder is obtained. The dry powder leaves loquat is macerated with methanol for 3 days, during immersion was done stirring several times, then filtered using a filter paper. Immersion is done 2 times until the filtrate is obtained clearly. Filtrate obtained is separated using a Rotary evaporator until a condensed paste is obtained in the form of pasta. Viscous extracts are stored inside the glass beaker and are covered with alluminium foil and then deposited into the freezer to prevent damage to the extract.

2.2 Screening Phytochemistry

The phytochemical screening is performed to detect secondary metabolites of the *E.japonica* leaves. Testing is conducted to detect alkaloid compounds, flavonoids, saponins, steroids and terpenoids.

2.3 Analysis of antioxidant activity of DPPH method

Measurement of DPPH Solution

10 mg of DPPH powder was dissolved into 50 mL of calibrate flask, inserted methanol until reaching the sign line so that the concentration gained 40 ppm. Measurements were performed at a wavelength of 400-800 nm to obtain maximum wavelengths. The final result is obtained at a maximum wavelength at 516 nm. It was subsequently used for the measurement of methanol *E.japonica*dan simplisia *E.japonica* extract.

Manufacturing of Raw Master solution of extract and Simplisia

As much as 25mg of methanol and Simplisia *E.japonica* extracts are weighed and put into a 5 mL calibrate flask with methanol, then the volume is chopped up to the sign line.

Measurement of antioxidant activity

The raw solution of the standard methanol extract was dipipet as much as 0.2:0.4:0.6 and 0.8 mL, put into a 5 mL calibrate flask (to get the concentration of 20:40:60 and 80 ppm). Each of the calibrate flask added 1.2 mL of the DPPH 0.5 mm raw Master solution, subsequently the volume was chopped with methanol and homogenized. The solution is incubated for 15 minutes, then measured by a spectrophotometer at a wavelength of 516 nm.
Determination of percent immersion is determined by the following formula:

$$\%\ \text{peredaman} = \frac{\text{Akontrol-Asampel}}{\text{Akontrol}} \text{x } 100\%$$

Description: $A_{kontrol}$: unsampled Absorbansi DPPH
A_{sampel}: DPPH Absorbansi with sample

Subsequent calculation results are inserted into a regression equation. The X-axis or abscissa is concentration (ppm) and the Y-axis or ordinariate is the percentage of radical immersion [5].

2.4 Thin-layer chromatography test (KLT)

Separation of the compounds contained in methanol extracts and Simplisia of *E.japonica* leaves are carried out using TLC on Silica plate gel with a plate length of 7.5 cm. Methanol extracts and Simplisia of *E.japonica* leaves are dissolved in n-hexa and ethyl acetate, Then it is taken along the plate at the upper limit of 1 cm and the lower limit is 2 cm. then eluted by using a solvent that has been complained. Elusi stopped when the movement of the development solution to the boundary line. The formed stain measured its Rf value. Stain spots are examined under UV rays at 245 nm wavelengths.

3. Results and Discussion

3.1 Making of Extract

The maceration of *E.japonica* leaves is done using methanol solvent. Samples were dismessed for 3x24 hours then maceration result is condensed with rotary evaporator until thick extract is obtained. The result of methanol extract obtained from 1.5kg Simplisia of *E.japonica* leaves is 150g.

3.2 Screening Phytochemistry

Screening phytochemicals against Simplisia and methanol leaves extract *E.japonica* is performed to identify the content of secondary metabolite of alkaloids, flavonoids, saponins, tannin, Streroids and terpenoids. The Phytochemical screening results are presented in table 1.

Table 1. The content of secondary metabolites of simplisia and methanol *E.japonica* extract

No	Components	Reagent Test	Result	
			Simplisia	Methanol Extract
1	Alcaloids	Bouchardart	+	+
		Wagner	-	-
		Maeyer	-	+
2	Flavonoids	$FeCl_3$	+	+
		NaOH 10%	-	-
3	Saponnin	Aquades	-	-
4	Steroid/Terpenoid	Salkowsky	-	-
		Liberman-Bouchard	-	+
5	Tannin	$FeCl_3$	-	+

3.3 Antioxidant Analysis by DPPH method

Antioxidants are a substance that at small concentrations is able to inhibit or prevent oxidation on substrates caused by free radicals [6]. Free radicals are highly reactive because they have unpaired electrons in the outer orbital so they are able to react with other body cell molecules by binding to these molecular electrons [7]. Free radicals are trapped as the cause of malfunction of the body's cells if continuously generated during the metabolic process, resulting in a trigger to degenerative diseases [8].

Results of measuring antioxidant activity contained in methanol extracts and *E.japonica* Leaves Simplisia obtained after incubation for 15 minutes. The test results of the antioxidant

activity of methanol and Simplisia *E.japonica* extract can be seen in Table 2, Figure 1 and Figure 2.

Table 2. Result of Antioxidant activity

No	Solution Test	linear regression	Correlation coefficient	IC_{50}	Category
1	Methanol Extract	y= 1,1 X + 6,7	r = 0,9772	39,4	Very strong
2	Simplisia	y= 0,61X + (-12,5)	r = 0,9772	102,4	Medium

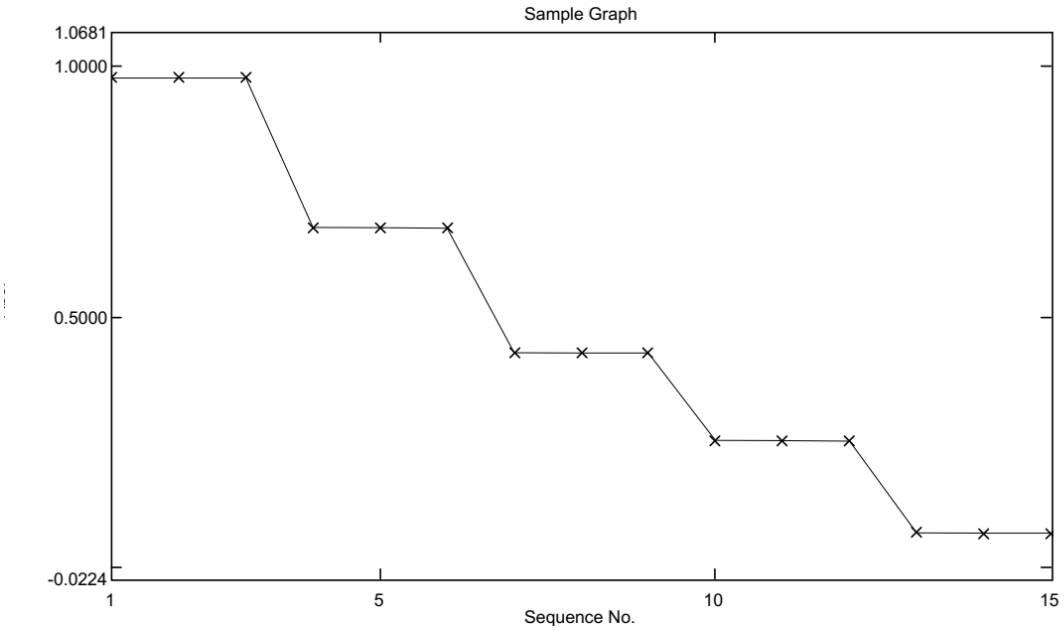

Figure 1. Effect of methanol extract *E.japonica* in DPPH

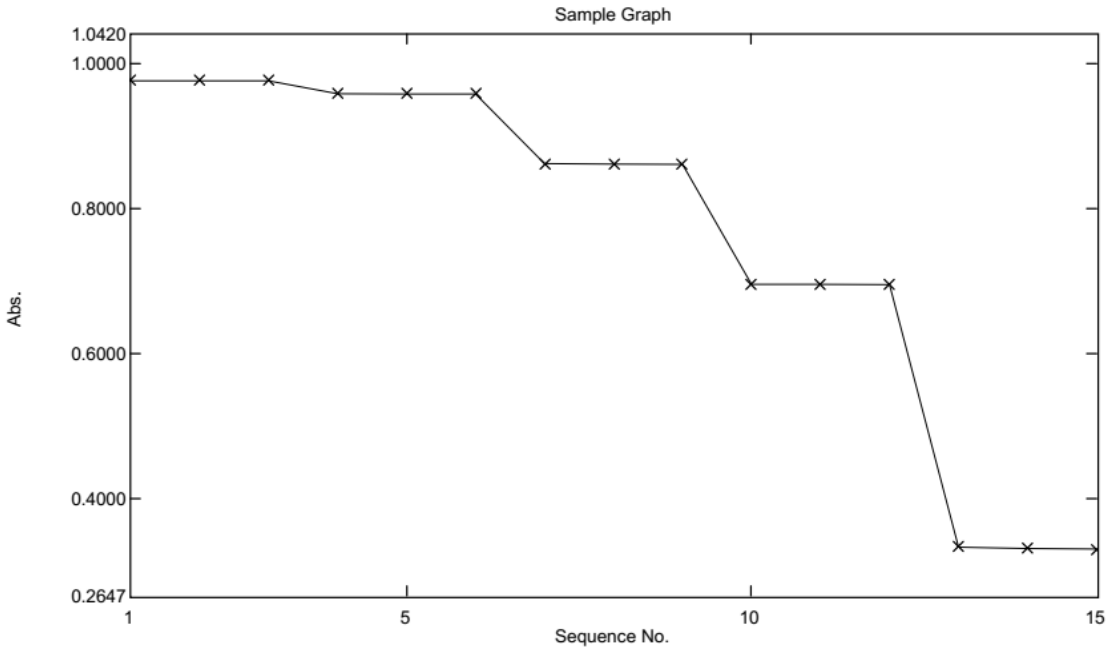

Figure 2. Effect of Simplisia E.japonica in DPPH

The results of the above studies have shown that methanol extracts and simplisia of *E.japonica* Leaves is the ability of different DPPH isolation activity. Antioxidants react with 1.1-difenil-2-Pikrilhydrazyl (DPPH) which stabilizes free radicals and reduced DPPH. DPPH will react with hydrogen atoms of the free radical reducer compounds forming a more stable 1.1-difenil-2-Pikrilhydrazine (DPPH-H). DPPH reagents that react with antioxidants will change color from purple to yellow, color intensity depending on the ability of antioxidants [8].

The results of the above studies have shown that methanol extracts and simplisia of *E.japonica* leaves is the ability of different DPPH isolation activity. Antioxidants react with 1.1-difenil-2-Pikrilhydrazyl (DPPH) which stabilizes free radicals and reduced DPPH. DPPH will react with hydrogen atoms of the free radical reducer compounds forming a more stable 1.1-difenil-2-Pikrilhydrazine (DPPH-H). DPPH reagents that react with antioxidants will change color from purple to yellow, color intensity depending on the ability of antioxidants [9].

The results of free radical isolation by methanol extract showed increased concentration in proportion to the increase in percent isolation. It is characterized by a change in the color of the purple solution to the yellow solution, which means that more hydrogen atoms of the *E.japonica* methanol leaves extract are paired with electrons on the DPPH free radicals. Results of free radical cancelling by the leaves Simplisia *E.japonica* also showed the increase in the concentration is proportional to the increase of percent of the isolation, but not as high as the results of the immersion by methanol leaves *E.japonica* extract. Activities Antioxidants from a compound can be classified according to the IC50 value obtained. If the IC50 value of an extract is below 50 ppm, its antioxidant activity is very strong. The value of IC50 is between 50-100 ppm and its antioxidant activity is strong. The value of IC50 is between 100-150 ppm and its antioxidant activity is moderate. The value of IC50 is between 150-200 ppm and its antioxidant activity is weak [9][10]. Methanol extract has antioxidant activity in a very strong category with a IC50 value of 34.9 ppm, while the leaves Simplisia *E.japonica* has antioxidant activity in medium category with IC50 value of 102.4 ppm.

3.4 Thin-layer chromatography (TLC) test

Thin-layer chromatography is a method of separation of chemical compounds based on the distribution of the motion phase and the silent phase. The Eluen used in this study is n-hexane: Ethyl acetate with a variation of solvent comparison (6:4, 7:3, 8:2, 9:1). Eluen is said to be good when able to separate compounds in quantities characterized by the appearance of stains. A separate stain is further measured by its Rf value. The farther the stain moves then the bigger the Rf value, instead the nearer the stain moves then the Rf value is smaller means the stain is held in the plate of silica and the sample is more polar. The Rf value (retention factor) can be seen in table 3.

Table 3. Result of Rf Value

Sample	Types of Eluen	Length of Substance	Rf Value
Simplisia	n-heksan:Ethyl acetate(6:4)	3,6	0,80
	n-heksan:Ethyl acetate(7:3)	2,9	0,64
	n-heksan:Ethyl acetate(8:2)	2,2	0,49
	n-heksan:Ethyl acetate(9:1)	0,8	0,18
Methanol	n-heksan:Ethyl acetate(6:4)	3,5	0,78

Extract	n-heksan:Ethyl acetate(7:3)	3,1	0,69
	n-heksan:Ethyl acetate(8:2)	2,3	0,51
	n-heksan:Ethyl acetate(9:1)	1,8	0,40

4. Conclusion

Methanol leaves *E.japonica* Extract is a secondary metabolite compound of alkaloids, flavonoids and steroid/terpenoids, while the leaves Simplisia *E.japonica* contains a compound of secondary metabolites of alkaloids and flavonoids. Methanol leaves *E.japonica* extract has a very strong antioxidant activity with a value of IC_{50} 39.4 ppm, while the leaves Simplisia *E.japonica* has an antioxidant medium category with a value of IC_{50} 102.4 ppm. The Rf value of methanol extract with a variation in solvent comparison (6:6, 7:3, 8:2, 9:1) is 0.78, 0.69, 0.51 and 0.40. The value of Simplisia leaves *E.japonica* with a variation in comparison of solvents (6:6, 7:3, 8:2, 9:1) are 0.80, 0.64, 0.49 and 0.18.

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