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Characterization and In Vitro Anti-cholesterol Activity Test of Ethanol Extract of Fruit *Ficus racemosa* L

Patimah, Dodi Irwandi*
Pharmaceutical and Food Analysis, Jakarta II Health Polytechnic Ministry of Health, Jakarta, Indonesia
*Corresponding Author: Dodi Irwandi Email: dodiirwandi@poltekkesjkt2.ac.id

Abstract**—** *The fruit of Ficus racemosa L., known in Indonesia as Loa, is believed to contain compounds that have biological activity. One of them is to lower cholesterol. This research aims to obtain initial information regarding the fruit ethanol extract's character, including secondary metabolite content, predicted compounds contained, water and ash content, and anti-cholesterol IC50. The methods used in predicting chemical compounds are using specific reagents and Gas Chromatography-Mass Spectrometry (GC-MS) instruments, water content using the Karl Fischer titration method and ash content using the gravimetric method, IC50 using the Visible spectrophotometric method. The test gives positive results containing alkaloid compounds, flavonoids, terpenoids, saponins, tannins, and glycosides with water and ash contents of 10.64% and 10.40%, respectively, and anti-cholesterol activity in vitro with an IC50 value of 522 µg/mL..*

Keywords**—** *Ficus racemosa L, GC-MS, anti-cholesterol.*

I. INTRODUCTION

Ficus racemosa L, also known as Loa in Indonesia, is found in various countries, including Thailand, Vietnam, Burma, Northern Australia, Nepal, Southern China, Bangladesh, India, Sri Lanka, Pakistan, and others. This plant thrives in moist soil at an altitude ranging from 100 to 700 meters above sea level and bears fruit throughout the year (1). Loa fruit is believed to possess anti-cholesterol activity (2).

Researchers have researched the biological activity of parts of the Loa plant. The fruit and leaves are antibacterial, the roots are a wound healer, and the bark is an antioxidant(3–5). There has been no research on the anti-cholesterol activity of the ethanol extract of Loa fruit. This research aims to obtain initial information on the chemical, water, and ash content and anti-cholesterol activity of the ethanol extract of Loa fruit (*Ficus racemosa* L).

II. MATERIALS AND METHODS

A. Materials

The following reagents were utilized: Liebermann-Burchad reagent, Molish reagent, Mayer's reagent, Dragendorf reagent, and cholesterol standard purity 99% from Sigma-Aldrich. The instruments used in the experiment were the Agilent Technologies 7890 Gas Chromatograph with Auto Sampler and 5975 Mass Selective Detector and Chemstation data system, rotary evaporator (Eyela), oven (Memmert UF 55), analytical balance (OHAUS PA214), Methrom 870 KF Titrino plus, and Furnace (Nuve MF 110).

B. Methods

Sampling and determination

Loa fruit was collected from South Jakarta and identified at the "Herbarium Bogoriense," Directorate of Scientific Collection Management at BRIN Cibinong.

The preparation of Loa fruit Simplicia

Loa fruit simplicia was prepared by cleaning the fresh fruit from impurities, cutting it into thin pieces, and drying it in the air without exposure to direct sunlight. Once dry, it is ground into powder and sieved using a 40-mesh sieve.

Preparation of extract

The extract was made by maceration using ethanol solvent. The ethanol extract was concentrated using a rotary evaporator until a thick methanol extract was obtained. The thick ethanol extract is then dried in an oven to produce a dry section.

Phytochemical screening of extracts

The screening method refers to Utami (6).

Identify the alkaloid group: several extracts were put into a test tube and dripped with 5 mL of 2 N HCl, heated, cooled, and then divided into 3 test tubes, 1 mL each. Each tube was added with each reagent. When Mayer's reagent is added, it is positive for alkaloids if it forms a white or yellow precipitate. When the Dragendrof reagent is added, it contains alkaloids and an orange precipitate forms.

Identify flavonoid groups: to determine whether flavonoids are present, a mixture of a specific amount of ethanol extract, 0.1 g of magnesium P powder, and ten drops of hydrochloric acid P is prepared. If the combination changes color from red-orange to purple-red, it indicates the presence of flavonoids. However, if the mixture turns orange-yellow, it suggests the presence of flavones, chalcone, and aurone.

Identify terpenoid and steroid groups: several extracts were placed in a test tube, mixed with 2 mL of ethyl acetate, and shaken. The ethyl acetate layer was separated and dripped onto a plate, and allowed to dry. Once dried, two drops of anhydrous acetic acid and one drop of concentrated sulfuric acid were added. If a red or yellow color appears, the test is positive for terpenoids. If a green color appears, it indicates the presence of steroids.

Identify saponin groups: a few extracts were mixed in a test tube, and 10 mL of hot water was added. After that, the mixture was cooled down and shaken for 10 seconds. The test for

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saponin was considered positive if the foam formed between 1-10 cm high within 10 minutes, and when one drop of 2N HCl was added, it did not cause the foam to disappear.

Identify tannin groups: to test for catechol tannins or pyrogallol tannins in a substance, take some extracts and mix them with 10 mL of hot water in a test tube. Boil the mixture for 5 minutes, then filter it. Next, add 3-4 drops of FeCl3 to the filtered solution. If the color of the solution turns blue-green or green-black, it indicates the presence of catechol tannins. On the other hand, if the color turns blue-black, it means that pyrogallol tannins are present.

Identify the group of glycosides: to detect glycosides, mix extracts with anhydrous acetic acid and sulfuric acid. A blue or green color indicates the presence of glycosides (Liebermann Burchard reaction). To identify sugar bonds, mix extracts with Molish reagent and sulfuric acid. A purple ring at the boundary confirms the presence of sugar bonds (Molish reaction).

Prediction test for compound content in Loa fruit extract using Gas Chromatography–Mass Spectrometry (GC–MS).

GC-MS compound analysis was carried out using the procedure at DKI Jakarta Health Laboratory, and then the results were compared with the compound database stored in the tool library. Ionization mode electron impact, electron energy 70 eV, column HP Ultra 2 Capillary Column Length (m) 30 x 0.20 (mm), 0.11 (µm) Film Thickness. Oven temperature, the initial temperature of 70ºC was held for 0 minutes, rising at 3ºC/min to 150ºC, held for 1 minute, and finally increasing to 20ºC/min to 280ºC held for 26 minutes, injection port temperature 250ºC, ion source temperature at 230ºC, interface temperature 280ºC, Quadrupole temperature 140ºC, carrier gas Helium, column mode constant flow, flow column 0.8 mL/minute, injection volume 3 µL.

Test the ash and water content: Test the water content of the extract using the Karl Fischer method and the ash content using the Gravimetric method.

Invitro test of extract anti-cholesterol activity

An in vitro test was conducted to determine the anti-cholesterol activity of an extract. The test followed the method used by Djamil and Farida with some modifications(7,8). A standard curve was created by making a concentration series of 20-100 µg/mL and adding 2.0 mL of acetic anhydrous and 0.1 mL of sulfuric acid. Then, a series of 200 – 1000 µg/mL extract concentrations was made, added with 2.0 mL of acetic anhydrous and 0.1 mL of sulfuric acid. The mixture was homogenized using a vortex, and the tube's outer layer was closed and allowed to stand according to the operating time until the color changed to green. The color results were then read using a UV-Vis spectrophotometer at a wavelength of 660 nm. For the blank, 5 mL of chloroform was used, plus 2 mL of acetic anhydrous and 0.1 mL of concentrated sulfuric acid. The negative control was 5 ml of 100 ppm cholesterol solution in chloroform plus 2 mL of anhydrous acetate and 0.1 mL of concentrated sulfuric acid.

The % cholesterol inhibition was calculated using the following formula: % Inhibition = Ab - As x 100 %, where Ab represents the absorption of cholesterol standard solution, and As represents the standard absorption of cholesterol after reacting with the extract. The IC50 value was obtained from y = a + bx, where y is 50 and x is IC50. The equation y = a + bx was obtained from the extract concentration and %IC relationship.

III. Result and Discussion

The results of determination test Number B-1011/ II.6.2/IR.01.02/5/2023 have confirmed that the sample is a Loa plant (*Ficus racemosa* L). A phytochemical screening test was performed on the ethanol extract of Loa fruit, and the results are shown in Table I. The test was conducted using Bagyalakshmi and Putra's method(9,10).

TABLE I. Phytochemical screening of Loa fruit (Ficus racemosa L) ethanol extract

No.	Group	Result
1	Alkaloid	+
2	Flavonoid	+
3	Terpenoid	+
4	Steroid	-
5	Saponin	+
6	Tannin	+
7	Glycosides	+

TABLE III. Chemical compounds of Loa fruit (Ficus racemosa L) ethanol extract

Retention Time	Quality	Chemical compounds	Content (%)
5.263	53	2,5-Piperazinedione, 3-methyl-6- (1-methylethyl)-	2,68
5.739	38	3-[(Cyclohexyl-methy]-emino)- methyl]-3H-benzooxazol-2-one	1,03
6.339	35	0-Methylisourea	1,74
7.104	97	4H-pyran-4-one, 2,3-dihydro-3,5- dihydroxy-o-methyl-	51,05
8.373	60	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	1,35
9.835	90	Catechol	4,19
30.983	99	n-Hexadecanoic acid	2,67
32.141	91	cis-Vaccenic acid	1,80
33.837	81	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1,49
38.940	99	.gamma. - Sitosterol	2,29
39.822	99	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	1,73
39.891	50	alpha.-Amyrin	1,11
40.602	92	12-0leanen-3-yl acetate, (3.aloha.)-	2,17
41.312	99	Lup-20 (29)-en-3-ol, acetate, (3.beta.)-	12,82

The GC-MS test was also conducted, and the chromatogram results are shown in Fig. 1. Based on compound predictions using the data library stored on the tool, 14 chemical compounds were found (Table II). The highest content of 51.05% was predicted to be the compound 4H-pyran-4-one, 2,3-dihydro-3,5- dihydroxy-o-methyl-. The following highest compound was 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-o-methyl- with 12.82% and Catechol with 41.19%. The remaining compounds had levels below 3%.

The water and ash contents of the extracts are summarized in Table III. In vitro anti-cholesterol activity testing gave results as summarized in Table IV, with an IC50 of 522 µg/mL. This

value was calculated using the regression equation between extract concentration and % inhibition (y = 22.169x - 585.51, R^2 = 0.9897).

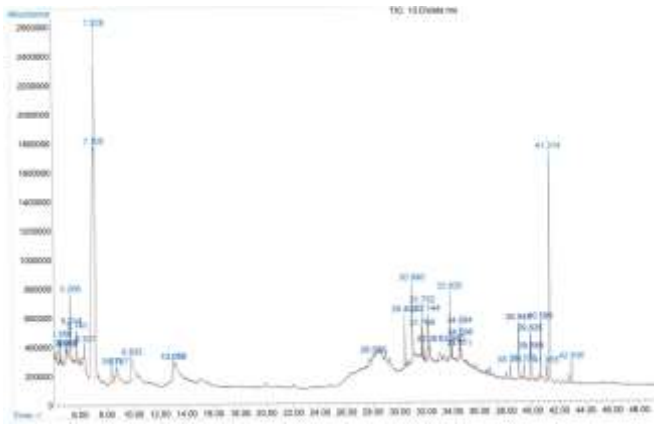

Fig. 1. GC Kromatogram of Loa fruit (*Ficus racemosa* L) ethanol extract

TABLE IIIII. Water and ash content of Loa Fruit exstract

Test	Content (%)
Water	10,64
Ash	10,40

TABLE IVV. IC50 anti-cholesterol of Loa fruit (*Ficus racemosa* L) ethanol extract

Extract concentrations (µg/mL)	% Inhibition	IC50 anti-cholesterol
200	37	522 µg/mL
400	43	
600	53	
800	62	
1000	73	

IV. CONCLUSION

Loa fruit (*Ficus racemosa* L) ethanol extract contains secondary metabolite compounds, including alkaloids, flavonoids, terpenoids, saponins, tannins, and glycosides. In the Gas Chromatography-Mass Spectroscopy test, 14 chemical compounds were found contained in the ethanol extract of Loa fruit. The water and ash contents were 10.64 and 10.40, respectively, anti-cholesterol IC50 522 µg/mL.

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