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Research Article

Antioxidant Activity of Methanol Fractions Stem Bark of Kayu Sarampa (*Xylocarpus moluccensis* (Lam.) M. Roen))

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ABSTRACT

Introduction: Methanol extract of X. moluccensis was found to be significantly effective in scavenging DPPH method. Therefore, this research is a follow-up research study from Budiarso et al (2020).. The methanol extract was then fractionated and tested for antioxidant activity. **Objective:** To assess antioxidants activity of methanolic fractions from stem bark of Kayu Sarampa. **Method:** The Stem bark was extracted with Reflux method using hexane, ethyl acetate, and methanol as solvent. The methanolic extract was fractionated using a chromatographic column were subjected to the antioxidant activity assay by the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and the ferric-reducing antioxidant power (FRAP) method. **Results:** F3 Fractions IC_{50} of *X. moluccensis* exhibits the highest DPPH scavenging activity compared with F2, F3, ascorbic acis as control positif, F5, and F4, wich are 4.64, 6.79, 9.69, 10.49, and 227.44 respectively and Ferric reducing power from methanolic fraction of *X. moluccensis* stembark F3 exhibits higher antioxidant power compared to F2, F1, F5, ascorbic acid and F4, respectively which are 667.8 µmol/gr, 607.8 µmol/gr, and 573.8 340.48 and 309.8 µmol/gr, respectively

Key words: Kayu Sarampa, DPPH, FRAP, Antioxidant.

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INTRODUCTION

Antioxidant was used to inhibit or minimalize oxidative damage. Two common methods which is used to evaluate antioxidant activity is 1,1-diphenyl-2-pilcrylhidrazyl (DPPH) and ferric reducing antioxidant power (FRAP)[1] This method measures synthetic radicals DPPH in a polar solute such as ethanol or methanol at room temperature which is scavenged by an antioxidant compound.[2] The ferric reducing antioxidant power (FRAP) assay is a typical ET-based method that measures the reduction of ferric ion (Fe3+)-ligand complex to the intensely blue-colored ferrous (Fe2+) complex by antioxidants in an acidic medium.[3]

Native people in Ratahan, Sulawesi Utara use batu Nyirih (*Xylocarpus moluccensis*) to treat diabetic patients. This plant is known as Kayu sarampa**.** *Xylocarpus* genus spread from India beach, Ceylon, Burma, Malaysia and Indonesia. Many researchers study about active components in Kayu Sarampa. It contains antibacterial, antidiabetes, antioxidant, antifilarial, antidiarrhea, antidepressant and cytotoxic activity.[4] Kayu sarampa can be used to treat fever, joint pain, headache, and disorders such as cholerae, constipation, and diarea. [5]

Methanol extract of *X. moluccensis* was found to be significantly effective in scavenging DPPH method.[6] Rouf (2007) report that methanol extracts of Xylocarpus granatum bark have a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.[7] The fruits of *X. moluccensis* contain limonoid which can change enzyme activity to metabolize glucose and increase glucose absorption by muscle tissue.[5] Therefore, this research is a follow-up research study from Budiarso et al (2020).[8] The methanol extract was then fractionated and tested for antioxidant activity.

MATERIAL

Plant material

Sample used in this study was the stem bark of *X. moluccensis* which was obtained from Ratatotok district, North Sulawesi and was identified by Herbarium Bogoriensis, Biological research centre, Indonesian Institute of Science.

Chemicals

Chemicals used in this study were dichloromethane, dimethyl sulphoxide (Merck cat. 3.17275, Germany), ethanol, ethyl acetate, methanol, n-hexane, 1.1-diphenyl-2-picrylhidrazyl (DPPH), ascorbic acid (Sigma-Aldrich, A5960), Ferrous sulfate heptahydrate (MERCK, Germany).

Sample preparation

The stem bark of *X. moluccensis*. from Ratatotok, North Sulawesi, was freshly picked, collected, sorted, and dried. The Stem bark were then crushed until they became smaller.

Extraction and Fractionation

One kilogram of stem bark powder of dried *X. moluccensis* was extracted using hexane. Extraction was initially done using reflux apparatus to obtain hexane extract. Hexane extract then was evaporated using a rotary evaporator to obtain a crude hexane extract (HE). It was stored under room temperature. The same way was done for ethyl acetic solvent to obtain ethyl acetate extract (EAE). Then, methanol was used to extract the residue to obtain methanolic extract (ME). The methanol extract was then fractionated and tested for antioxidant activity.

Determination of free radical 1.1-diphenyl-2-picrylhidrazyl (DPPH) scavenging activity

DPPH radical scavenging activity was done according to the procedure declared by Gracia et al (2014) with slight modification.[9] The test solution from 5 concentration series of 20 µL pipette was put into 96 wells, added 180 µL of DPPH, vortexed until homogeneous, incubated at room temperature for 30 minutes and the absorbance was measured at a wavelength of 517 nm. The absorbance of the solution was measured for 5 minutes before incubation time ends. Color change from purple to yellow means free radical scavenging efficiency. Free radical scavenging activity was calculated as the percentages of color decreasing of DPPH solution using the following equation:

$$\textit{Free radical scavenging activity (\%)} = \left(1 - \frac{\textit{sample absorbance}}{\textit{control absorbance}}\right) \times 100\%$$

After the inhibition percentage of each concentration was obtained, linear regression was made so that the equation y = a + bx was obtained, where x is the concentration (µg/mL) and y is the percentage of inhibition (%). Antioxidant activity is expressed by 50% Inhibitory Concentration or IC_{50}, which is the concentration of the sample that can reduce DPPH radicals by 50% from the initial concentration.

FRAP assay test

This test was based on the microplate reader method described by Xiao F. *et al*.[9] Acetate buffer (300 mM, pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) 10 mM in 40 mM HCl, $FeCl_3 \bullet 6H_2O$ (20 mM). The working FRAP reagent was prepared by mixing the three solutions in the ratio of 10:1:1. Methanolic fractions of stem bark of *X. moluccensis* (20 µL, consentration 500 mg/L) was mixed with 280 µL of working FRAP reagent, incubated ay 37°C for 30 minutes in the dark and the absorbance was measured at 598 nm after vertexing.The solutions of $FeSO_4 \bullet 7H_2O$ ranging from 100 to 2000 µM were prepared and used for the preparation of the calibration curve of known Fe^{2+} concentration. The parameter equivalent concentration was defined as the concentration of antioxidant have a Ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4 \bullet 7H_2O$.[7]

Statistical analysis

All the experimental data do triplicates and the results are expressed as mean ± SD. IC_{50} was analyzed using Probit. Antioxidant power were analyzed using One way ANOVA analysis followed by Duncan Multiple Range Test. Analyzes were performed using SPSS software version 20.

RESULTS AND DISCUSSION

X. moluccensis was extracted sequentially using reflux with hexane, ethyl acetic, and methanol as the solvent. Relux is an extraction using heat. The methanolic extract was fractionated using a chromatographic column with silica gel G_{60} as the stationary phase and n-hexane, ethil acetate and methanol as the mobile phase.

Table 1 shows the yield of methanolic fractions. A polar bioactive component from kayu sarampa extract is dissolved in methanol. The yield of the extract is depending on the effectivity of solvent used for extraction. High yield of extract means high extracted component.

Free Radicals DPPH Scavenging Activity

The evaluated *X. moluccensis* concentration were 2.5, 5.0, 7.5, 10.0, and 12.5 mg/L, respectively. Free radical DPPH commonly used as substrate to evaluate antioxidative activity of antioxidant. Antioxidant properties of *X. moluccensis* extract reacted with DPPH, the solution color changed from purple to yellow. The change in solution color affected to DPPH absorbance. The higher concentration of antioxidant component in solution, the lower the absorbance of DPPH. The change in solution color is caused by a component that donates hydrogen atom to DPPH radical. Antioxidant reducted DPPH radical to more stable form namely DPPH-H (2,2-diphenyl-1-picrylhidrazine).[3] Lai *et al*. (2001) stated that antioxidant activity using radical DPPH mostly increase in a concentration-dependent manner.[10]

IC_{50} value represents the ability of *X. moluccensis* fracions to scavenge 50% of DPPH free radical. The lowest IC_{50} value of an extract represents high antioxidant activity.[11] IC_{50} was obtained from a regression linear equation in Figure 1 which plotted x axis (concentration) with y axis (free radical DPPH scavenging activity), so a regression equation and IC_{50} obtained is presented in Table 3.

Table 2 showed that F3 Fractions IC_{50} of *X. moluccensis* exhibits the highest activity compared with F2, F3, F5, and F4, respectively. IC_{50} value of an fraction represens its potential to inhibit free radicals. A strong level IC_{50} categorized with IC_{50} value 50-100 mg/L, midle level category 100-250 mg/L, and low level category was 250-500 mg/L.[12]

Ferric reduction antioxidant power

The FRAP method is a method of testing antioxidant activity through the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+} in the presence of 2.4,6-tri (2-pyridyl)-s-triazine (TPTZ) in an acidic atmosphere which produces intensive blue colour from the Fe^{2+}-TPTZ complex and causes an increase in absorbance when it is measured using a microplate reader at a maximum absorption of 593 nm. In the test using the FRAP method, ferrous sulfate heptahydrate (FSH) is used as a standard. First, the standard FSH calibration curve is made. The antioxidant activity is measured based on the sample equality with the AFS standard. The linear regression equation of FSH obtained y = 0.0005 x + 0.05441. Antioxidant power of sarampa extract is shown in following table 4.

Data is presented as the mean ± standard deviation (N=3). Means with different letters are significantly different (p<0,05).

Table 3 shows that F3 from methanolic fraction of *X. moluccensis* stembark exhibits higher antioxidant power compared to F2, F1, F5, ascorbic acid and F4, respectively which are 667.8 µmol/gr, 607.8

Table 1: The yield of methanolic fractions *X. moluccensis*.

	F1	F2	F3	F4	F5
Extract weight (g)	1.21	2.16	2.27	4.22	5.55
Yield %	7.8	14.0	14.7	27.4	36.1

Table 2: Regression equation and IC_{50} of *X. moluccensis* extracted with different solvents.

	F1	F2	F3	F4	F5	Ascorbic acid
Regression Equation	y= 3.4835x + 16.891	y = 5.5513x + 13.375	y = 5.0745x + 26.408	y=0,2158x + 0,9182	y= 3.9792x + 4.76	y = 0.3788x + 46.026
IC_{50} (µg/L)	9.69	6.79	4.64	227.44	11.36	10.49

Table 3: Antioxidant power using FRAP.

Sample	Antioxidant Power (FRAP) (µmol Fe^{2+}/g)
Control (Ascorbic Acid)	340.48 ± 0.02
F1	516.0 ± 0.06
F2	607.8 ± 0.08
F3	667.8 ± 0.05
F4	309.8* ± 0.06
F5	573.8 ± 0.07

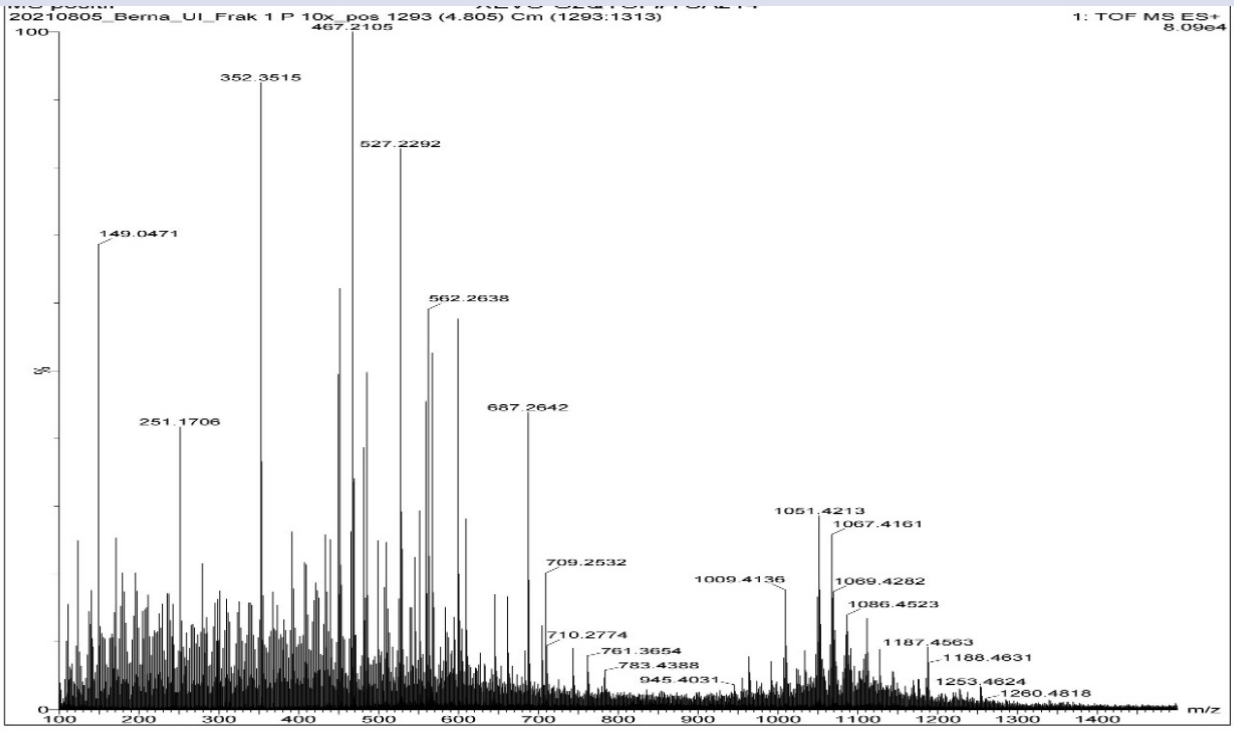

Figure 1. Candidate mass compound $C_{35}H_{42}O_{14}$.

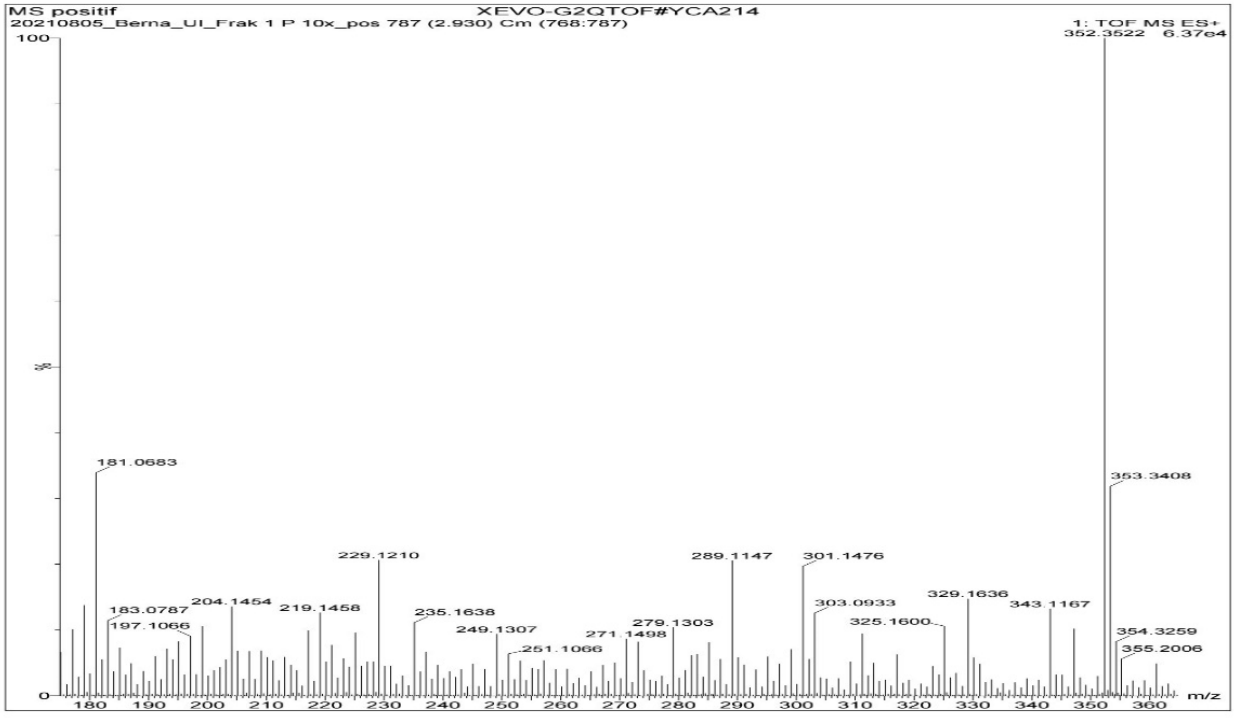

Figure 2. Candidate mass compound $C_{15}H_{10}O_7$.

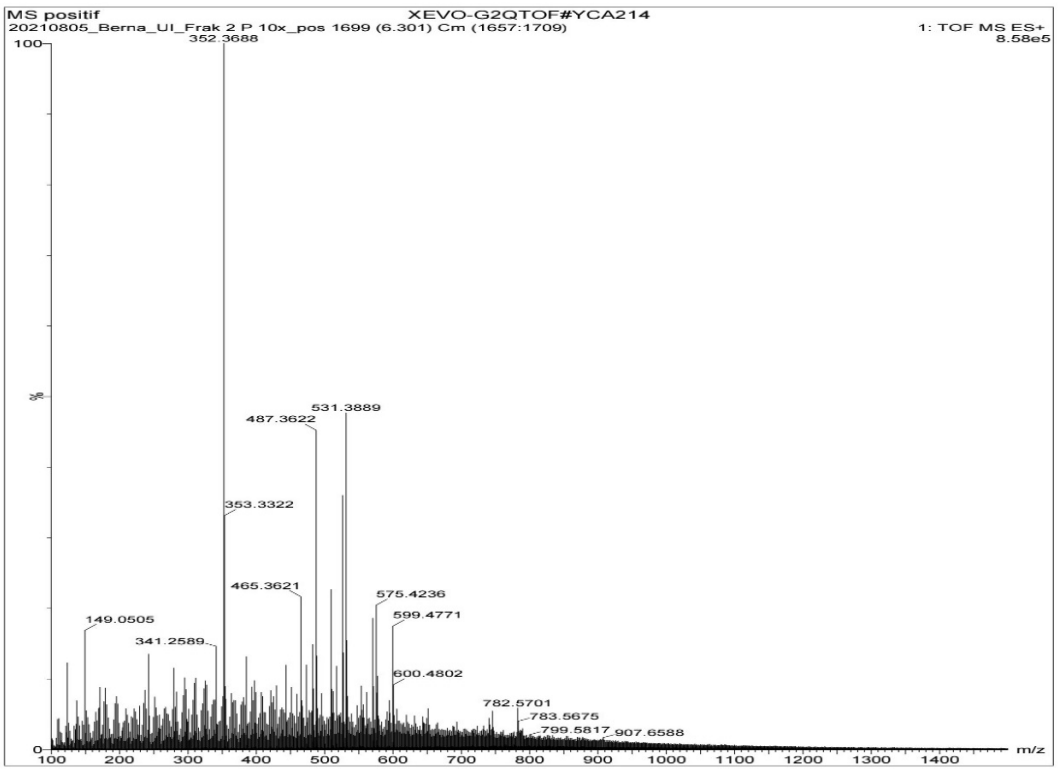

Figure 3. Candidate mass compound $C_{29}H_{38}O_9$.

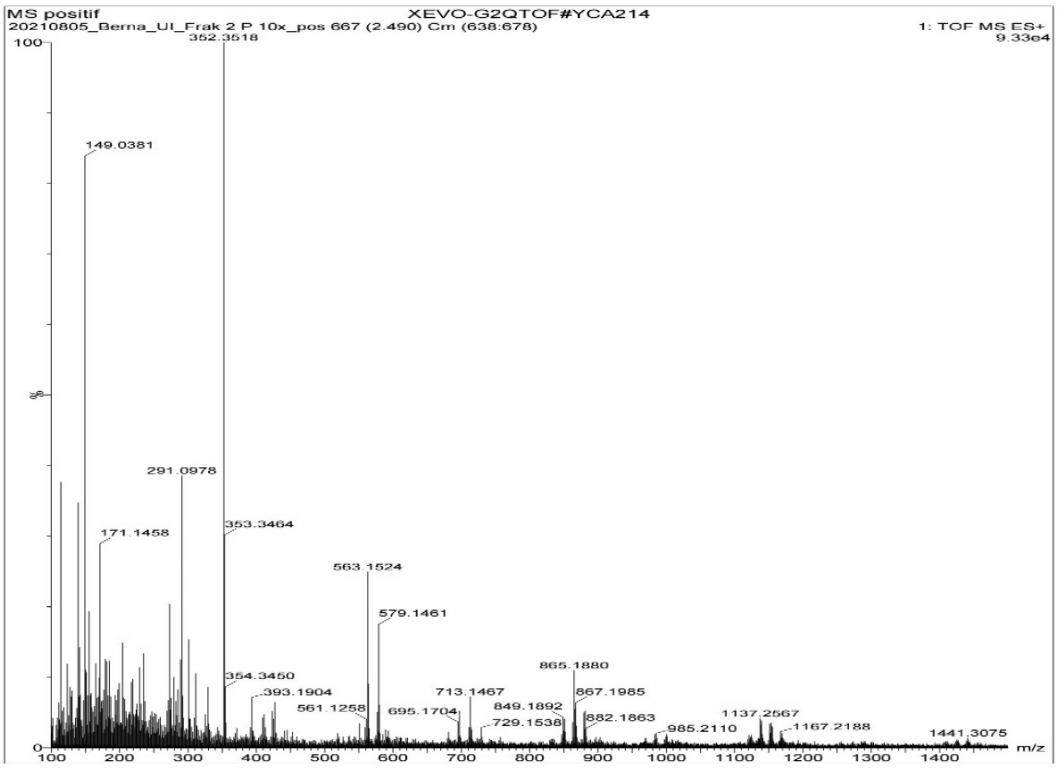

Figure 4. Candidate mass compound $C15H_{14}O_6$.

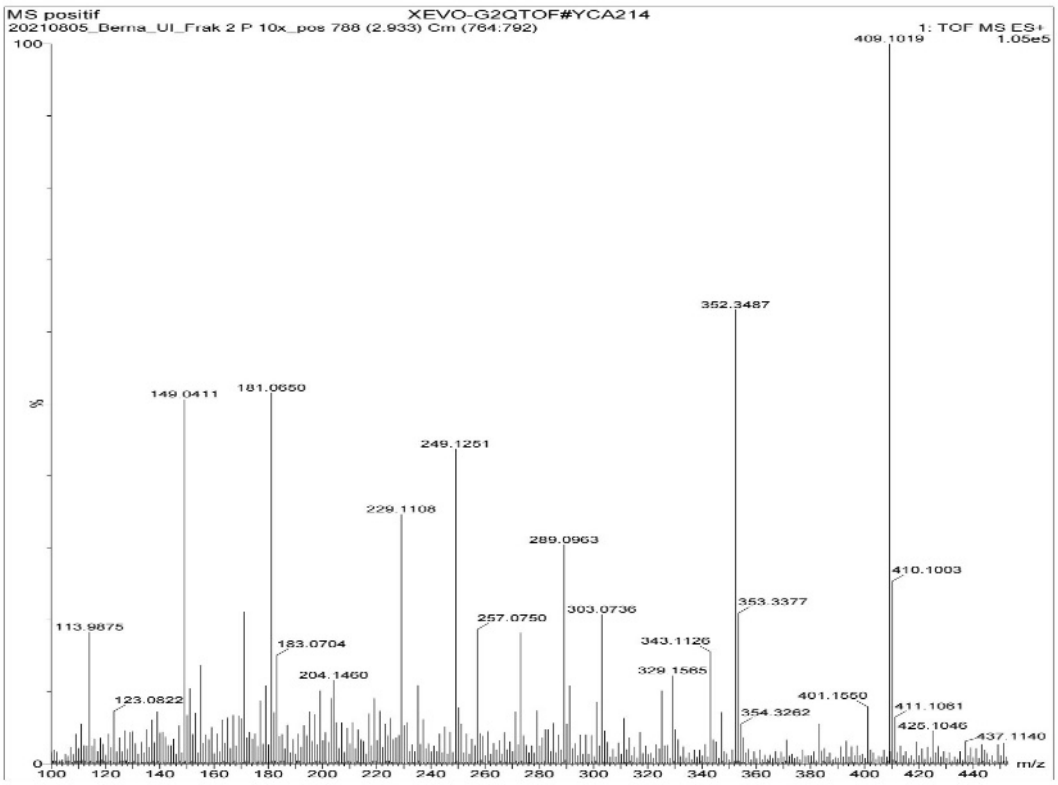

Figure 5. Candidate mass compound $C_{15}H_{10}O_7$.

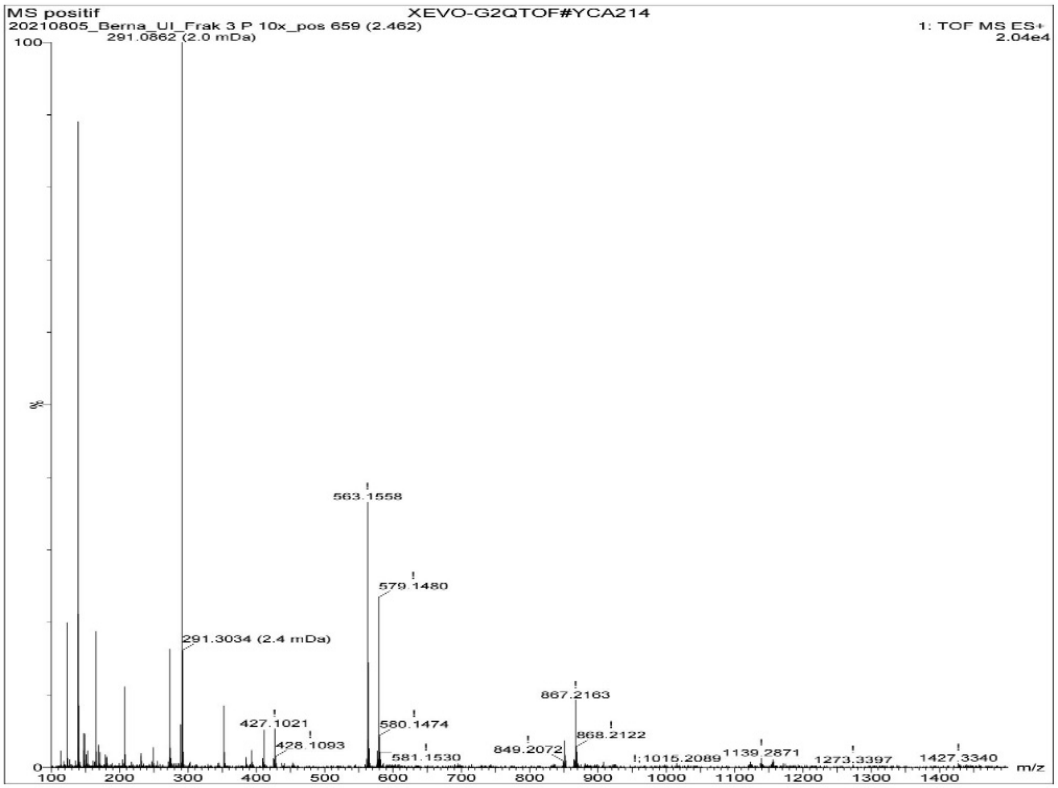

Figure 6. Candidate mass compound $C_{15}H_{14}O_6$.

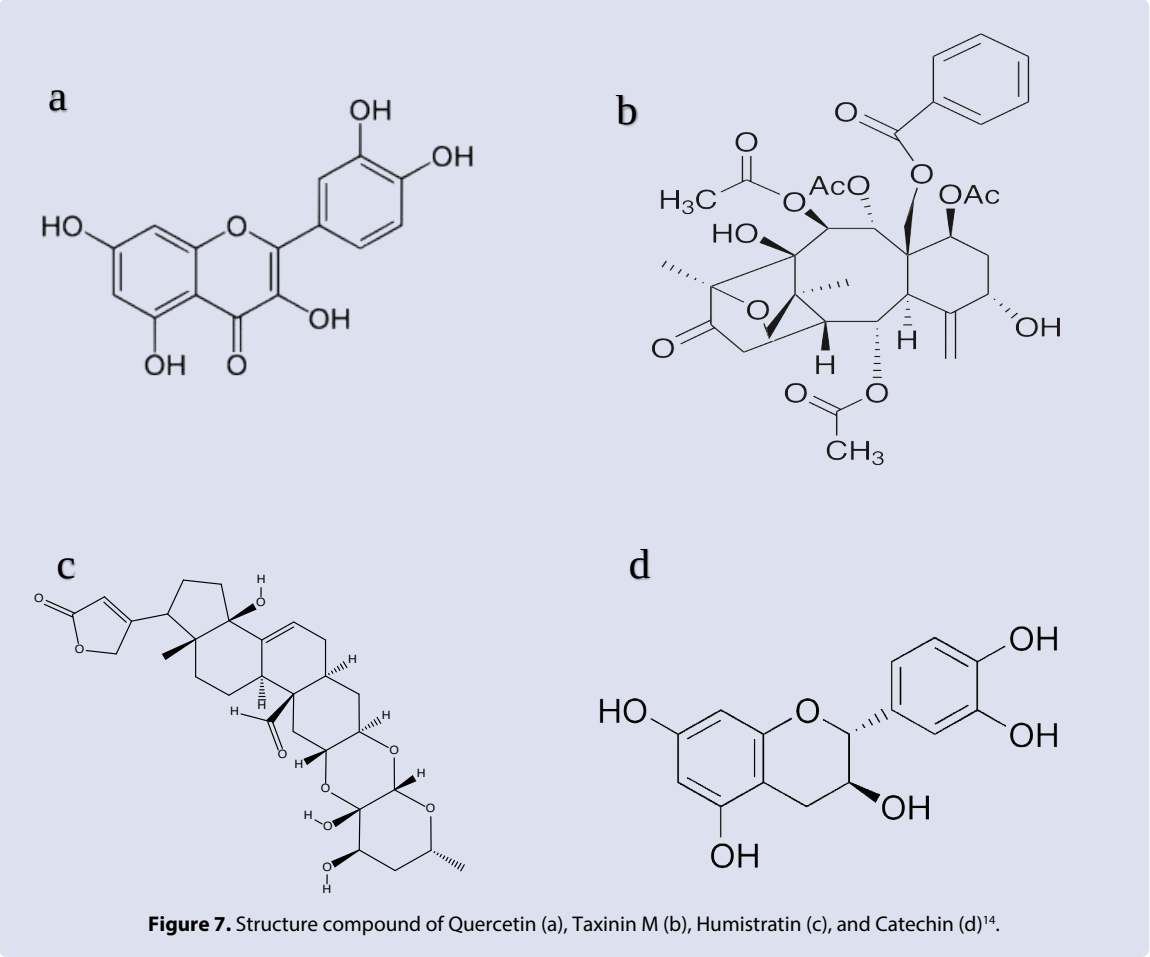

Figure 7. Structure compound of Quercetin (a), Taxinin M (b), Humistratin (c), and Catechin (d)[14].

µmol/gr, and 573.8 340.48 and 309.8 µmol/gr, respectively. Statistical analysis reveals that antioxidant power of samples extracted with different solvent are significantly different ($p<0,05$). Based on the result presented in Table 3, The higher total antioxidant content in extract means more compounds can reduce Fe3+ to Fe2+ (blue), and compounds that reduce Fe3+ is an antioxidant compound. Prior *et al* 2005 states that methanol is a polar compound which is easy to position the hydrogen atoms of a compound or hydroxyl groups to form hydrogen bonds because the bonds would facilitate the transfer of protons (hydrogen atoms antioxidants). Flavonoids, a subclass of polyphenols, are a group of phytochemicals that are among the most potent and abundant antioxidants.[13]

LC-MS Result of Methanol Fractions of Kayu Sarampa Stem Bark

The Methanol Fractions of Kayu Sarampa stem bark was identified using LC-MS. The results of LC-MS are seen in Figure 1-7.

The results of LC-MS showed that the methanol fractions of the stem bark contained several compounds, namely compounds with candidate mass $C_{15}H_{10}O_7$ (Quercetin) is a plant flavonol from the flavonoid group of polyphenols, $C_{35}H_{42}O_{14}$ (Taxinin M) is a taxane compound, $C_{29}H_{38}O_9$ (Humistratin) is a steroid glycoside., $C_{15}H_{14}O_6$ (Catechin) is a flavan-3-ol, a type of natural phenol and antioxidant.[14]

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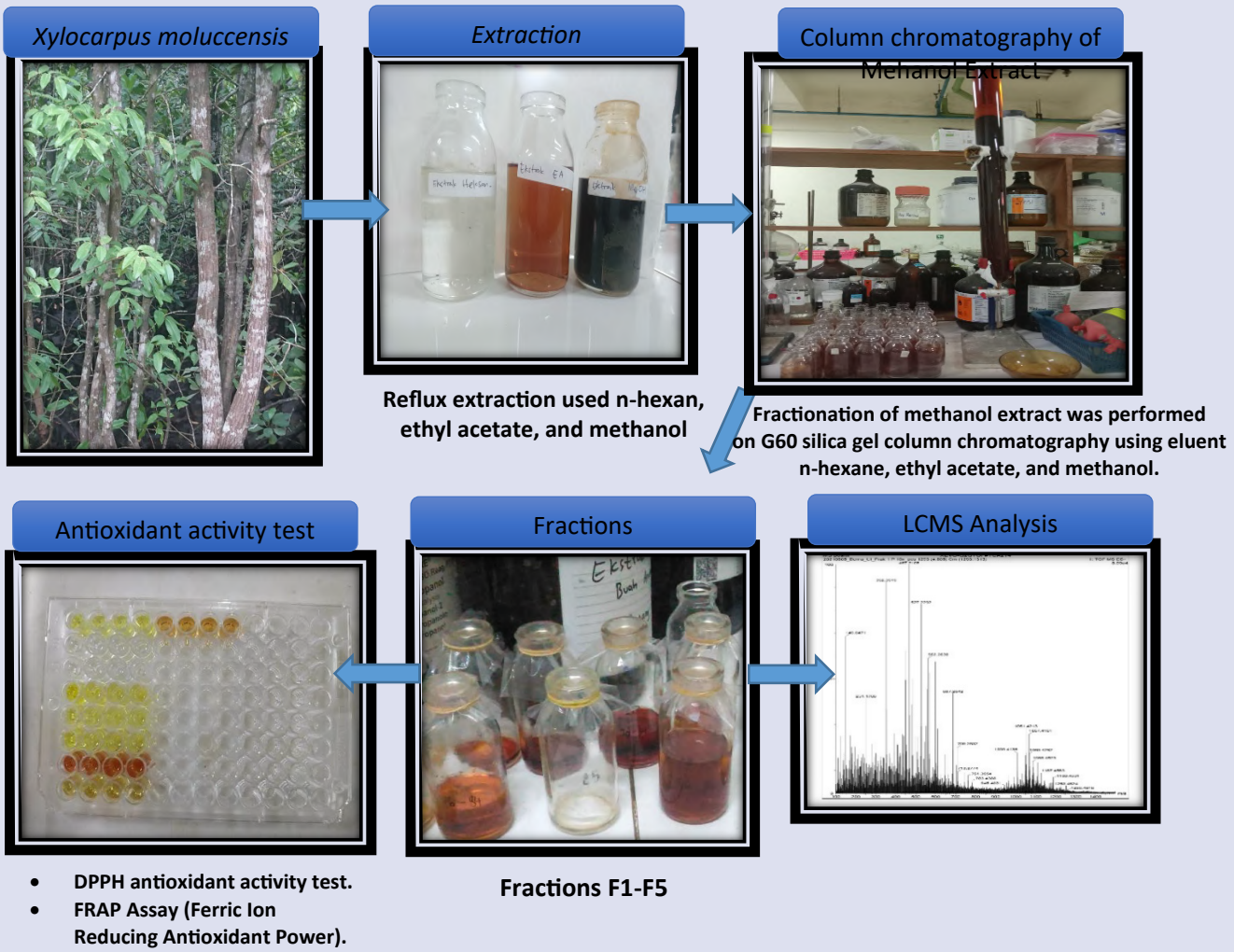
GRAPHICAL ABSTRACT



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