Determination of Total Phenolic, Flavonoid Content Andfree Radical Scavenging Activity of Etanol Extract Sawo Stem Bark (*Manilkara Zapota* (L.))

Rahmiwati Hilma[1], Herliani[1], M.Almurdani[2]

[1]Chemistry Dept of Universitas Muhammadiyah Riau
[2]Chemistry Dept of Riau University

*Corresponding Email : rahmiwatihilma@umri.ac.id

Abstract. Sawo (Manilkarazapota (L.) van Royen) was a plant used traditionally by the community to treat various diseases. The purpose of this study was to determine the content of total phenols, flavonoids and the activity of free radical scavenging ethanol extract of the stem bark of *M. zapota* (L.) van Royen. The stem bark extraction sample was carried out by maceration method using ethanol. Test of total phenol and flavonoids content using the Folin-Ciocalteu method, free radical scavenging activity using DPPH (2,2-diphenyl-1-pikril-hidrazil) method. From the phytochemical test results showed that positive extracts contain phenolic and flavonoid. The results showed that the yield of stem bark ethanol extract was 25.11% with a total phenolic content was 12.97 mgGAE/g extract. Total flavonoid levels was 8.75 mgQE/g extract. The activity of free radical scavenging extract (IC_{50}) values of 19.69 ppm.

1. Introduction

Sawo (Manilkara zapota (L.) van Royen) is a plant that bears fruit throughout the year and spreads widely in Indonesia. Sawo is included in the family tree of Sapotaceae. It is one of the plants that is widely used in traditional medicine, including decoction of skin and fruit used for fever and diarrhea, and bark tea used for dysentery [1,2].

Sawo can provide pharmacological effects due to the content of active metabolites contained in this plant. The active metabolite compounds contained in sawo are alkaloids, flavonoids, tannins, triterpenoids, saponins and glycosides [1]. The results of previous studies stated that sawo leaves and bark also contained active metabolites, namely alkaloids, flavonoids, phenolics and saponins [3,4].

Several studies on M. zapota (L.) Van Royen have been carried out, including: ethanol bark of brown bark has antibacterial activity against S.epidermidis and K. pneumonia [4]. Also, fruit juice (sawo juice) can inhibit the growth of S. thyposa bacteria [5]. Furthermore, ethanol extracts of young sawo fruit are active against E. coli bacteria [6].

Some phenolic compounds have been isolated from this plant, including myricetin-3-O-α-L-rhaminopyranoside, Apigenin-7-O-α-L-rhaminopyranoside, and Caffeic acid, which are found in leaves and manilkorasid (saponin compounds) isolated from brown bark [7].

Phenolic compounds are natural ingredients that are widely used today. Their ability as an active biological compound provides a great role for human life. One of them is as an antioxidant for prevention and treatment of degenerative diseases, cancer, premature aging and disruption of the body's immune system [8].

Flavonoids are almost present in all parts of the plant including the fruit, roots, leaves and outer skin of the stem [9]. A number of medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-allergic, and anticancer activities [10].

Given the importance of the function of phenolic compounds and flavonoids, the purpose of this study was to determine the total phenolic and flavonoid levels contained in the plants of M. zapota (L.) Van Royen and the effect on free radical scanvenging activity. Thus, the utilization of plants M. zapota (L.) Van Royen can be maximized to be used as an alternative herbal treatment in healing various diseases and their use can be accounted for by the community.

2. Research Methodology

This research was carried out for ± 6 (six) months at the Chemistry Laboratory of Universitas Muhammadiyah Riau, Pekanbaru.

2.1 Tools and Materials Used

The tools used in this experiment were rotary evaporator, analytic scale, knife, cutting board, glass bottle, parchment paper, autoclave, microplate reader, micro pipette, drop pipette, water bath, UV-Vis spectrophotometer, cloth, tissue and other glass tools.

The ingredients used were sawo bark (Manilkara zapota (L.) van Royen), ethanol 96%, DPPH 80µg / ml solution, ABTS solution, gallic acid, Folin-Ciocalteu 0.25 N, 7.5% Na2CO3, quartetine, 5% NaNO2, AlCl3. 6H2O 10%, 1M NaOH, Methanol, Chloroform, DMSO, Shrimp Larvae, Sea Water.

2.2. Research Procedures

Sampling

Sawo samples were taken from Kampar, Kampar District, Riau Province. The parts used were the leaves and bark of sawo (M. zapota (L.) van Royen).

Sample Preparation

The dark green leaves and the bark of sawo (Manilkara zapota (L.) van Royen), were cleaned first from the inherent dirt, then were dried aerated. After that, dry sorting was done to remove dirt that was left behind during the drying process, cut into small pieces, made into powder and weighed.

***Manufacture of sawo Stem Bark Extract* (*M. zapota* (L.) van Royen).**

The simplicia powder of sawo leaves and bark was extracted using maceration method with ethanol solvent. Simplicia powder 400 grams of leaves and 120 grams of sawo bark were put into dark colored bottles separately. Then the simplicia was soaked using ethanol solvent for 5 days and occasionally shaken. Next, the maceration was filtered and the pulp was macerated again. The repetition was carried out three times. The collected filtrate was then thickened using a rotary evaporator to obtain a thick extract. After that, the extract was weighed and the yield value was calculated.

Phytochemical Test

Preliminary test of Total Phenolic and Flavonoid content was carried out on ethanol extract of sawo bark samples (M. zapota (L.) according to method [11]:

a. Phenolic Test

A total of 0.5 grams of thick extract of M. zapota (L.) Van Royen stem bark from ethanol solvent was placed on porcelain pellets, then added with methanol, and stirred until homogeneous. After that, FeCl3 was added. Phenolic presence was characterized by the color formation of green, yellow, orange, or red.

b. Flavonoid Test

A total of 0.5 grams of thick extract of M. zapota (L.) Van Royen stem bark from ethanol solvent, each of which was put into a test tube. Then 5 drops of ethanol were added, and shaken until homogeneous. After that, Mg tape and 5 drops of concentrated HCl were added. The presence of flavonoids was indicated if it produced the color of yellow, orange, and red.

***Analysis of total phenolic and flavonoid bark of sawo plant* (*M. zapota* (L.) van Royen)**

a. Making of Galatic Acid Calibration Curves

Main solution of 1000 ppm gallic acid was made. 2 mg of Gallat Acid was weighed and put into 2 ml of measuring flask. From the main solution that had been made, a series of 4, 8, 12, 16 and 20 ppm was made. Then 10 µl of Folin-Ciocalteu 0.25 N was added, reagent allowed to stand for 5 minutes then 20µl of Na2CO3 was added. 7,5% changed the color to blue. It was then incubated for 30 minutes. The solution with these variations in levels reads its absorption in a UV spectrophotometer with a maximum absorption wavelength of 765 nm. Then from the absorbance, the regression equation is determined.

b. Sample Measurement

A total of 100 µl of sample, gallic or blank acid were mixed with 10 µl of Folin-Ciocalteu 0.25 N reagent, allowed to stand for 15 minutes. After that, as much as 20 µl of 7.5% Na2CO3 was added to each well, which caused the color changes to blue. The mixture was incubated for 30 minutes in a dark place before absorbance was measured at a wavelength of 765 nm. Phenolic levels were calculated by entering the results of the level of absorption into the standard curve that had been made.

c. Making a Quercetin Calibration Curve

1000 ppm quercetin main solution was made. 2 mg of quercetin was put into 2 ml of measuring flask. From the main solution that had been made, a series of 8, 16, 24, 32 and 40 ppm was created. Then 60 µl of 5% NaNO2 was added, stood for 5 minutes. Then 50 µl AlCl3 of 10% H2H2O was added. which created yellow color. Let it stand for 30 minutes before finally adding 30 µl of 1 M. NaOH. It was incubated for 5 minutes. The solution with these variations in levels reads its absorption on the microplate reader with a maximum absorption wavelength of 510 nm. Then from the absorbance, the regression equation is determined.

d. Sample Measurement

A total of 100 µl of sample was mixed with 60 µl of 5% NaNO2, let stand for 5 minutes, added 50 µl AlCl3. 10% H 2 O 2. There was a change in color to yellow. It was allowed to stand for 5 minutes and finally 30 µl of 1 M NaOH was added. Then the mixture was incubated in a dark place at room temperature for 5 minutes. The mixture absorbance was measured at a wavelength of 510 nm. Flavonoid levels were calculated by entering the results of the level of absorption into the standard curve that had been made.

***Activity test for sawo free radical scavenging* (M. zapota (L.) van Royen)**

The measurement of antioxidant activity was carried out by the stable free radical method DPPH (1,1-diphenyl-2-picrylhydrazil radical-scavenging). The sample consisted of leaf and bark extract. Each sample was piped to 50µl, then added 80µl of DPPH to 80µg / ml concentration and put into the first well on the microplate. Next, the second well was piped DPPH concentration of 80µg / ml as much as 80µl. Then 50µl methanol was added. The third well was pipetted 50µl in the sample blank and the fourth well was pipetted 50µl in DPPH blank. It was incubated for 30 minutes in a dark place. Then the absorbance of the sample was measured using a microplate reader at a wavelength of 520 nm.

3. Results and Discussion

3.1. *Extraction and Partitions*

From the sample of sawo bark (M. zapota (L.) van Royen) as much as 0.35 kg, 120 gr dry samples which had been mashed up were obtained. Extraction was carried out by maceration method using 96% ethanol which was distilled for 3x24 hours. After filtering and concentrating using a rotary evaporator, 30,135 gr thick bark ethanol extract was obtained. The extract yield obtained was 25.11%.

3.2. Phytochemical test results

Phytochemical test was conducted as a preliminary test qualitatively to determine the secondary metabolite content of plants. Phytochemical test results of fresh bark and sawo bark extract showed the presence of phenolic compounds and flavonoids. Test results on bark extract also showed the presence of phenolic compounds and flavonoids.

Table 1. Phytochemical Test Results of sawo bark (M. zapota (L.) van Royen)

Phytochemicals	Reactor	Fresh stem bark	Stem Bark extract	Observation result
Total phenolic	$FeCl_3$	+	+	Blue to black
Flavonoid	Metal Mg + HCl p	+	+	Pink to reddish

3.3. Analysis of total phenolic and flavonoid bark of sawo plant (M. zapota (L.) van Royen)

In this study, determining the levels of total phenol compounds in samples was done by using gallic acid (GAE) as a standard solution. Gallic acid was used as a standard solution because it is one of the natural and stable phenols, and is relatively cheap compared to others. Gallic acid is included in the phenolic compound derived from hydroxybenzoic acid which is classified as simple phenolic acid. Galic acid is the choice as a standard for the availability of stable and pure substances [9]. Galic acid is reacted with the Folin-Ciocalteu reagent to produce a yellow color which indicates that it contains phenol, after which it is added with a solution of Na2CO3 to produce blue [10]. Phenolic compounds react with the Folin-Ciocalteu reagent only in an alkaline atmosphere so that proton dissociation occurs in phenolic compounds to phenolic ions, so that Na2CO3 solution is added [8].

The standard solution of gallic acid was measured with variations in concentrations of 20, 40, 60, 80 and 100 µg / mL measured at a maximum wavelength of 744.8 nm. Then the value of absorbance of standard solution of gallic acid was obtained at each concentration. A linear line equation was found which would be used to determine the total phenolic content in samples of ethanol extract of stem bark of M. zapota (L.) Van Royen.

The results of absorption measurement of standard gallic acid solution were put into Microsoft Excel to obtain a calibration curve of standard gallic acid solution in the form of a graph of concentration versus absorption curve (Figure 1)

Vol 3-Sep2018
ISSN: 2541-3023

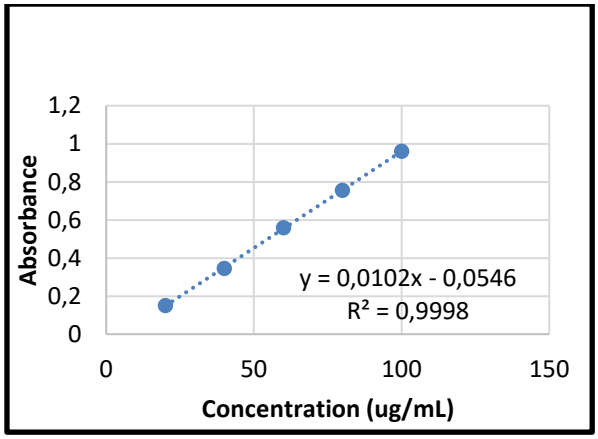

Figure 1. Standard Curve Error Curves

Based on the graph, it can be seen that the calibration curve with regression equation for absorption of gallic acid at concentrations of 20,40, 60, 80, and 100 µg / mL is y = 0,010x - 0,054. In the standard solution of phenol compounds, a linear relationship between absorbance and concentration is obtained. In absorbance measurements which are indicated by the correlation coefficient (r) of 0.999, this value (r) is close to 1 which indicates that the regression equation is linear.

Phenolic compounds and flavonoids are secondary metabolites found in plants. The results obtained in this study indicate a significant level of phenolic compounds in ethanol extract, sawo bark (Tab. 2).

Table 2. Results of measurements of total phenolic levels of Sawo bark extract

Sample	Replication	Absorbance	Average Absorbance	Initial phenolic content (mg/mL)	Total phenolic (mgGAE/g eks)
EKBS*	1	0,587			
	2	0,574	0,582	0,052	12,977
	3	0,584			

*EKBS = Sawo Stem Bark Extract

The antioxidant activity of this extract is probably caused by the presence of phenolic compounds possessed by extracts [9]. In previous studies, phenolic compounds were known to have various biological effects as antioxidants, protecting cell structures, anti-inflammatory, and as antiseptics [3].

To determine the level of total flavonoid compounds in the sample, quercetin (QE) is used as a standard solution. In the measurement of total flavonoid levels, the addition of AlCl3 can form a complex, so that the wavelength shift towards visible (visible) marked with a solution produces a more yellow color. The addition of potassium acetate to maintain the wavelength is in the visible area [8].

Quercetin standard solutions were measured with variations in concentrations of 20, 40, 60, 80 and 100 µg / mL measured at a maximum wavelength of 431 nm. The absorbance value of the quartetin standard solution was obtained at each concentration. A graph of the concentration curve versus the absorbance of the linear line equation (Figure 2) was obtained.

Vol 3-Sep2018
ISSN: 2541-3023

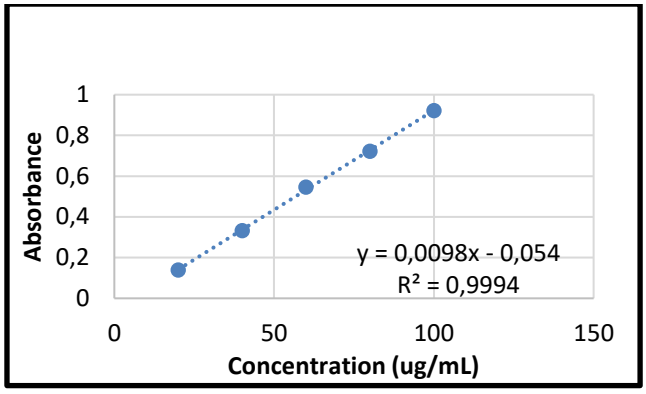

Figure 2. Quercetin Standard Curves

From the graph of the standard quercetin solution, the linear regression equation y = 0.009x - 0.054 was obtained. A standard solution of flavonoid compounds obtained a linear relationship between absorbance and concentration. In the absorbance measurement which is indicated by the correlation coefficient (r) of 0.999, this value (r) is close to 1 which indicates that the regression equation is linear which will be used to determine the total flavonoid levels in the M. zapota bark ethanol extract sample (L .) Van Royen

Table 3. Results of measurements of the flavonoid levels of Sawo bark extract

Sample	Replication	Absorbance	Average	Initial phenolic content (mg/mL)	Total phenolic (mgGAE/g eks)
EKBS*	1	0,397			
	2	0,394	0,396	0,035	8,755
	3	0,396			

*EKBS = Sawo Stem Bark Extract

The benefits of flavonoids include protecting the cell structure, increasing the effectiveness of vitamin C, anti-inflammatory, preventing bone loss and as an antibiotic [12]. According to research by Kurniasari (2006), a number of medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-allergic and anticancer activities.

3.4. Activity test of free radical scavenging of bark extract of M. zapota (L.) van Royen)

The test activity of free radical scavenging by DPPH method was done to determine how much activity a sample was to inhibit DPPH stable radicals by donating hydrogen atoms. Samples that have antioxidant activity will reduce DPPH to DPPH-H which is marked by a purple to yellow color change [9].

Table 4. Results of measurement of free radical scavenging activity of Sawo bark extract

Sample	Concentration (ug/mL)	ln Kons	Repetition 1	Repetition 2	Repetition 3	Average	Abs Sample	% Inhibition	IC50 (ug/mL)
EKBS	1000	6,908	0,118	0,112	0,112	0,114	0,012	96,747	
	500	6,215	0,142	0,141	0,143	0,142	0,039	88,826	
	250	5,521	0,176	0,176	0,173	0,175	0,073	79,491	
	125	4,828	0,209	0,202	0,209	0,207	0,104	70,533	19,698

62,5	4,135	0,238	0,233	0,233	0,235	0,132	62,612
31,25	3,442	0,254	0,258	0,251	0,254	0,151	57,049

*EKBS = Sawo Stem Bark Extract

From the table, it can be seen that there is a correlation between the total content of total polyphenols and flavonoids on the activity of free radical scavenging, where the high levels of total polyphenols and flavonoids in the extract also provide high activity for free DPPH Scavenging radicals.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that:
1. Extract of M. zapota (L.) Van Royen stem bark contains phenolic group compounds of 12.97 mgGAE / g extract calculated against Galatic Acid.
2. Extract of M. zapota (L.) Van Royen stem bark contains flavonoid group compound of 8.75 mgQE / g extract calculated against quercetin.
3. Activity of free radical scavenging extract produces IC50 value of 19.69 ppm.

BIBLIOGRAPHY

[1] Milind, P. dan Preeti. 2015. Chickoo: A Wonderful Gift From Nature. *Int. J. Res. Ayurveda Pharm* 6(4): 544-550.

[2] Nuraini, D. N. 2011. *Aneka Manfaat Biji-Bijian.* Gava Media. Yogyakarta.

[3] Prihardini. dan A. S. Wiyono. 2015. Pengembangan dan Uji Antibakteri Ekstrak Daun Sawo (*Manilkara zapota*) Sebagai Lotio Terhadap *Staphylococcus aureus*. *Jurnal Wiyata* 2(1): 87-92.

[4] Nurhayati, U. 2015. Aktivitas Antibakteri Ekstrak Etabol Kulit Batang Sawo (*Manilkara achras*) Terhadap Staphylococcus epidermidis Dan Klebsiella Pneumonia Serta Bioautografinya. Skripsi Universitas Muhammadiyah Surakarta. Surakarta.

[5] Mustary, M., M. N. Djide, I. Mahmud, dan N. Hasyim. 2011. Uji Daya Hambat dan Analisis KLT-Bioautografi Perasan Buah Sawo (*Achras zapota* Linn) terhadap Bakteri Uji Salmonella thyposa. *Jurnal MKMI* 7(1): 25-27.

[6] Arsyad, M. dan A. R. Annisa. 2013. Konsentrasi Hambat Minimum (KHM) Ekstrak Etanol Buah Sawo (*Achras zapota L.*) terhadap Pertumbuhan Bakteri *Escherichia coli. Jurnal Ilmiah Ibnu Sina* 1(2): 211-218.

[7] Baky, M. H., A. M. Kamal., M. R. Elgindi. dan E. G. Haggag. 2016. A Review on Phenolic Compounds From Family Sapotaceae. *Journal Of Pharmacognosy and Phytochemistry* 5(2): 280-287.

[8] Rebaya, A., Belghith, S. I., Baghdikian, B., Leddet, V.M., Mabrouki, F., Olivier, E., Cherif, J.K., Ayadi, M.T. 2015. Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of *Halimium halimifolium* (Cistaceae) Journal of Applied Pharmaceutical Science Vol. 5 (01), pp. 052-057.

[9] Sembiring, E. N., Elya, B. Sauriasari, R.2018. Phytochemical Screening, Total Flavonoid and Total Phenolic Content and Antioxidant Activity of Different Parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacogn J. 10(1): 123-127

[10] Ukieyanna, E. (2012). Aktivitas antioksidan, kadar fenolik, dan flavanoid total tumbuhan suruhan *(Peperomia pellucid L. Kunth)*. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor

[11] Marjoni, Riza. 2016. *Dasar-dasar fitokimia*. CV Trans info media. Jakarta timur.

[12] Noreen H, Semmar N, Farman M, McCullagh JSO, 2017. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*, *Asian Pacific Journal of Tropical Medicine*, 5(1): 161-165