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ANTIOXIDANT ACTIVITY TEST AND INFRARED PROFILE OF HEXANE, ETHYL ACETATE, AND ETHANOL EXTRACT OF BREADFRUIT LEAVES (*Artocarpus altilis*)

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ABSTRACT

The emergence of various degenerative diseases caused by highly active and dangerous chemical compounds known as free radicals requires the use of antioxidants to inhibit oxidation reactions and prevent cell damage by binding free radicals and highly reactive molecules. Breadfruit (*Artocarpus altilis*) leaves are rich in phytochemical secondary metabolites. This study aimed to determine the antioxidant activity and correlate the functional group obtained by infrared profile in the hexane, ethyl acetate, and ethanol extracts of breadfruit leaves with variations in solvent polarity. This study was conducted using an experimental method using breadfruit (*Artocarpus altilis*) leaves as test materials. The extract was obtained by the maceration method with variations of solvent polarity, namely hexane, ethyl acetate, and ethanol. The examination of antioxidant activity by Diphenyl Picrylhydrazyl (DPPH) method to determine the Inhibitory Concentration 50% (IC_{50}) values and determination of functional groups using the Fourier Transform Infrared (FTIR) method. The antioxidant activity is indicated by Inhibitory Concentration of 50% (IC_{50}) of hexane, ethyl acetate, and ethanol extracts of breadfruit leaves, respectively 139.24 µg/mL (moderate), 47.26 µg/mL (very strong), and 129.31 µg/mL (moderate). The functional group obtained for hexane extract were C–H, C=O, C=C, C–O; for ethyl acetate extract were O–H, C–H, C=O, C=C, C–O; and for ethanol extract were O–H, C–H, C=O, C=C, C–O.

Keywords: Antioxidant Activity, Infrared Profile, Extract, Polarity, Breadfruit, *Articarpus altilis*

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INTRODUCTION

Indonesia is one of the tropical countries that is rich in biodiversity because there are many plants from the Moraceae family, one of which is breadfruit (*Artocarpus altilis*).[1] On the island of Sumatera, this plant has been used as a cultivated plant by the community for generations and is used as a herbal remedy for treating various diseases such as cancer,[2] liver,[3] diabetes mellitus,[4] fever, inflammation,[5] antioxidants, and relieving asthma.[6] The secondary metabolites content in breadfruit (*Artocarpus altilis*) leaves is in the form of phenolic compounds, saponins, tannins, alkaloids, flavonoids, polyphenols, hydrocyanic acid, acetylcholine, and riboflavin.[7] Free Radicals are foreign compounds that enter the body and damage the body's immune system. These free radicals can arise due to various complex chemical processes in the body, environmental pollutants, radiation from chemical substances, toxins, fast food, and foods fried at high temperatures.[8] If the amount is excessive, free radicals will trigger pathological effects that can attack anything, especially those that are vulnerable such as lipids, and proteins, and have implications for the emergence of various degenerative diseases.[9] As a solution to overcome the risk of free radicals, the use of antioxidants is needed to inhibit oxidation reactions and prevent cell damage by binding free radicals and highly reactive molecules.[10] The body needs important substances that help protect the body from free radical attacks, namely antioxidants or substances containing antioxidants. Antioxidants are substances that can neutralize free radicals. This is because antioxidants can provide electron pairs to free electrons that are

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radicals.[11] Exogenous antioxidants according to their source are divided into natural antioxidants and synthetic antioxidants. Natural antioxidants are antioxidants that come from natural sources that are formed from reactions that occur in the processing process or are isolated from natural sources that cannot be eaten and used as food additives.[12] synthetic antioxidants are antioxidants that come from the results of chemical reaction synthesis and are then produced for commercial purposes. Examples of natural antioxidants are vitamin A, vitamin C, and vitamin E. Meanwhile, examples of synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ).[13] There are various methods or ways to test antioxidant activity, one of the most commonly used is the diphenyl picrylhydrazyl (DPPH) method. The diphenyl picrylhydrazyl (DPPH) method is a conventional method that has also long been used to determine the activity of antioxidant compounds.[14] There are many advantages to using the DPPH method, namely this method is easy to use, fast, and quite precise. The diphenyl picrylhydrazyl (DPPH) method is a simple, fast method that only requires an ultraviolet-visible (UV - Vis) spectrophotometer to assess the radical scavenging activity of several compounds.[15] Fourier Transform Infrared (FTIR) is a powerful tool that can be used to detect functional groups, identify compounds, and analyze mixtures of samples being analyzed without damaging the sample. The infrared region in the electromagnetic wave spectrum starts from a wavelength of 4000 cm^{-1} to 650 cm^{-1}.[16] The working principle of Fourier Transform Infrared (FTIR) is the interaction between energy and matter.[17] This research aimed to test the antioxidant activity of breadfruit (*Artocarpus altilis*) leaf extracts with different solvent polarities and relate it to the functional groups of breadfruit leaf extracts with different solvent polarities.

EXPERIMENTAL

Tools and Materials

Tools used in this research were balance (Tanita), analytical balance (Shimadzu), drying cabinet (Memmert), blender (Philips), glassware (Iwaki), ultraviolet-visible spectrophotometer (Merck), infrared spectrophotometer (Agilent).

Materials used in this research were breadfruit (*Artocarpus altilis*) leaves, hexane (Merck), ethyl acetate (Merck), ethanol (Merck), and Diphenyl Picrylhidrazil (SmartLab).

Plant Identification

Identification of breadfruit (*Artocarpus altilis*) was carried out at the Herbarium Madanense of the University of Sumatera Utara, by matching the characteristics of the fruits, leaves, stems, and roots with pictures, taxonomy, and descriptions of plant groups. The aim is to find out and ensure the truth of the identity of the plants that will be used during the research.

Sample Preparation

The sampling process was done purposively, that is, without comparing the same plants from other areas. The plants used were fresh breadfruit (*Artocarpus altilis*) leaves taken in the morning at the University of Tjut Nyak Dhien, Medan Helvetia, Medan, Sumatera Utara, 20123, Indonesia. The breadfruit (*Artocarpus altilis*) leaves were collected, sorted, washed, dried, and milled.

Extract Preparation

The extract preparation used in this research was modified from previous research by Isnansetyo et al. in 2022 with the procedure: amber vessels were prepared separately for hexane, ethyl acetate, and ethanol, inserted 50 g of breadfruit (*Artocarpus altilis*) leaves powder, added 500 ml of solvent, stirred 3 times a day, closed and left for 4 days protected from light, filtered using filter paper, collected macerate from each vessel, evaporated on a rotary evaporator (hexane at temperature 40°C and pressure 360 mBar; ethyl acetate temperature 40°C with pressure 240 mBar; and ethanol at temperature 15-20°C and pressure 240 mBar), resulted in extract from each solvent.[18]

Antioxidant Activity Test

The extract preparation used in this research was modified from previous research by Zebua et al. in 2024 with the procedure: testing of antioxidant activity of breadfruit leaf extract (*Artocarpus altilis*) includes the preparation of diphenyl picrylhydrazyl (DPPH) stock solution with a concentration of 200 ppm, preparation of diphenyl picrylhydrazyl (DPPH) working standard solution with a concentration of 40 ppm,

determination of maximum wavelength, determination of operating time, preparation of stock solution of extract and ascorbic acid, measurement of free radical scavenging activity of diphenyl picrylhydrazyl (DPPH) with concentration, and data analysis.[19]

Preparation of Diphenyl Picrylhydrazyl Solution

Diphenyl Picrylhydrazyl was weighed as much as 20 mg, inserted into a 100 mL volumetric flask, added 60 mL of methanol, shaken until dissolved, diluted with methanol to the mark line, and shaken until homogeneous (obtained diphenyl picrylhydrazyl (DPPH) stock solution with a concentration of 200 µg/mL). Diphenyl Picrylhydrazyl (DPPH) stock solution is taken as 2 mL, inserted into a 10 mL volumetric flask, diluted with methanol to the marked line, and shaken until homogeneous (obtained diphenyl picrylhydrazyl (DPPH) solution with a concentration of 40 µg/mL), analyzed with an ultraviolet-visible spectrophotometer to obtained maximum wavelength (between 400 nm to 800 nm) and operating time (between 10 minutes to 60 minutes).

Preparation of Extract Solution

Each extract was weighed as much as 100 mg, inserted into a 100 mL volumetric flask, added 60 mL of methanol, shaken until dissolved, diluted with methanol to the mark line, and shaken until homogeneous (obtained extract stock solution with a concentration of 1000 µg/mL). Extract stock solution is taken in various volumes, inserted separately into a 10 mL volumetric flask, added 2 mL diphenyl picrylhydrazyl (DPPH) stock solution, diluted with methanol to the marked line, shaken until homogeneous (obtained extract solution with various concentrations and diphenyl picrylhydrazyl (DPPH) solution with a concentration of 40 µg/mL), left for operating time, analyzed with ultraviolet-visible spectrophotometer to obtain absorbance for further calculation of the scavenging percentage and antioxidant activity.

$$Scavenging\ Percentage = \frac{DPPH\ Absorbance - Sample\ \&\ DPPH\ Absorbance}{DPPH\ Absorbance} \times 100\%$$

The scavenging percentage is plotted against concentration, obtained by the equation Y = a × X + b, where X abscissa is extract concentration and Y ordinate is a scavenging percentage. The Inhibitory Concentration of 50% (IC_{50}) is calculated by equation.

Infrared Analysis

The extract preparation used in this research was modified from previous research by Nerdy et al. in 2024 with the procedure: approximately 5 mg of sample was placed in Fourier Transform Infrared with Attenuated Total Reflectance mode, measured the spectrum at wave numbers 4000 cm^{-1} – 650 cm^{-1}.[20]

RESULTS AND DISCUSSION

The plants used in the study were identified at the Herbarium Medanense at the University of Sumatera Utara and the identification results obtained with the identification number 1644 / MEDA / 2023 were Breadfruit (*Artocarpus altilis*). A total of 2 kg of breadfruit (*Artocarpus altilis*) leaves after being dried at a temperature of 30 ° C to 45 ° C obtained 200 grams of dry powder. The results of maceration of 50 grams of dry powder from breadfruit (*Artocarpus altilis*) leaves obtained different amounts of thick extract according to the polarity of each solvent. For maceration with hexane solvent, 2.57 g of thick extract was obtained; with ethyl acetate solvent, 4.15 g of thick extract was obtained; and with ethanol solvent, 9.18 g of extract was obtained.

Antioxidant Activity Test

Measurement of the maximum absorbance of Diphenyl Picrylhydrazyl (DPPH) solution with a concentration of 40 µg/mL in ethanol by using an ultraviolet-visible (UV - Vis) spectrophotometer obtained the maximum absorbance results at a wavelength of 515 nm. The wavelength that provides maximum absorbance of the Diphenyl Picrylhydrazyl (DPPH) solution obtained in this study is by previous studies, namely 515 nm.[21] Measurement at the maximum wavelength will provide better sensitivity.[22] Determination of the operating time of the Diphenyl Picrylhydrazyl (DPPH) solution with a concentration of 40 ppm in ethanol by using an ultraviolet-visible (UV - Vis) spectrophotometer obtained a stable time starting from the 15th minute to the 45th minute. Determination of operating time for colored solutions is very important to determine the stable time of the solution which is indicated by no decrease or no increase

in maximum absorbance.[23] The study was continued by determining the antioxidant activity of hexane, ethyl acetate, and ethanol extract of breadfruit (*Artocarpus altilis*) leaves with varying concentrations. Tables-1, 2, and 3 respectively show the data of the antioxidant activity test of hexane, ethyl acetate, and ethanol extract of breadfruit (*Artocarpus altilis*) leaves.

Table-1: Data of Antioxidant Activity Test of Hexane Extract of Breadfruit (*Artocarpus altilis*) Leaves

Concentration (µg/mL)	Absorbance (AU)	Scavenging (%)	Inhibitory Concentration 50% (µg/mL)
0	1,1214	0	139.24
50	0,9245	17,5584	
75	0,8145	27,3676	
100	0,7154	36,2047	
125	0,6173	44,9527	
150	0,5186	53,7542	
175	0,4187	62,6627	

Table-2: Data of Antioxidant Activity Test of Ethyl Acetate Extract of Breadfruit (*Artocarpus altilis*) Leaves

Concentration (µg/mL)	Absorbance (AU)	Scavenging (%)	Inhibitory Concentration 50% (µg/mL)
0	1.1312	0	47.26
10	1.0084	10.8557	
20	0.8938	20.9866	
30	0.7610	32.7263	
40	0.6566	41.9554	
50	0.5342	52.7758	
60	0.4140	63.4017	

Table-3: Data of Antioxidant Activity Test of Ethanol Extract of Breadfruit (*Artocarpus altilis*) Leaves

Concentration (µg/mL)	Absorbance (AU)	Scavenging (%)	Inhibitory Concentration 50% (µg/mL)
0	1.0026	0	129.31
50	0.8022	19.9880	
75	0.7190	28.2865	
100	0.6138	38.7792	
125	0.5202	48.1149	
150	0.4186	58.2486	
175	0.3242	67.6641	

Based on the data from the antioxidant activity test against hexane, ethyl acetate, and ethanol extracts of breadfruit (*Artocarpus altilis*) leaves with varying concentrations, it can be seen that the higher extract concentration, resulted in the lower Diphenyl Picrylhydrazyl (DPPH) absorbance value, indicated the greater scavenging activity against Diphenyl Picrylhydrazyl (DPPH) as a free radical.[24] The analysis continued with a plot between extract concentration and scavenging percentage resulting from the antioxidant activity test. Figures-1, 2, and 3 respectively show the Relationship curve of hexane, ethyl acetate, and ethanol extract concentration of breadfruit (*Artocarpus altilis*) leaves against scavenging percentage resulting from antioxidant activity test.

From the relationship curve of various extract concentrations of breadfruit (*Artocarpus altilis*) leaves against scavenging percentage resulting from the antioxidant activity test, the regression equation and determination coefficient were obtained. The regression equation for hexane extract was $Y = 0.3587 \times X + 0.0511$ with the determination coefficient (R^2) being 0.9998. The regression equation for ethyl acetate extract was $Y = 1.0536 \times X + 0.2058$ with the determination coefficient (R^2) being 0.9996. The regression equation for ethanol extract was $Y = 0.3863 \times X + 0.0463$ with a determination coefficient (R^2) was 0.9997.

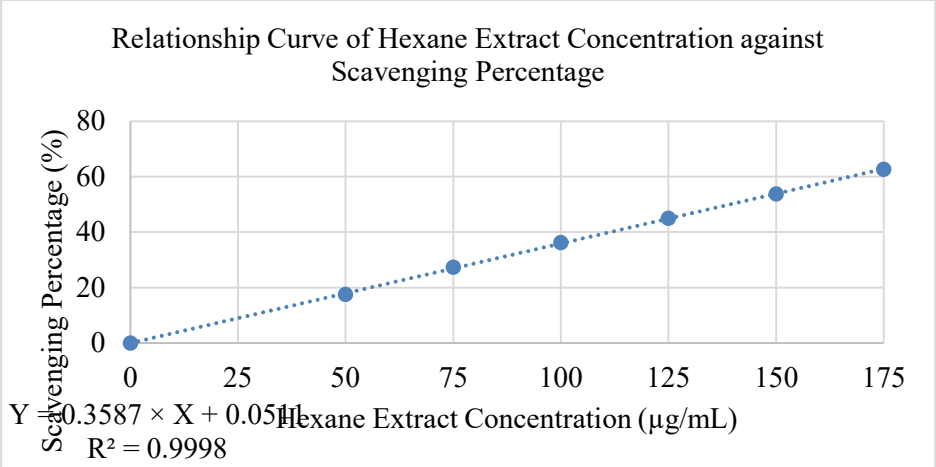

Fig.-1: Relationship Curve of Hexane Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves Against Scavenging Percentage Resulting from Antioxidant Activity Test

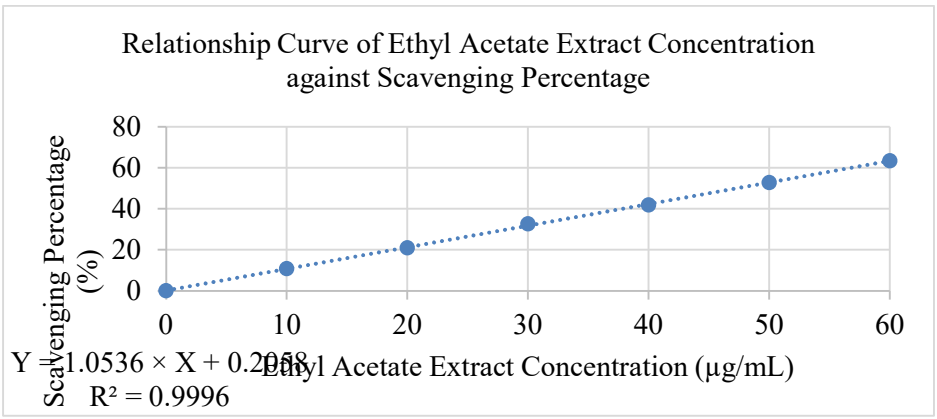

Fig.-2: Relationship Curve of Ethyl Acetate Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves Against Scavenging Percentage Resulting from Antioxidant Activity Test

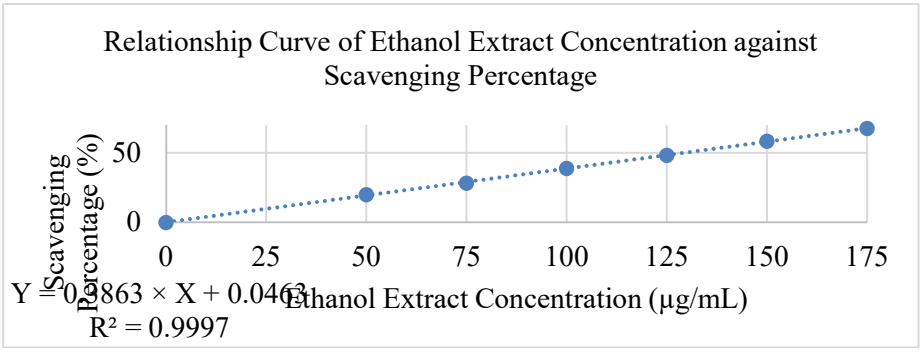

Fig.-3: Relationship Curve of Ethanol Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves Against Scavenging Percentage Resulting from Antioxidant Activity Test

The results obtained for all extracts obtained a determination coefficient of not less than 0.99, which indicates that the ordinate (Y) can be determined by the abscissa (X).[25] The research continued with the calculation of the Inhibitory Concentration 50% (IC_{50}) value to find out the category of antioxidant activity of hexane, ethyl acetate, and ethanol extract of breadfruit (*Artocarpus altilis*) leaves. Based on the calculation results, the Inhibitory Concentration 50% (IC_{50}) value was obtained for hexane, ethyl acetate, and ethanol extract respectively 139.24 µg/mL, 47.26 µg/mL, and 129.31 µg/mL. These results indicate that hexane extract and ethanol extract have a "Moderate" antioxidant activity and ethyl acetate extract has a "Very Strong" antioxidant activity.[26]

Infrared Profile Analysis

Each hexane, ethyl acetate, and ethanol extracts of breadfruit (*Artocarpus altilis*) leaves were analyzed by Fourier Transform Infrared (FTIR) at wavenumber 4000 cm^{-1} to 650 cm^{-1} to determine the functional group profile of each extract. Figures-4, 5, and 6 respectively show the infrared spectrum of hexane, ethyl acetate, and ethanol extract concentration of breadfruit (*Artocarpus altilis*) leaves.

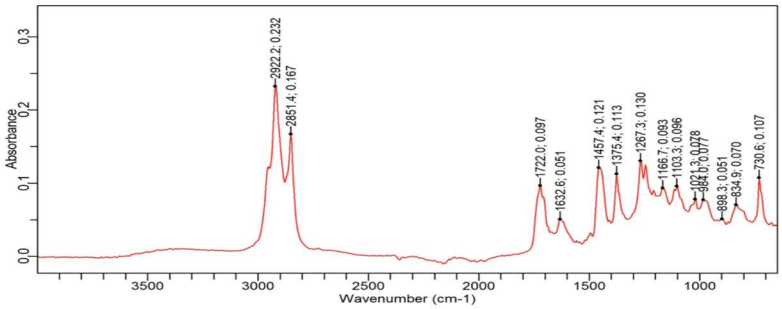

Fig.-4: Infrared Spectrum of Hexane Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves

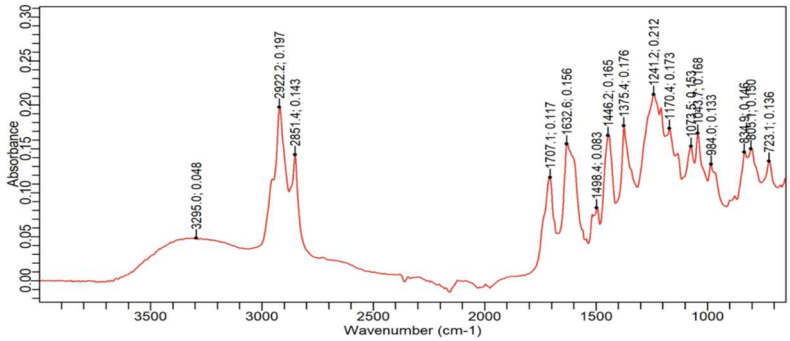

Fig.-5: Infrared Spectrum of Ethyl Acetate Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves

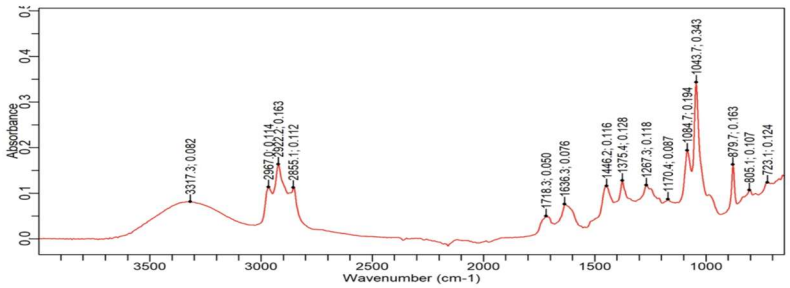

Fig.-6: Infrared Spectrum of Ethanol Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves

The infrared spectrum obtained from the extract was analyzed for the functional groups of each extract. The infrared method is a powerful method for analyzing functional groups in an unknown sample. Tables-4, 5, and 6 show the functional groups found in hexane, ethyl acetate, and ethanol extract concentrations of breadfruit (*Artocarpus altilis*) leaves.

Table-4: Functional Groups Found in Hexane Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves

No	Wavenumber (cm^{-1})	Functional Group
1	2922.2	C–H
2	1722.0	C=O
3	1632.6	C=C
4	1021.3	C–O

Table-5: Functional Groups Found in Ethyl Acetate Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves

No	Wavenumber (cm^{-1})	Functional Group
1	3295.0	O–H
2	2922.2	C–H
3	1707.0	C=O
4	1632.6	C=C
5	1170.4	C–O

Table-6: Functional Groups Found in Ethanol Extract Concentration of Breadfruit (*Artocarpus altilis*) Leaves

No	Wavenumber (cm^{-1})	Functional Group
1	3317.3	O–H
2	2967.0	C–H
3	1718.3	C=O
4	1636.6	C=C
5	1170.4	C–O

The results of functional group analysis on hexane, ethyl acetate, and ethanol extracts of breadfruit (*Artocarpus altilis*) leaves show that there is no hydroxyl group (O–H) in the hexane extract. In the entire extract, there is a carbonyl group (C=O), C–O group, C–H group, and C=C group. The most active secondary metabolite compounds as antioxidants in plants are flavonoids which are identical to phenolic groups and polyphenolic groups.[27] In the hexane extract there is no hydroxyl group, so it can be indicated that the hexane extract does not contain flavonoid compounds. This result is by the nature of flavonoids which are insoluble in non-polar solvents so that they cannot be extracted in non-polar hexane solvents.[28] The hexane extract also has the lowest antioxidant activity with the highest Inhibitory Concentration 50% (IC_{50}) value compared to ethyl acetate extract and ethanol extract. This result is also supported by infrared spectrum data showing that in the hexane extract, there is no hydroxyl group, so it can be indicated that the hexane extract does not contain flavonoid compounds. The most active secondary metabolite compounds as antioxidants in plants are flavonoids which are identical to phenolic groups and polyphenolic groups.[29] The absence of flavonoid compounds will have an impact on the weak antioxidant activity of an extract. This result is by the nature of flavonoids which are insoluble in non-polar solvents so that they cannot be extracted in non-polar hexane solvents.[30] The ethyl acetate extract also has the highest antioxidant activity with the lowest Inhibitory Concentration 50% (IC_{50}) value compared to hexane extract and ethanol extract. This result is also supported by infrared spectrum data showing that the ethyl acetate extract has a hydroxyl group, so it can be indicated that the ethyl acetate extract contains flavonoid compounds.[31] Besides that, flavonoids are polar and semipolar compounds that will easily dissolve in semipolar solvents.[32]

CONCLUSION

The antioxidant activity test on the ethyl acetate extract of breadfruit leaves is classified as very strong with an Inhibitory Concentration 50% (IC_{50}) value of 47.26 µg/mL. The results of the antioxidant activity test on the hexane and ethanol extract of breadfruit leaves are classified as moderate with an Inhibitory Concentration 50% (IC_{50}) value respectively 139.24 µg/mL and 129.31 µg/mL. The functional group in the hexane extract of breadfruit leaves were C–H, C=O, C=C, and C–O; in ethyl acetate extract of breadfruit leaves were O–H, C–H, C=O, C=C, C–O; and in ethanol extract of breadfruit leaves were O–H, C–H, C=O, C=C, C–O functional groups.

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CONFLICT OF INTERESTS

The authors (Nilsya Febria Zebua, Nerdy, Nerdy, Abdi Wira Septama, Shadiq Suwailim, and Refi Bahrianur) declare there is no conflict of interest in this research.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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