PHARMACON
Jurnal Farmasi Indonesia
Pharmaceutical Journal of Indonesia

Pharmacon: Jurnal Farmasi Indonesia
Vol. 22 No1, 2025, pp. 27–34
DOI:10.23917/pharmaconv22i1.10546
p-ISSN: **1411-4283**, e-ISSN: **2685-5062**

Antioxidant Activity of DPPH and ABTS Methods, from Jackfruit Leaf Extract and Fraction (*Artocarpus heterophyllus*) with ELISA Reader

Istiara Subekti[1], Tanti Azizah Sujono[2*], Andi Suhendi[3]
[1] Students of the Master of Pharmacy Study Program, Muhammadiyah University of Surakarta
[2] Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Muhammadiyah University of Surakarta
[3] Bagian Kimia Farmasi, Fakultas Farmasi Universitas Muhammadiyah Surakarta

Corresponding author: **tas198@ums.ac.id*

ARTICLE HISTORY:
Submitted : 2025-05-21
Accepted : 2025-06-20
Published : 2025-06-30

KEYWORDS:
Antioxidant; *Artocarpus heterophyllus;* Jackfruit

Citation:
Subekti, F.M,, Sujono, T.A., Suhendi, A. (2025). Antioxidant Activity of DPPH and ABTS Methods, from Jackfruit Leaf Extract and Fraction (*Artocarpus heterophyllus*) with ELISA Reader. Pharmacon: Jurnal Farmasi Indonesia, 22(1), 27-34. https://doi.org/10.23917/pharmacon.v22i1.10546

ABSTRACT

Oxidative stress caused by the accumulation of free radicals is a key factor in the development of degenerative diseases. Jackfruit leaves (*Artocarpus heterophyllus*), which are rich in polyphenolic compounds, have the potential as natural antioxidants. This study aims to activate the antioxidant activity of jackfruit leaf extracts and fractions. The study was conducted by extraction using the maceration method with 70% ethanol solvent and fractionated by vacuum liquid chromatography into polar, semi-polar and non-polar fractions. Antioxidant activity was measured in vitro, using 3 methods including DPPH, ABTS and FRAP which were read with an ELISA Reader with vitamin C as the reference standard. The results showed that the crude extract had strong antioxidant activity with an IC_{50} value of 31.93 µg/mL (DPPH) and 29.59 µg/mL (ABTS), as well as the highest antioxidant equivalent ascorbic acid (AEAC) levels in the FRAP test. The polar fraction showed moderate antioxidant activity, while the semi-polar and non-polar fractions showed weak activity. These findings suggest that polar compounds such as flavonoids and phenolics play a role in the antioxidant effects of jackfruit leaves. In conclusion, jackfruit leaf extract and its polar fractions have significant antioxidant activity and show promise for further development as natural antioxidant agents in nutraceutical formulations aimed at preventing oxidative stress-related diseases.

INTRODUCTION

Free radicals are molecules that have one or more unpaired electrons, making them highly reactive and unstable. Because of this property, they tend to react with other molecules such as lipids, proteins, or DNA, which can damage cell structures and trigger chain reactions that are detrimental to body tissues. (Martemucci et al., 2022). The accumulation of free radicals in the body can trigger oxidative stress which plays a role in the development of various degenerative diseases. (Rusmana et al., 2017).

Antioxidants work by neutralizing free radicals, thereby reducing the risk of cell and tissue damage (Sreeja et al., 2021). Antioxidants can be synthetic or natural substances. Natural antioxidants can come from plants or animals. Natural antioxidants are considered better for long-term use and prevention because of their more natural and multifunctional nature because they have a complex polyphenolic structure, which allows them to affect various molecular pathways simultaneously, thus providing a more comprehensive therapeutic effect (Divya, 2019).

Jackfruit leaves (*Artocarpus heterophyllus*) are one of the plants that contain phenolics and flavonoids that can inhibit lipid peroxidation (Ajiboye et al., 2018) The antioxidant activity of these compounds plays a role in increasing the performance of antioxidant enzymes such as

superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) which are responsible for maintaining the integrity of cell membranes and protecting against damage caused by free radicals or reactive oxygen species (ROS). (Sekhon-Loodu & Rupasinghe, 2019).

To identify bioactive compounds with the highest antioxidant activity, jackfruit leaf extract can be separated into several fractions based on differences in solvent polarity. This fractionation aims to separate groups of compounds according to their chemical properties, thus facilitating the identification and evaluation of the biological activity of each fraction (Ali et al., 2019). This process allows for more specific and accurate identification and isolation of bioactive compounds, as well as increasing the purity of the compounds before further biological activity testing (Poojar et al., 2017). This fractionation process is important because fractionation also reduces the complexity of the crude extract, ensuring safety, effectiveness, and consistency of pharmacological activity in therapeutic applications (Barba-Ostria et al., 2022)

Antioxidant activity assessment can be done through various in vitro methods, such as DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power). These three methods have different working principles, so that simultaneous testing can provide a more comprehensive picture of the antioxidant capacity of a sample. (Irawan et al., 2022). Therefore, this study is important to be carried out to determine the antioxidant activity of extracts and fractions systematically in order to identify which extracts or fractions have the most significant activity and to understand the contribution of bioactive compounds in them.

METHODS

Extraction and Fractionation

Jackfruit leaf powedered is mashed and then macerated for 48 hours with 70% ethanol at a ratio of 1:10 (Ajiboye et al., 2018). The maceration soak is then filtered and the liquid extract or macerate is evaporated with a rotary evaporator until thick. Fractionation is carried out using the chromatography method for thick extracts from jackfruit leaves. Before fractionation, TLC analysis is carried out to determine the right eluent, using a silica plate GF254 stationary phase and a mobile phase of methanol, chloroform, ethyl acetate and n-hexane. After obtaining the optimal eluent ratio, the sample in the form of a thick extract is also fractionated using vacuum liquid chromatography (VLC).

The optimization results obtained a combination of solvent in mobil phase, n-hexane, h-hexane: ethyl acetate (9:1), n-hexane: chloroform (8:2), ethyl acetate: chloroform (1:1), chloroform: methanol (1:1) and methanol. Each solvent was then flowed into the sample and the fraction results were tested by TLC to identify the same RF valueA sample or extract of 25 grams is impregnated with silica imreg g60 (30-70 mesh) as much as 2 times the weight of the extract and is inserted into the VLC column. Elution is carried out with a solvent with an increasing polarity level according to the results of the previous optimization. Each elution was collected as much as 200 mL and spotted on a silica plate to see its chromatography profile. Fractions that have similar chromatography profiles are combined as one fraction. Evaporation of the solvent in the fraction is carried out with a rotary evaporator to obtain a thick fraction Itsna, et al 2014).

DPPH Method

A 150 µg/mL DPPH solution was prepared, then a standard vitamin C solution was made with a concentration of 0.5–10 µg/mL, as well as sample solutions (extract, polar fraction, semi-polar fraction, and non-polar fraction) in various concentrations, namely 25–200 µg/mL, 50–300 µg/mL, and 500–1000 µg/mL. The standard solution or sample solution was reacted with DPPH solution in a ratio of 1:4 (50 µl:150 µl), then homogenized, transferred to a 96-well microplate, and incubated for 30 minutes in the dark. Furthermore, the solution mixture was measured using an ELISA reader at a wavelength of 517 nm (Irawan et al., 2022)

ABTS Method

Prepare 10 mL of ABTS solution with a concentration of 7 mM and 10 mL of potassium persulfate solution with a concentration of 2.45 mM, using methanol as a solvent. The formation of radicals is carried out by mixing the two solutions, then incubating for 16 hours in the dark at room temperature. Next, prepare a standard solution of vitamin C with a

concentration range of 0.25–10 µg/mL, as well as sample solutions in the form of extracts, polar fractions, semi-polar fractions, and non-polar fractions with concentration variations of 25–200 µg/mL, 50–300 µg/mL, and 500–1000 µg/mL, respectively. The standard solution and sample are reacted with the ABTS solution in a ratio of 1:4 (50 µl:150 µl), homogenized, transferred to a 96-well microplate, then incubated for 30 minutes in the dark. The solution measurement is carried out using a reader ELISA at a wavelength of 734 nm (Sukweenadhi et al., 2020)

FRAP Method

Prepare FRAP reagent by dissolving 187 mg of sodium acetate trihydrate in 250 mL of acetate buffer solution at pH 3.6, 270 mg of iron(III) chloride hexahydrate (FeCl3 6H2O) in 100 mL of distilled water, and 150 mg of TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 50 mL of 40 mM HCl solution. Mix 25 mL of sodium acetate solution, 2.5 mL of FeCl3 solution, and 2.5 mL of TPTZ solution, then add distilled water until the total volume reaches 100 mL to obtain ready-to-use FRAP reagent. Make a vitamin C solution with a concentration variation of 25–80 µg/mL, and samples in the form of extracts, polar, semi-polar, and non-polar fractions with a concentration range of 100–600 µg/mL each. Standard solution or sample is reacted with FRAP reagent with a ratio of 1:3 (50 µl:100 µl) at room temperature for approximately 15 minutes. Measurement of reaction results is carried out using an ELISA reader at a wavelength of 593 nm (Firuzi et al., 2005)

RESULT AND DISCUSSION

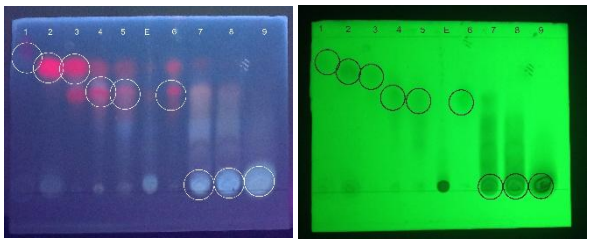

Figure 1. TLC Separation of fraction leaf extract (*Artocarpus heterophyllus*), mobile phase chloroform methanol (8 :2), stationary phase silica gel GF254, a. visualitation under UV 366 nm, b. visualitation under UV 254

This study began with the extraction process where the extraction results of jackfruit leaves *Artocarpus heterophyllus* obtained a yield of 15.9%. The fractionation process began with mobile phase optimization to determine the best compotition of solvent system that provided the best solvent characteristics on TLC (Paprika, et al 2025) (**Figure 1**). The fractionation process yielded nine collections, which were grouped based on Rf values into polar, semi-polar, and non-polar fractions

The polarity classification of the fractionation results was carried out based on the Rf value obtained by the thin layer chromatography (TLC) method. The RF value of each container was 0.86 for the first container, 0.75 for containers 2 and 3, 0.64 for containers 4,5 and 6 and 0.11 for containers 7, 8 and 9. Compounds with low Rf values showed strong interactions with the polar stationary phase, indicating that the compound was polar. Conversely, compounds with high Rf values showed weak interactions with the stationary phase, indicating that the compound was non-polar (Pratama et al., 2018). The polar, semi-polar and non-polar fractions received yields of 5.3%, 7.7% and 32% respectively.

Determination of antioxidant activity in this study was carried out using three different methods, namely DPPH, ABTS, and FRAP. Absorbtion of test solution measured using ELISA reader instrument following previous study (Irawan et al., 2022; Sembiring et al., 2016; Sukweenadhi et al., 2020). ELISA reader offer several advantages compared to the uv-vis spectrophotometer, such as time efficiency, fewer sample and reagent requirements, and a good level of precision (Suhendi et al., 2018). The antioxidant activity test used in this study used 3 types, because they all have different principles.

The DPPH method assesses antioxidant activity through electron transfer that reduces DPPH radicals, indicated by a change in color from dark purple to paler as measured spectrophotometrically. This mechanism is dominated by electron transfer, and involves a small amount of hydrogen atom transfer (Shahidi & Zhong, 2015).

The ABTS method measures antioxidant activity based on the ability of the compound to reduce the blue-green $ABTS^{+}$ cation radical with an absorption peak at 734 nm. This radical is formed from the oxidation of ABTS by agents such as peroxide or persulfate. Reaction with antioxidants decreases the color intensity, which is observed spectroscopically and the decrease

in color indicates the antioxidant potential of the sample (Munteanu & Apetrei, 2021).

The FRAP method assesses antioxidant activity based on the compound's ability to reduce Fe^{3+} ions to Fe^{2+}. The Fe^{2+} ions formed will form a colored complex with TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), which has a maximum absorption at 595 nm and can be measured spectrophotometrically. The higher the reduction ability of a compound, the greater its antioxidant activity (Firuzi et al., 2005)

One of the parameters often used to assess antioxidant potential is the IC_{50} value, which is the concentration of a sample that is able to inhibit 50% of free radicals. The lower the IC_{50} value, the higher the antioxidant activity.(Irawan et al., 2022), Antioxidant activity based on DPPH and ABTS methods is classified into five categories: very strong (IC_{50} < 50 µg/mL), strong (50–100 µg/mL), moderate (100–150 µg/mL), weak (150–200 µg/mL), and very weak (>200 µg/mL). In addition to the IC_{50} value, the FRAP method uses the AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) unit to indicate the reduction capacity of the sample which is stated as equivalent to vitamin C (Sukweenadhi et al., 2020)

Table 1. Results of antioxidant activity tests using the DPPH method

Sampel	Linear regression equation	IC_{50} value (µg/mL)
Vitamin C	y = 4.2879 ± 0.17 x + 47.058 ± 1.96 R^2 = 0.9754	0.68 ± 0.428
Extract	y = 0.2139 ± 0.01 X + 42.697 ± 1.59 R^2 = 0.9234	31.93 ± 5.011
Fraksi Polar	y = 0.2328 ± 0.005 x + 17.894 ± 1.254 R^2 = 0.9902	137.95 ± 7.189
Fraksi Semi Polar	y = 0.102 ± 0.001 x + 17.771 ± 0.89 R^2 = 0.975	664.52 ± 14.195
Fraksi Non Polar	y = 0.1142 ±0.006 x - 42.417 ± 6.65 R^2 = 0.9674	809.01 ±13.240

Note : Results are presented as mean ± SD of IC_{50} (50 inhibitory concentration, n = 3)

The method used is valid because the results of the positive control show the same activity as previous research or are compounds with very strong antioxidant activity (Irawan et al., 2022). Vitamin C has very strong antioxidant activity with the working principle of acting as an antioxidant by donating protons or electrons to neutralize free radicals. In the DPPH and ABTS methods, this reaction is characterized by a decrease in color intensity due to radical reduction by vitamin C. While in the FRAP method, vitamin C reduces the Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ, which is indicated by an increase in absorbance due to the formation of a blue complex. These three methods reflect the ability of vitamin C as an antioxidant through the electron donation mechanism (Sukweenadhi et al., 2020) Besides that, the results of the consistency test are indicated by the R value for all samples above 0.9.

In the DPPH method, vitamin C as a positive control showed very strong antioxidant activity with an IC_{50} value of 0.68 ± 0.428 µg/mL. Crude jackfruit leaf extract showed strong activity with an IC_{50} value of 31.93 ± 5.011 µg/mL. The polar fraction had an IC_{50} value of 137.95 ± 7.189 µg/mL, which was included in the moderate category. However, the semi-polar and non-polar fractions showed very high IC_{50} values, 664.52 ± 14.195 µg/mL and 809.01 ± 13.240 µg/mL, respectively, indicating very weak antioxidant activity (**Table 1**). This is in line with research (Insanu et al., 2022) s that the antioxidant activity of jackfruit leaf samples extracted using polar solvents produces the highest antioxidant activity compared to semi-polar and non-polar solvents.

Table 2. Antioxidant Activity Test Results with the ABTS method

Sampel	Linear regression equation	IC_{50} value (µg/mL)
Vitamin C	y = 4,228 ± 0.19 x + 44,053 ± 0.371 R^2 = 0.9297	1.402 ± 0.401
Extract	y = 0.3719 ± 0.009 x + 44.731 ± 0.2009 R^2 = 0.9621	29.592 ± 4.920
Fraksi Polar	y = 0.155 ± 0.009 x + 36.344 ± 1.909 R^2 = 0.9951	87.507 ± 6.891
Fraksi Semi Polar	y = 0.090 ± 0.0098 x + 13.526 ± 4.507 R^2 = 0.9763	404.634 ± 11.287
Fraksi Non Polar	y = 0.0702 ±0.004 x + 15.138 ± 2.845 R^2 = 0.997	586.901 ± 14.878

Note : Results are presented as mean ± SD of IC50 (50 inhibitory concentration, n = 3)

Antioxidant activity testing using the ABTS method was carried out to evaluate the ability of samples to neutralize $ABTS^+$ radicals. The test results are shown through the IC_{50} value of each sample listed in **Table 2**. Vitamin C showed the highest antioxidant activity, followed by the

crude extract which was classified as having strong activity. The polar fraction showed moderate activity, while the semi-polar and non-polar fractions had relatively weak activity. These findings reflect the differences in the ability of each fraction to neutralize $ABTS^{+}$ radicals. Previous research has conducted antioxidant activity tests using the ABTS method but with a sample of protein concentrate from jackfruit leaves which showed that the protein hydrolysate produced using the pancreatin enzyme for 120 minutes had an ABTS radical scavenging activity of 98.20% (Calderón-Chiu et al., 2021).

Table 3. Results of Antioxidant Activity Test using the FRAP method

Sampel	Concentration (µg/mL)	AEAC
Extract	600	167.18±9.31
	500	173.42±2.16
	400	169.93±3.98
	300	158.30±13.94
	200	125.95±9.98
	100	64.24±3.48
Polar Fraction	600	128.51±5.52
	500	113.80±1.76
	400	96.23±0.76
	300	78.28±2.17
	200	53.95±3.32
	100	32.64±1.02
Semi Polar Fraction	600	61.72±3.07
	500	57.88±1.93
	400	44.77±2.00
	300	36.35±0.98
	200	28.91±0.80
	100	16.88±0.54
Non Polar Fraction	600	19.04±1.32
	500	16.88±0.80
	400	15.00±0.23
	300	14.07±0.83
	200	12.09±0.76
	100	9.64±0.56

Note : Results are presented as mean ± SD of the linear regression of Vitamin C (50 inhibitory concentration, n = 3)

Antioxidant activity with the FRAP method is expressed by the equivalent value to the comparator or in research is vitamin C which is expressed as AEAC or (Ascorbic Acid Equivalent Antioxidant Capacity). The determination of the AEAC value is based on the standard linear regression value with the results of the linear regression calculation of vitamin C in the FRAP method test being y = 134.88x + 0.4299 with a coefficient of determination value of 0.9804.

The FRAP method was used to measure the ability of compounds to convert iron ions from the form of Fe^{3+} to Fe^{2+}. Data on the antioxidant activity of each sample based on this method are presented in **Table 3**. The crude extract showed the highest antioxidant activity with the largest AEAC value at all concentrations, especially at a concentration of 500–600 µg/mL. The activity of the polar fraction was moderate, followed by the semi-polar fraction which had lower activity, while the non-polar fraction had the lowest AEAC value at all concentrations, indicating the smallest reduction ability.

Overall, antioxidant activity decreased with increasing polarity of the fraction, meaning that compounds with higher polarity contributed more to the reduction process of Fe^{3+} ions to Fe^{2+}Previous studies using the FRAP method on *Artocarpus heterophyllus* plants focused on the skin and fruit stalks, with variations in extraction techniques such as maxeration, percolation, and soxhletation using 70% ethanol polar solvents. The results showed that the extract obtained through the maxeration method had the highest antioxidant activity compared to percolation and soxhletation. This finding indicates that cold extraction techniques, such as maxeration, are more effective in maintaining and extracting bioactive compounds that play a role in antioxidant activity. This is reinforced by the highest total phenolic and flavonoid content in the maxeration sample, which confirms the important role of both compounds in antioxidant activity. (Daud et al., 2017)

The third test method showed a consistent pattern, where jackfruit leaf extract showed the highest antioxidant activity, followed by the polar fraction. While the semi-polar and non-polar fractions showed the lowest activity. The IC_{50} values in the DPPH and ABTS methods and the AEAC values from the FRAP method showed that the intact extract had an effectiveness close to vitamin C as a positive control. The higher antioxidant activity in the intact extract was likely due to the synergistic effect between bioactive compounds that were reduced after the fractionation process. This consistent pattern confirms that polar antioxidant compounds, such as flavonoids and phenols, are

more soluble in polar solvents such as ethanol, in accordance with their chemical properties. (Calderón-Chiu et al., 2021).

The difference in results between the DPPH and ABTS methods is likely due to differences in antioxidant reaction mechanisms and the types of solvents used. The DPPH method is more suitable for compounds that are soluble in methanol, while ABTS can be applied to compounds with various levels of polarity. Meanwhile, the FRAP method measures the total reduction capacity of a compound through its ability to reduce Fe^{3+} ions under acidic conditions. (Munteanu & Apetrei, 2021). In general, the results of the study showed that The highest antioxidant activity was detected in the intact extract and polar fraction, which is most likely due to the content of bioactive compounds such as phenolic compounds, flavonoids, and other antioxidants that interact synergistically. These results are consistent with previous studies that reported that ethanol extract of jackfruit leaves has the highest flavonoid concentration and shows the lowest IC_{50} value when compared to semi-polar and non-polar fractions (Adnyani et al., 2017).

CONCLUSIONS

This study proves that jackfruit leaf extract (*Artocarpus heterophyllus*) has high antioxidant activity, with the highest effectiveness in the intact extract, followed by the polar fraction. Semi-polar and non-polar fractions showed low activity. This indicates that active compounds such as phenolics and flavonoids are more abundant in the polar fraction and work synergistically in the intact extract. Differences between test methods (DPPH, ABTS, and FRAP) reflect differences in reaction mechanisms and compound solubility. In conclusion, jackfruit leaves exhibit promising antioxidant properties, especially within their extract and polar fraction components. This suggests their potential for further exploration in in vivo studies and supports their prospective use in the formulation of nutraceutical products aimed at preventing oxidative stress-related diseases

ACKNOWLEDGMENT

The author would like to thank the Muhammadiyah University of Surakarta and all the supervising lecturers who have provided guidance, direction, and facilities that support the smooth running of this research as well as technical assistance provided during the testing process.

AUTHORS' CONTRIBUTIONS

The first author Istiara Subekti is responsible for the implementation of the research, starting from sampling, data processing, to the preparation of the manuscript. The second author Tanti Azizah Sujono and the third author Andi Suhendi act as supervisors who provide direction, scientific supervision, and review of the content and structure of the research manuscript.

CONFLICT OF INTERESTS

No conflict of interest in this publication.

ETHICAL CONSIDERATION

This study was an in vitro study that did not involve human subjects or living animals, so it did not require approval from an ethics committee . All procedures were carried out in accordance with the ethical standards of laboratory research and followed the guidelines in force at the institution where the study was conducted.

BIBLIOGRAPHY

Adnyani, N. M. R. D., Parwata, I. M. O. A., & Negara, I. M. S. (2017). Potential of Jackfruit Leaf Extract (*Artocarpus heterophyllus Lam*.) as a Natural Antioxidant. Jurnal Kimia, 162. https://doi.org/10.24843/jchem.2017.v11.i02.p10

Ajiboye, B. O., Ojo, O. A., Adeyonu, O., Imiere, O., Oyinloye, B. E., & Ogunmodede, O. (2018). Ameliorative activity of ethanolic extract of *Artocarpus heterophyllus* stem bark on alloxan-induced diabetic rats. Advanced Pharmaceutical Bulletin, 8(1). https://doi.org/10.15171/apb.2018.017

Ali, A., Chua, B. L., & Chow, Y. H. (2019). An insight into the extraction and fractionation technologies of the essential oils and bioactive compounds in Rosmarinus officinalis L.: Past, present and

future. TrAC - Trends in Analytical Chemistry, 118, 338–351. https://doi.org/10.1016/j.trac.2019.05.040

Calderón-Chiu, C., Calderón-Santoyo, M., Herman-Lara, E., & Ragazzo-Sánchez, J. A. (2021). Jackfruit (*Artocarpus heterophyllus* Lam) leaf as a new source to obtain protein hydrolysates: Physicochemical characterization, techno-functional properties and antioxidant capacity. Food Hydrocolloids, 112(August 2020). https://doi.org/10.1016/j.foodhyd.2020.106319

Daud, M. N. H., Fatanah, D. N., Abdullah, N., & Ahmad, R. (2017). Evaluation of antioxidant potential of *Artocarpus heterophyllus* L. J33 variety fruit waste from different extraction methods and identification of phenolic constituents by LCMS. Food Chemistry, 232, 621–632. https://doi.org/10.1016/j.foodchem.2017.04.018

Divya, C. (2019). Comparative Study of Synthetic vs . Natural Antioxidants in Inflammatory Diseases. 4, 65–68.

Firuzi, O., Lacanna, A., Petrucci, R., Marrosu, G., & Saso, L. (2005). Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. Biochimica et Biophysica Acta - General Subjects, 1721(1–3), 174–184. https://doi.org/10.1016/j.bbagen.2004.11.001

Girsang, E., Lister, I. N. E., Ginting, C. N., Sholihah, I. A., Raif, M. A., Kunardi, S., Million, H., & Widowati, W. (2020). Antioxidant and antiaging activity of rutin and caffeic acid. Pharmaciana, 10(2), 147. https://doi.org/10.12928/pharmaciana.v10i2.13010

Insanu, M., Pramasatya, H., Buddhisuharto, A. K., Tarigan, C., Zahra, A. A., Haniffadi, A., Sabila, N., & Fidrianny, I. (2022). Unused Parts of Jackfruit (*Artocarpus heterophyllus*): Prospective In Vitro Antioxidative Activity. Open Access Macedonian Journal of Medical Sciences, 10(A), 1529–1536. https://doi.org/10.3889/oamjms.2022.9274

Irawan, C., Putri, I. D., Sukiman, M., Utami, A., Ismail, Putri, R. K., Lisandi, A., & Pratama, A. N. (2022). Antioxidant Activity of DPPH, CUPRAC, and FRAP Methods, as well as Activity of Alpha-Glucosidase Inhibiting Enzymes from Tinospora crispa (L.) Stem Ultrasonic Extract. Pharmacognosy Journal, 14(5), 511–520. https://doi.org/10.5530/pj.2022.14.128

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. Oxygen, 2(2), 48–78. https://doi.org/10.3390/oxygen2020006

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. International Journal of Molecular Sciences, 22(7). https://doi.org/10.3390/ijms22073380

Paprika, S. (n.d.). Isolation And Characterization Of B -Sitosterol And B -Citraurin From Saponified Paprika Oleoresin. 23(1), 89–101.

Poojar, B., Ommurugan, B., Adiga, S., Thomas, H., Sori, R. K., Poojar, B., Hodlur, N., Tilak, A., Korde, R., Gandigawad, P., In, M., Sleep, R., Albino, D., Rats, W., Article, O., Schedule, P., Injury, C. C., Sori, R. K., Poojar, B., … Gandigawad, P. (2017). Methodology Used in the Study. *Asian Journal of Pharmaceutical and Clinical Research*, *7*(10), 1–5. https://doi.org/10.4103/jpbs.JPBS

Pratama, M. R. F., Suratno, S., & Mulyani, E. (2018). Profile of Thin-Layer Chromatography and UV-Vis Spectrophotometry of Akar Kuning Stem Extract (Arcangelisia flava). Borneo Journal of Pharmacy, 1(2), 72–76. https://doi.org/10.33084/bjop.v1i2.367

Sekhon-Loodu, S., & Rupasinghe, H. P. V. (2019). Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. Frontiers in Nutrition, 6(April), 1–11. https://doi.org/10.3389/fnut.2019.00053

Sembiring, E., Sangi, M. S., & Suryanto, E. (2016). Aktivitas Antioksidan Ekstrak Dan Fraksi Dari Biji Jagung (Zea mays L.). Chemistry Progress, 9(1), 14–20.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. Journal of Functional Foods, 18, 757–781. https://doi.org/10.1016/j.jff.2015.01.047

Sreeja Devi, P. S., Kumar, N. S., & Sabu, K. K. (2021). Phytochemical profiling and antioxidant activities of different parts of *Artocarpus heterophyllus* Lam. (Moraceae): A review on current status of knowledge. Future Journal of Pharmaceutical Sciences, 7(1). https://doi.org/10.1186/s43094-021-00178-7

Suhendi, A., Hanwar, D., Santoso, B., Wicaksono, A. N., & Widiana, L. (2018). Antioxidant activity of non polar and semipolar fractions of ethanol extract of Zingiber zerumbet smith leaves by spectrophotometer and ELISA reader. Journal of Pharmaceutical Sciences and Research, 10(3), 439–441.

Sukweenadhi, J., Yunita, O., Setiawan, F., Kartini, Siagian, M. T., Danduru, A. P., & Avanti, C. (2020). Antioxidant activity screening of seven Indonesian herbal extract. Biodiversitas, 21(5), 2062–2067. https://doi.org/10.13057/biodiv/d210532

Unuofin, J. O., & Lebelo, S. L. (2020). Antioxidant Effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes: An Updated Review. Oxidative Medicine and Cellular Longevity, 2020. https://doi.org/10.1155/2020/1356893