

Open Access Full Text Article



Research Article

Ethyl acetate extract of breadfruit leaves (*Artocarpus altilis*) improves lipid profile and reduces F2-isoprostane in dyslipidemia-induced Wistar rats (*Rattus norvegicus*)

Shinta Eka Kusuma Dewi * Anak Agung Gde Putra Wiraguna Gde Ngurah Indraguna Pinatih

Faculty of Medical and Health Sciences Udayana University, Bali, Indonesia

Article Info:



Article History:

Received 06 Dec 2023

Reviewed 10 Jan 2024

Accepted 03 Feb 2024

Published 15 Feb 2024

Cite this article as:

Dewi SEK, Wiraguna AAGP, Pinatih GNI, Ethyl acetate extract of breadfruit leaves (*Artocarpus altilis*) improves lipid profile and reduces F2-isoprostane in dyslipidemia-induced Wistar rats (*Rattus norvegicus*), Journal of Drug Delivery and Therapeutics. 2024; 14(2):122-126

DOI: <http://dx.doi.org/10.22270/jddt.v14i2.6377>

Abstract

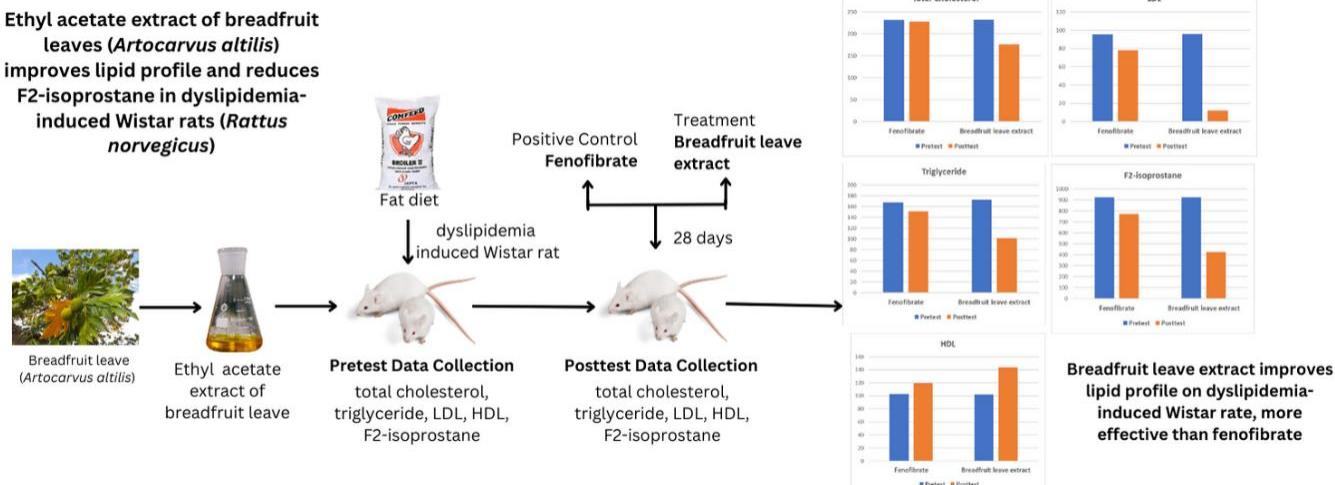
Dyslipidemia is closely related to the process of lipid peroxidation. In the non-enzymatic peroxidation process of arachidonic acid, an esterification process occurs and produces a metabolite in the form of isoprostane. The most common isoprostane found is F2-isoprostane. In traditional medicine, breadfruit leaves (*Artocarpus altilis*) have been widely used due to their antioxidant compounds, such as flavonoids, phenols, tannins, saponins, and other secondary metabolites. This study aims to evaluate the activity of breadfruit leaf ethyl acetate extract in improving the lipid profile and F2-isoprostane in dyslipidemia-induced Wistar rats (*Rattus norvegicus*). This study was an experimental study with a pretest-posttest control group design with two treatment groups: fenofibrate 1.65 mg (P1) and breadfruit leaf ethyl acetate extract (760 mg) (P2) for 28 days. Total cholesterol, triglyceride, HDL, LDL, and F2-isoprostane levels were measured before and after treatment expressed in mg/dL. The data were analyzed using a parametric t-paired and t-unpaired test with a 95% confidence level. The study found that breadfruit leaf extract was better than fenofibrate at lowering total cholesterol (pre 172.41 ± 4.93; post 101.24 ± 2.09), triglycerides (pre 172.41 ± 4.93; post 101.24 ± 2.09), LDL (pre 95.78 ± 8.00; post 12.03 ± 10.06), F2-Isoprostane (pre 924.86±10.40; post 432.99±14.17), and raising HDL (pre 102.29 ± 10.40; post 143.89 ± 5.99). In conclusion, ethyl acetate extract is better than fenofibrate in improving lipid profiles and reducing F2-isoprostane in dyslipidemia-induced animal tests.

Keywords: breadfruit leaves (*Artocarpus altilis*), dyslipidemia, F2-Isoprostane, fenofibrate, lipid profile, total cholesterol

*Address for Correspondence:

Shinta Eka Kusuma Dewi, Faculty of Medical and Health Sciences Udayana University, Bali, Indonesia

Graphical Abstract



INTRODUCTION

Dyslipidemia is a disorder that occurs due to lipid or fat metabolism disorders and is known as one of the important risks of cardiovascular diseases such as diabetes mellitus, obesity, and hypertension. Poor lifestyles such as lack of physical activity, stress, and consumption of foods high in fat and carbohydrates also play a role as triggers for dyslipidemia and other cardiovascular disorders.

Dyslipidemia is closely related to the aging process. Ageing increases the risk of developing dyslipidemia. A study in China found that the prevalence of dyslipidemia in adults was high, about 56.8%, exceeding the percentage in adolescents. Biological aging is also often associated with cardiovascular disease¹. Dyslipidemia also accelerates the aging process, where there is an increase in the lipid peroxidation process in dyslipidemia conditions². Increased lipid peroxidation is one of the markers of oxidative stress that accelerates the aging process, as lipids in biological membranes and lipoproteins are the main targets of peroxidation³.

A process called esterification happens when arachidonic acid is peroxidated without the help of enzymes. This makes metabolites like isoprostane. The most commonly found isoprostane is F2-isoprostane. Elevated levels of F2-isoprostane can be used as an indicator of lipid peroxidation in individuals with high levels of LDL (low-density lipoprotein)⁴. F2-isoprostane is one type of isoprostane compound that often appears in the process of lipid peroxidase, which is the initial stage of dyslipidemia. Compared to malondialdehyde (MDA), F2-isoprostane can be measured more precisely to subpicomolar levels, is relatively stable, has no diurnal variation, and can be detected in all types of normal biological tissues⁵.

Pharmacological management of dyslipidemia is currently done by administering statins as the first line. However, the administration of fenofibrate is also recommended in dyslipidemia therapy⁶. Fenofibrates reduce lipid levels by increasing the triglyceride hydrolysis of lipoprotein lipase into fatty acids, so that triglyceride and LDL levels in serum will decrease while HDL (high-density lipoprotein) will increase⁷. Fibrates act against the nuclear transcription factor PPAR-alpha. Fibrates also reduce apolipoprotein C-III (lipoprotein lipase inhibitor) and increase apolipoprotein A-I, fatty acid transport protein, and lipoprotein lipase, so as to increase the catabolism of tryglicerida-rich plasma origin and reduce the formation of VLDL (Very Low Density Lipoprotein)⁸.

In addition to conventional medicines, traditional medicines are increasingly used to treat various pharmacological conditions. Plants are one of the sources of traditional medicine that are widely explored and used. Various types of plants contain metabolites that are biologically active. One of the plants that can be used is breadfruit (*Artocarpus altilis*). The people of Indonesia frequently use breadfruit leaves as traditional medicine because they are one of the natural ingredients with good medicinal properties⁹. Breadfruit leaves contain various antioxidant compounds such as flavonoids, saponins, hydrocyanic acid, polyphenols, acetylcolin, riboflavin, ethanol, phenols, and tannins¹⁰.

Flavonoid compounds can be used to help reduce the occurrence of dyslipidemia, one of which is by increasing the work of lipoprotein lipase¹¹. Lipase in the pancreas is a lipase that is quite large in hydrolyzing fat, ranging from 50% to 70%¹². Flavonoids can also inhibit the cholesterol ester transfer protein, which mediates the exchange of triglycerides from LDL with cholesterol esters from HDL, chylomicrons, and VLDL, so as to increase HDL levels and reduce LDL. Flavonoids also have anti-inflammatory effects by suppressing the action of

cytokines such as tumor necrosis factor α (TNF- α). A decrease in TNF- α will increase insulin sensitivity, increase fatty acid oxidation in the liver, and inhibit cholesterol synthesis by hepatic cells¹³. Flavonoids can also improve lipid profiles by reducing HMG-CoA reductase activity. Isoflavones, flavones, and flavanones reduce cholesterol levels through inhibition of cholesterol formation and increased expression of low-density lipoprotein (LDL) receptors¹⁴.

Breadfruit leaves (*Artocarpus altilis*) contain flavonoid compounds, polyphenols, and other metabolites that can improve lipid profiles and overcome dyslipidemia. With reference to various studies, this study was conducted to evaluate the activity of breadfruit leaf extract (*Artocarpus altilis*) in improving total cholesterol, triglyceride, LDL, HDL, and F2-isoprostane levels in male Wistar rats (*Rattus norvegicus*) with dyslipidemia.

MATERIALS AND METHODS

Research design

This was a pure experimental study using a pre-test-post-test control group design with two treatment groups: administration of fenofibrate and ethyl acetate extract of breadfruit leaves. Total cholesterol, LDL, HDL, triglycerides, and F2-isoprostane levels were observed.

Sample preparation

Breadfruit leaves (*Artocarpus altilis*) were obtained in Tumbu village, Karangasem sub-district (Bali, Indonesia). Breadfruit leaf samples were taken from young breadfruit leaves that were not too old; old breadfruit leaves were not chosen because some compounds from old breadfruit leaves will decompose into energy sources according to the theory of photosynthesis. Leaf samples were cleaned of dirt and cut into pieces, baked at 50 °C for 24 hours, and blended finely with the addition of 5 liters of water. The ready sample was macerated for 24 hours using a 90% ethyl acetate distiller with periodic stirring. The filtrate obtained was concentrated with a vacuum rotary evaporator and water bath until a thick extract was obtained.

Animal Experiment

The experimental animals used in this study were male Wistar rats (*Rattus norvegicus*) aged 10–12 weeks. A total of 36 rats were randomly divided into two groups, namely the group of test animals given fenofibrate 1.65 mg orally for 28 days (P1) and ethyl acetate extract of breadfruit leaves 760 mg orally for 28 days (P2). This study was approved by the ethics committee with certificate number B/209/UN14.2.9/PT.01.04/2022, Faculty of Veterinary Medicine, Udayana University. The experimental animals were first adapted for 1 week in a laboratory environment with a temperature of 28–32°C, 30% humidity, and lighting every 12 hours (06.00–18.00) before being given research treatment. During adaptation, rats were given standard feed (BR-2 Comfeed, which contains 19% crude protein and 5% crude fat) as much as 12 grams/200g BW/day and drinking water through a sonde. For 28 days after the adaptation process, rats were given high-fat feed at 2 ml/200g BW/day through a round to induce dyslipidemia. The high-fat diet given was a standard diet with the addition of duck egg yolk (which contains 17 grams of protein, 35 grams of fat, and 884 mg/100 grams of cholesterol). The dyslipidemia condition was characterized by total cholesterol levels \geq 200 mg/dL with a weight of 150–200 grams. After achieving the dyslipidemia condition, the rats were then randomly divided into two groups, weighed, and 1.2 ml of blood was taken through the eye vein (medial canthus sinus orbitalis) for pretest total cholesterol, LDL, triglycerides, and HDL. 2 days later, all rats were again taken 0.6 ml of blood through the eye vein (medial canthus sinus orbitalis) for the F2-isoprostane pre-test. For the

next 28 days, rats were given fenofibrate (P1) and ethyl acetate extract of breadfruit leaves (P2). After 28 days of administration, all rats in the two groups were weighed, and 1.8 ml of blood was taken through the eye vein (medial canthus sinus orbitalis) for posttest total cholesterol, LDL, triglycerides, HDL, and F2-isoprostane examination. In the blood collection of rats, rats were first fed for 10–12 hours before blood collection. Then the rats were anesthetized using ketamine HCL at a dose of 40–80 mg/kgBB and xylazine 5–10 mg/kgBB intramuscularly in the thigh. Furthermore, rat blood was taken through the eye vein (medial canthus sinus orbitalis).

Data collection and analysis

Measurements were made on blood serum. The measurement of total cholesterol, LDL, triglycerides, and HDL was carried out using the Glyserol Peroxidase Phosphat Acid method through colorimetric enzymatic reactions using a Cobas analyzer. The examination procedure refers to Sambodo, Tethool and Rumetor (2015). Results are expressed in mg/dL. F2-isoprostane measurement was performed by the ELISA method using a kit from Bioassay Technology Laboratory (Shanghai, China) (Cat No. E4805Hu) following the manufacturer's procedure. Results were expressed as mg/dL. The study data in the form of total cholesterol, triglyceride, LDL, and F2-isoprostane levels were statistically analyzed using the

parametric unpaired T test to compare the effects in both groups. Total cholesterol, triglyceride, LDL, and F2-isoprostane levels in the pretest and posttest groups were tested with a paired parametric T test to determine the effect of fenofibrate and extract administration. The test was conducted using the SPSS for Windows version 21 program with a confidence level of 95%. The significance value (p) <0.05 in parametric testing stated that there was a difference between groups.

RESULT AND DISCUSSION

Breadfruit (*Artocarpus altilis*) has long been known for its use as food and medicinal plants and is also known to have antioxidant effects, especially on its leaves^{10,16}. Phytochemical testing in this study found that the ethyl acetate extract of breadfruit leaves contained flavonoids of 12,410.29 mg/100g and phenols of 6,668.78 mg/100g. This study showed that the administration of breadfruit leaf extract was proven to be able to improve lipid levels and F2-isoprostane in the blood serum of test animals (Table 1). The administration of breadfruit leaf extract significantly reduced total cholesterol, triglycerides, LDL, and F2-isoprostane and increased HDL compared to fenofibrate. Better than fenofibrate, breadfruit leaf extract had an ameliorative effect.

Tabel 1: Lipid and F2-isoprostane serum level

Parameter	Fenofibrate (mg/dL)		Breadfruit leave extract (mg/dL)	
	Pretest	Posttest	Pretest	Posttest
Total cholesterol	231.83 ± 8.34 ^a	228.12 ± 9.97 ^{b*}	232.56 ± 6.27 ^a	176.17 ± 11.66 ^{c**}
Triglyceride	167.99 ± 11.10 ^a	151.59 ± 1.45 ^{b**}	172.41 ± 4.93 ^a	101.24 ± 2.09 ^{c**}
HDL	102.65 ± 9.01 ^a	119.66 ± 1.79 ^{b**}	102.29 ± 10.40 ^a	143.89 ± 5.99 ^{c**}
LDL	95.58 ± 8.67 ^a	78.15 ± 10.61 ^{b**}	95.78 ± 8.00 ^b	12.03 ± 10.06 ^{c**}
F2-Isoprostane	923.04 ± 8.47 ^a	772.27 ± 14.67 ^{b**}	924.86 ± 10.40 ^b	432.99 ± 14.17 ^{c**}

Results are expressed as the mean ± standard deviation. Results with different letters (a,b,c) in each parameter group indicate significant differences based on statistical analysis (p < 0.05). The sign (*) on the posttest value indicates no significant difference (p > 0.05) with the pretest value in each test group, while the sign (**) indicates a significant difference (p < 0.05).

Before (pretest) and after (posttest) treatment, fenofibrate and ethyl acetate extracts of breadfruit leaves were used to compare the changes in lipid profiles and F2-isoprostane levels. The results showed that there were significant differences in all observation parameters and treatment groups, except for the total cholesterol parameter with fenofibrate administration (indicated by *). Both fenofibrate and ethyl acetate extracts of breadfruit leaves resulted in a significant decrease in triglyceride, LDL, and F2-isoprostane levels and a significant increase in HDL. Administration of fenofibrate did not significantly reduce total cholesterol levels, but administration of an ethyl acetate extract of breadfruit leaves significantly reduced total cholesterol levels. Although there was a significant difference in the administration of fenofibrate and ethyl acetate extract of breadfruit leaves on triglycerides, HDL, LDL, and F2-isoprostane levels, the difference produced through the administration of ethyl acetate extract of breadfruit leaves was higher than the administration of fenofibrate, as shown in the posttest value of each parameter in both treatment groups. Statistical testing showed a significant difference (p<0.05) in the posttest values of all parameters (indicated by the letters b and c of the posttest values).

Isoprostane is a metabolite of the non-enzymatic peroxidation of arachidonate. The form most commonly found in plasma is F2-isoprostane, so it is widely used as a marker of oxidative

stress⁵. In this study, the ethyl acetate extract of breadfruit leaves was able to reduce F2-isoprostane levels better than the administration of fenofibrate. The study found that when fenofibrate was given, F2-isoprostane levels went down, but not as much as when ethyl acetate extract of breadfruit leaves was given. Several experimental studies and clinical studies state the antioxidant effects found in fibre-group drugs. The mechanism involved is activating peroxisome proliferator-activated receptor alpha protein (PPAR-alpha), thus decrease the blood cholesterol and increase fatty acid oxidation. However, a study by Scheffer et al. (2013) showed a significant decrease in urinary F2-isoprostane levels with the administration of atorvastatin instead of fibrates¹⁷. Not many experimental studies have examined the effect of fenofibrate on reducing F2-isoprostane.

The results of this study are similar to previous research showing that giving breadfruit leaf water can reduce total cholesterol, LDL, HDL, and triglyceride levels¹⁸. The activity produced by breadfruit leaf extract is closely related to secondary metabolite compounds in the extract, especially flavonoid and phenolic compounds. Polyphenols have a protective effect against cardiovascular disease through their ability to inhibit LDL oxidation potently¹¹. The flavonoid content can also improve lipid profiles. Research on diabetic mice shows improvements in lipid profiles through the

mechanism of decreasing HMG-CoA (3-hydroxy 3-methylglutaryl coenzyme A) reductase activity and increasing plasma LPL (lipoprotein lipase) activity and LCAT (lecithin-cholesterol acyltransferase) activity. Increasing LPL activity causes a decrease in LDL cholesterol levels. This activity also plays a role in reducing LDL triglyceride levels. This activity also plays a role in reducing triglyceride levels because LPL gene activity is the main enzyme that plays a role in eliminating triglycerides from blood circulation ^{14, 19}. Meanwhile, LCAT activity plays a role in cholesterol esterification and the movement of cholesterol from the HDL surface to the core. After taking up cholesterol from peripheral tissues and macrophages, HDL facilitates the delivery of cholesterol to the liver.

Polyphenols are potential LDL oxidation inhibitors and can absorb pro-oxidation components, including heavy metals such as iron. Metabolite compounds such as ascorbic acid, carotenoids, and phenolic compounds effectively inhibit lipid peroxidation through the lipoxygenase deactivation mechanism, considering free radicals and reactive oxygen species (ROS) by propagating the reaction cycle and chelating heavy metal ions. Flavonoid and phenolic components can protect lipids and vitamin C from oxidative processes ²⁰.

Breadfruit leaves are known as powerful antioxidants. Flavonoids and tannins are components of the phenolic class reported to have high antioxidant activity. Flavonoids are thought to work on the myeloperoxidase (MPO) pathway, where MPO inhibition occurs. This inhibition prevents MPO from undergoing oxidation, which can damage proteins, lipids, nucleic acids, and even the oxidation of LDL and HDL in the arteries. The antioxidant effect of these flavonoids can be a protective factor against cardiovascular disease ¹⁴.

This study's phytochemical examination of breadfruit leaf ethyl acetate extract showed the content of phenols, flavonoids, and tannins. Breadfruit leaf ethyl acetate extract has a high antioxidant content. Antioxidants are essential in reducing the effects of free radicals, which play a role in ageing ²¹. It is hoped that the ability of breadfruit leaf ethyl acetate extract to improve the lipid profile can provide an alternative therapy for the problem of dyslipidemia and various degenerative diseases that may arise due to dyslipidemia, such as obesity and cardiovascular disease. The antioxidant ability of breadfruit leaves, which in this study was marked by a decrease in F2-isoprostane levels, is expected to increase the protective effect against the incidence of cardiovascular disease. The results of this study showed that oral administration of breadfruit leaf ethyl acetate extract reduced total cholesterol, triglyceride, LDL, and F2-isoprostane levels and increased HDL levels in male Wistar rats with dyslipidemia better than fenofibrate.

CONCLUSION

Administration of breadfruit leaf extract to Wistar rats with induced dyslipidemia showed that the extract was able to improve the lipid profile. Administration of the extract effectively reduces total cholesterol, triglyceride, LDL, and F2-isoprostane levels and increases HDL levels. Compared to administering fenofibrate as a positive control, breadfruit leaf extract produced more activity.

Conflict of interest

There are no conflicts of interest in connection with this paper

REFERENCES

- [1] Lin CF, Chang YH, Chien SC, et al. Epidemiology of Dyslipidemia in the Asia Pacific Region. *Int J Gerontol* 2018; 12: 2-6. <https://doi.org/10.1016/j.ijge.2018.02.010>
- [2] Pamungkas T. Efek ekstrak daun sukun (*Artocarpus altilis*) terhadap kadar kolesterol total tikus putih (*Rattus norvegicus*). Universitas Muhammadiyah Malang, 2016.
- [3] Ito F, Sono Y, Ito T. Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: Oxidative Stress in Diabetes, Atherosclerosis, and Chronic Inflammation. *Antioxidants* (Basel, Switzerland); 8. Epub ahead of print March 2019. <https://doi.org/10.3390/antiox8030072> PMid:30934586 PMCid:PMC6466575
- [4] Davies SS, Roberts LJ 2nd. F2-isoprostanes as an indicator and risk factor for coronary heart disease. *Free Radic Biol Med* 2011; 50: 559-566. <https://doi.org/10.1016/j.freeradbiomed.2010.11.023> PMid:21126576 PMCid:PMC3058898
- [5] Ward WF, Qi W, Van Remmen H, et al. Effects of age and caloric restriction on lipid peroxidation: measurement of oxidative stress by F2-isoprostane levels. *J Gerontol A Biol Sci Med Sci* 2005; 60: 847-851. <https://doi.org/10.1093/gerona/60.7.847> PMid:16079206
- [6] Erwinanto, Santoso A, Putranto JNE, et al. Pedoman Tatalaksana Dislipidemia. 1st ed. Jakarta, Indonesia: Perhimpunan Dokter Spesialis Kardiovaskular Indonesia, 2013. Epub ahead of print 2013. <https://doi.org/10.1136/bcr.09.2008.0970> PMid:21686532 PMCid:PMC3027967
- [7] Nugroho CA. Pengaruh ekstrak rosela (*Hibiscus sabdariffa*) terhadap kadar trigliserida tikus putih diabetes. *Widya War* 2013; 37: 269-280.
- [8] Asih RS, Alifiar I, Purwandy Y. Pengaruh Kronofarmakologi Terhadap Kadar Kolesterol Total Dan Trigliserida Dalam Darah Pasien Pengguna Obat Golongan Statin Dan Fibrat. *JFL J Farm Lampung* 2021; 9: 78-83. <https://doi.org/10.37090/jfl.v9i2.335>
- [9] Soifoini T, Donno D, Jeannoda V, et al. Phytochemical Composition, Antibacterial Activity, and Antioxidant Properties of the *Artocarpus altilis* Fruits to Promote Their Consumption in the Comoros Islands as Potential Health-Promoting Food or a Source of Bioactive Molecules for the Food Industry. *Foods* (Basel, Switzerland); 10. Epub ahead of print September 2021. <https://doi.org/10.3390/foods10092136> PMid:34574246 PMCid:PMC8468414
- [10] Muhammad Z. Pengaruh Pemberian Ekstrak Daun Sukun (*Artocarpus Altilis*) Terhadap Jumlah Anakan Mencit (*Mus Musculus*). Universitas Islam Negeri Raden Inten, Lampung, 2021.
- [11] Dzomba P. Anti-obesity and antioxidant activity of dietary flavonoids from *Dioscorea steriscus* tubers. *J Coast Life Med*. Epub ahead of print 2014. <https://doi.org/10.12980/JCLM.2.201414B8>
- [12] Ahn M-S. Current Concepts of Premature Ventricular Contractions. *J lifestyle Med* 2013; 3: 26-33.
- [13] Hussain T, Tan B, Murtaza G, et al. Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy. *Pharmacol Res* 2020; 152: 104629. <https://doi.org/10.1016/j.phrs.2020.104629> PMid:31918019
- [14] Zeka K, Ruparelia K, Arroo R, et al. Flavonoids and Their Metabolites: Prevention in Cardiovascular Diseases and Diabetes. *Diseases* 2017; 5: 19. <https://doi.org/10.3390/diseases5030019> PMid:32962323 PMCid:PMC5622335
- [15] Sambodo P, Tethool AN, Rumetor SD. Anti-Cholesterol Effect of n-Hexane Fraction of *Biophytum petersianum* Klotsch in (Efek Antikolesterol Fraksi n - Heksana Rumput Kebar Pada Hewan Model Hiperlipidemia). *J Kedokt Hewan* 2015; 9: 59-60. <https://doi.org/10.21157/j.ked.hewan.v9i1.2793>
- [16] Latifah. Identifikasi golongan senyawa flavonoid dan uji aktivitas antioksidan pada ekstrak rimpang kencur *Kaemferia galanga* L. dengan metode dpph (1,1-difenil-2-pikrilhidrazil). Universitas Islam Negeri Maulana Malik Ibrahim Malang, 2015.
- [17] Scheffer PG, Schindhelm RK, van Verschuer VMT, et al. No effect of atorvastatin and simvastatin on oxidative stress in patients at high risk for cardiovascular disease. *Neth J Med* 2013; 71: 359-365.

[18] Adaramoye OA, Akanni OO. Effects of Methanol Extract of Breadfruit (*Artocarpus altilis*) on Atherogenic Indices and Redox Status of Cellular System of Hypercholesterolemic Male Rats. *Adv Pharmacol Sci* 2014; 2014: 605425. <https://doi.org/10.1155/2014/605425> Mid:24592277 PMCid:PMC3926424

[19] Fajaryanti N, Nurrochmad A, Fakhrudin N. Evaluation of antihyperlipidemic activity and total flavonoid content of *Artocarpus altilis* leaves extracts. *Int J Pharm Clin Res* 2016; 8: 461-465.

[20] Halimatussakkidiah, Amna U, Mardina V. Antioxidant activity of methanol extract of *Diplazium esculentum* (Retz.) Sw. leaves collected from Aceh. *IOP Conf Ser Mater Sci Eng*; 725. Epub ahead of print 2020. <https://doi.org/10.1088/1757-899X/725/1/012082>

[21] Pangkahila W. *Tetap Muda, Sehat, dan Berkualitas: Konsep Anti Aging Medicine*. Jakarta, Indonesia: Kompas Media Nusantara, 2017.