Journal of Advances in Medicine and Pharmaceutical Sciences
p-ISSN: 2964-268X, e-ISSN: 2963-4253

Case Report Paper

Activity Test of Matoa Leaves on Angiotensin II as an Increasing SOD and GPx

Octaviana Dyah Oentari[1*], Jason Merari Peranginangin[1], Ika Purwidyaningrum[1]

[1] *Faculty of Pharmacy, Universitas Setia Budi. Surakarta, Indonesia.*

Article History
Received:
03.07.2024

Revised:
25.07.2024

Accepted:
06.08.2024

***Corresponding Author:**
Octaviana Dyah Oentari

Abstract: The leaves of the matoa are plants that can be used for hypertension. Matoa leaves were assumed to have thought to have hypertensive activity because they contain flavonoids (quercetin-3-O-rhamnosida). Flavonoids can lower blood pressure which is modulated by the Renin-Angiotensin-Aldosterone System (SRAA). Was to fine out about the giving process of matoa leaf extract and fractions in increasing levels of SOD and GPx. This research used the maceration method with 96% ethanol solvent and fractionated by liquid-liquid method using n-hexane fraction solvent, ethyl acetate fraction, and water fraction. In this study, 21 male white rats with Wistar strain were divided into 7 groups, group I named as normal control, group II as negative control (CMC-Na 1%), group III as positive control (Irbesartan), and Group IV (matoa leaf extract), Group V (n-hexane fraction group VI (ethyl acetate fraction) group VII (water fraction). The data obtained were analyzed with the Shapiro-Wilk test, Levene's test and ANOVA. The results showed that the matoa leaf extract a dose 300mg/kg BB lower blood pressure in angiotensin II induced rats increase SOD and GPx levels in the liver induced by Angiotensin II

Keywords: Angiotensin II, Fraction, GPx, *Pometia pinnata*, SOD.

Journal of Advances in Medicine and Pharmaceutical Sciences, vol. 3, no. 2, pp. 54-59, November 2024. **DOI: 10.36079/lamintang.jamaps-0302.752**

1. Introduction

Hypertension is one of the most common cardiovascular diseases and often occurs around us. Blood pressure in normotensive people increases in the morning, and gradually decreases during the day, then peaks again at night to show a greater decrease at midnight [1]. In 2018, the prevalence of hypertension in Indonesia showed a large figure of 34.1% with as many as 70% of sufferers experiencing mild hypertension with the high prevalence rate, research was conducted on several medicinal plants to lower blood pressure [2].

This hypertension occurs because angiotensin I is converted into angiotensin II by angiotensin converting enzyme (ACE). In the body contains angiotensinogen which is produced by the liver and then returned by the renin enzyme can be converted into angiotensinogen I and will be converted into angiotensin II by ACE in the lungs [3]. When the intrinsic arterial pressure in the kidney, it can cause many molecules and proteins in the juxtaglomerular to break down and release renin. Angiotensin II is a very strong vasoconstrictor and can cause other effects as well and affect circulation. And as long as angiotensin II is in the blood, angiotensin II has two main effects, namely increasing arterial pressure [4].

In general, the treatment of hypertension using angiotensin receptor blockers (ARBs) which are effective in the treatment of hypertension and heart failure can be developed as agents that block more RAAS and reduce the adverse effects of ACE inhibitors associated with high cough frequency and other adverse effects. The ARB group can effectively lower blood pressure and has a dose curve with a flat response, if increasing this dose above a low dose or also will not drastically lower blood pressure. Like ARBs and ACE Inhibitors, most have a fairly long half-life for once-daily administration. But candesatran, eprosartan, and losartan have the shortest half-lives and require doses given twice a day to effectively lower blood pressure. ARBs have the lowest side effects compared to other antihypertensive drugs. The use of natural ingredients that can treat and prevent hypertension is widely carried out by researchers. Recently, research on medicinal plants has been scientifically studied, and has the ability to cure various diseases [5].

One of the medicinal plants that has properties as an antihypertensive is matoa leaves. Matoa leaves contain flavonoid compounds and phenolic compounds [6]. Flavonoid compounds are usually found in plants and have antioxidant, antiallergic, antiviral and anti-inflammatory effects while phenolic compounds are compounds that are efficacious as antidiarrheals and antibacterials [7].

Matoa leaves contain quercetin-3-O-rhamnoside and kaempferol-3-O-rhamnoside compounds, this is known by the research of Hansen et al, 1996 using the Erythroxylum laurifolium plant which also contains compounds (quercetin) which have activity as quercetin-3-O-rhamnoside ACE Inhibitor with IC50 0.67 mm and kaempferol-3-O-rhamnoside (afzelin) with IC50 2.8 mM, so it is suspected that the compounds responsible for antihypertension in matoa leaves are quercetin-3-O-rhamnoside and kaempferol-3-O-rhamnoside [8]. The cause of hypertension is oxidative stress, namely where there is an imbalance between the formation of Reactive Oxygen Secies (ROS) and the antioxidant system that lasts a long time. The reaction of free radicals and phospholipids of epithelial cell membranes, one of which is malondialdehyde (MDA) [9].

The formation of MDA can be prevented by antioxidant compounds originating from our bodies, namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). But the initial defense in the body to overcome oxidative stress is SOD. So, the presence of SOD is very important in the body to ward off free radicals [10]. Oxidative stress, which is where the imbalance between the number of antioxidants and free radical molecules in the body can cause dysfunction of β cells of the islets of Langerhans, and cause degeneration and cell death [11]. Meanwhile, the selection of the fraction method used in this study refers to the states that extracts and fractions of matoa leaves are effective in lowering blood pressure in mice induced by NaCl and prednisone. This fractionation aims to separate a main group of compounds contained based on the polarity of the solvent [12]

Hypertension is often called the “silent killer” because it can happen to anyone. Apart from stroke and tuberculosis, hypertension is one of the diseases with the greatest risk factors for death in Indonesia. This disease usually does not directly kill the sufferer, but rather triggers other diseases that are classified as severe and deadly and give symptoms that continue for a target organ. A person is said to have hypertension if their blood pressure is high or exceeds the normal blood pressure value of 140/80 mmHg [13]

The mechanism of hypertension occurs due to the change of angiotensin I to angiotensin II by angiotensin converting enzyme (ACE). ACE plays an important role in regulating blood pressure. Blood contains angiotensinogen produced in the liver which is then converted by the renin enzyme

Journal of Advances in Medicine and Pharmaceutical Sciences, vol. 3, no. 2, pp. 54-59, November 2024. **DOI: 10.36079/lamintang.jamaps-0302.752**

into angiotensin I and will be converted into angiotensin II by ACE found in the lungs [14].

In general, the use of Angiotensin Receptor Blockers (ARB) is the most effective in treating hypertension. However, the use of natural ingredients to treat and prevent hypertension has been widely carried out by researchers in the world. One of the medicinal plant studies that has been scientifically studied as an antihypertensive is matoa leaves. Matoa is a native Papuan plant known to contain phenolic and flavonoid compounds. It was shown that matoa leaf extract also has diuretic and antihypertensive effects. Ethanol extracts from matoa leaves, skin and seeds were also studied to have diuretic activity which is one of the antihypertensive drug groups in male Wistar rats. Extracts of matoa leaves, skin and seeds can affect sodium and potassium levels in urinary excretion. In this study it was reported that the effective dose of ethanol extract of matoa leaves for diuretic effects was 100 mg/kg bb [15].

2. Method

This research used maceration method with 96% ethanol solvent and fractionated by liquid-liquid method using n-hexane fraction solvent, ethyl acetate fraction, water fraction. In this study, 21 male white rats of Wistar strain were used as test animals divided into 7 groups, namely group I as normal control, group II as negative control (CMC-Na 1%), group III as positive control (Irbesartan), group IV (matoa leaf extract), Group V (n-hexane fraction), group VI (ethyl acetate fraction), group VII (water fraction).

3. Finding and Discussion

The results of the fraction with n-hexane solvent obtained a fraction weight of 0.60 g, this shows that in the matoa leaf fraction it is suspected that there are 0.60 g of non-polar compounds with a fraction yield of 0.04% which shows the comparison of the number of fractions produced from the fractionation of matoa leaves, the fraction with 41 ethyl acetate solvent obtained a fraction weight of 2.39 g, this shows that in the matoa leaf fraction it is suspected that there are 2.39 semipolar compounds with a fraction yield of 0.16% which shows the comparison of the number of fractions produced from the fractionation of matoa leaves, the fraction with water solvent obtained a fraction weight of 2.00 g, this shows that in the matoa leaf fraction it is suspected that there are 2.00 polar compounds with a fraction yield of 0.13% which shows the comparison of the number of fractions produced from the fractionation of matoa leaves. The results of the fractionation of matoa leaf ethanol extract obtained 0.598 grams of n-hexane fraction, 2.385 grams of ethyl acetate fraction and 1.995 grams of water fraction.

Table 1. Fractions Produced from The Fractionation of Matoa Leaves

No	Name	Fraction Weight (g)	Yield (%)
1	*n*-Hexane	0.60	0.04
2	Ethyl Acetate	2.39	0.16
3	Water	2.00	0.13

The TLC results of the extract and fraction of matoa leaves can be seen in the Table 2. The plate shows five spots. where each leftmost spot shows a comparison. Then the second is the ethanol extract. then the n- hexane fraction. the ethyl acetate fraction. and the last rightmost is the water fraction. Identification of thin layer chromatography (TLC) aims to determine the chemical content of matoa leaves. The compounds identified are flavonoids

Table 2. Extract and Fraction of Matoa Leaves

Sample	Light	UV 366	UV 254	Detector
Extract	Light Brown	Blue Fluorescence	Yellow	Citrobrat
n-hexane fraction	-	-	-	Citrobrat
Ethyl acetate fraction	Light Brown	Blue Fluorescence	Yellow	Citrobrat
Water fraction	Light Brown	Blue Fluorescence	Yellow	Citrobrat
Quercetin	Light Brown	Blue Fluorescence	Yellow	Citrobrat

Journal of Advances in Medicine and Pharmaceutical Sciences, vol. 3, no. 2, pp. 54-59, November 2024. **DOI: 10.36079/lamintang.jamaps-0302.752**

Systolic and diastolic blood pressure were measured using the CODA device. Increased systolic and diastolic blood pressure indicated the effects of angiotensin II induction given for 14 days.

Table 3. Systolic Blood Pressure

Group Treatment	Systolic Blood Pressure					
	Group Treatment	Group Treatment	Group Treatment	Group Treatment	Group Treatment	Group Treatment
Normal control	130.3±3.2	Normal control	130.3±3.2	Normal control	130.3±3.2	Normal control
Negative control	114.3±10.0	Negative control	114.3±10.0	Negative control	114.3±10.0	Negative control
Positive control	117.0±13.1	Positive control	117.0±13.1	Positive control	117.0±13.1	Positive control
Extract	122.7±4.0	Extract	122.7±4.0	Extract	122.7±4.0	Extract
n-hexane fraction	126.7±2.1	n-hexane fraction	126.7±2.1	n-hexane fraction	126.7±2.1	n-hexane fraction
Ethyl acetate fraction	126.3±2.5	Ethyl acetatefraction	126.3±2.5	Ethyl acetate fraction	126.3±2.5	Ethyl acetate fraction
Water fraction	117.0±5.2	Water fraction	117.0±5.2	Water fraction	117.0±5.2	Water fraction

The results of the systolic blood pressure obtained were continued to the Shapiro-Wilk test to determine whether the data was normally distributed (sig>0.05) the results of systolic blood pressure showed normally distributed data. then continued with the Levene test to determine whether the data was homogeneous (sig>0.05) the results of systolic blood pressure showed homogeneous data. then the ANOVA test was carried out to determine whether the data tested was normally distributed (sig<0.05) and saw homogeneous variance (sig>0.05) continued with Post Hoc Tukey to determine whether there were significant differences between groups. The results of the Post Hoc Tukey showed that the normal group and the negative group were significantly different from the positive group and the extract group. n-hexane fraction. ethyl acetate fraction. water fraction were significantly different from the normal group and the negative group.

Based on statistical analysis in the EDM group. the n-hexane fraction. ethyl acetate fraction and water fraction against the negative group (CMC) were significantly different (p<0.05) which means that CMC has no activity in lowering blood pressure after administration of angiotensin II induction which will cause an increase in blood pressure while in the positive group there was no significant difference (p>0.05) which means that it is suspected that EDM. n-hexane fraction. ethyl acetate fraction and water fraction have activity in lowering systolic blood pressure which is almost the same as Irbesartan's activity in lowering systolic blood pressure and in the normal group it is significantly different (p<0.05) which means that after administration of angiotensin II induction. there was an increase in blood pressure different from the normal group that was not given induction which did not experience an increase in blood pressure.

The results of the percentage of systolic blood pressure lowering activity in the treatment group are suspected due to the active substance contained in matoa leaves. namely flavonoids (quercetin). Quercetin is included in the flavonoid compound. Flavonoids are polyphenol compounds in the form of glycosides that bind a sugar group so that flavonoids are included in polar compounds. Water fraction is a polar solvent that can attract polar substances such as flavonoids. saponins. and tannins in the extraction process. According to reported quercetin can lower blood pressure and reduce symptoms of hypertension in male white rats given angiotensin induction

The results of diastolic blood pressure showed differences between test groups. The results of diastolic blood pressure obtained were continued to the Shapiro-Wilk test to determine whether the data was normally distributed (sig>0.05) the results of diastolic blood pressure showed normally distributed data. then continued with the Levene test to determine whether the data was homogeneous (sig>0.05) the results of diastolic blood pressure showed homogeneous data then ANOVA test was carried out to determine whether the data tested was normally distributed (sig<0.05) and homogeneous variance was seen (sig>0.05) continued with Post Hoc Tukey to determine significant differences between groups. The results of Post Hoc Tukey showed that the normal group and the negative group were significantly different from the positive group and the extract group. n-hexane fraction. ethyl acetate fraction. water fraction were significantly different from the normal group and negative group. Results of SPSS analysis of diastolic blood pressure.

Journal of Advances in Medicine and Pharmaceutical Sciences, vol. 3, no. 2, pp. 54-59, November 2024. **DOI: 10.36079/lamintang.jamaps-0302.752**

Based on statistical analysis in the EDM group. the n-hexane fraction. ethyl acetate fraction and water fraction against the negative group (CMC) were significantly different ($p<0.05$) which means that CMC has no activity in lowering blood pressure after administration of angiotensin II induction which will cause an increase in blood pressure while in the positive group there was no significant difference ($p>0.05$) which means that it is suspected that EDM. n-hexane fraction. ethyl acetate fraction and water fraction have activity in lowering diastolic blood pressure which is almost the same as Irbesartan's activity in lowering diastolic blood pressure and in the normal group it is significantly different ($p<0.05$) which means that after administration of angiotensin II induction there was an increase in blood pressure different from the normal group that was not given induction which did not experience an increase in blood pressure. The results of the percentage of diastolic blood pressure lowering activity in the treatment group are suspected due to the active substance contained in matoa leaves. namely flavonoids (quercetin). Quercetin is included in the flavonoid compound. Flavonoids are polyphenol compounds in the form of glycosides that bind a sugar group so that flavonoids are included in polar compounds. Water fraction is a polar solvent that is able to attract polar substances such as flavonoids. saponins and tannins in the extraction process SOD The levels of SOD and GPx in the liver tissue of mice based on the table above can be seen that compared to the normal control group. groups II to VII experienced a decrease. The decrease in SOD and GPx levels occurred because both enzymes protect cells in the liver from damage caused by free radicals after being induced by Angiotensin II. A very large decrease occurred in the negative control. However. based on the results of statistical tests. there were differences in each group. both in SOD levels and GPx levels.

This shows that the administration of matoa leaf extract can affect SOD and GPx levels in the liver tissue of mice. The decrease in SOD and GPx levels in the water fraction group was not the same as the negative control. indicating that the water fraction can act as an antioxidant and work together with the SOD and GPx enzymes in reducing oxidation reactions caused by free radicals in mouse tissue. The antioxidant system in mammalian cells such as SOD and GPx functions to inhibit free radicals so that there is protection in the liver organ induced by angiotensin II.

SOD is a metalloenzyme found in subcellular and contains copper or zinc and manganese which functions as an antioxidant in catalyzing superoxide anion into hydrogen peroxide and oxygen. while GPx catalyzes hydrogen peroxide into non-toxic compounds. In the water and ethyl acetate fractions. the activity is almost the same as Irbesartan. The treatment is thought to be due to the active substance in matoa leaves. namely flavonoids (quersertin). Quersertin is included in flavonoid compounds . Flavonoids are polyphenol compounds in the form of glycosides that bind one sugar group so that flavonoids are polar compounds. The water fraction is a polar solvent that can attract polar substances such as flavonoids. saponins and tannins in the extraction process [16].

How ever. based on the results of statistical tests. there are differences in each group. both in SOD levels and GPx levels. This shows that giving matoa leaf extract can affect SOD and GPx levels in rat liver tissue. The decrease in SOD and GPx levels in the matoa leaf extract group at a dose of 300 mg/kg BW which was not the same as the negative control showed that the leaf extract can act as an antioxidant and work together with the SOD and GPx enzymes in reducing oxidation reactions caused by free radicals in the liver tissue of mice.

The antioxidant system in mammalian cells such as SOD and GPx functions to inhibit free radicals so that there is protection in the liver organ induced by CCl4. SOD is a metalloenzyme found in subcellular and contains copper or zinc and manganese which function as antioxidants in catalyzing superoxide dismutase anion into hydrogen peroxide and oxygen. while GPx catalyzes hydrogen peroxide into non-toxic compounds.

Oxidative stress conditions that can cause endothelial dysfunction in hypertension. The availability of NO as a natural vasodilator in the body is disrupted by ROS which are produced in large quantities in the body [17]. Which state that increased lipid peroxidation in hypertension is related to ROS which interferes with NO. Higher MDA levels in the hypertension group were due to increased ROS. ROS in the body can increase the activity of Angiotensin Converting Enzyme (ACE) which functions to form Angiontensin II. In addition to being a vasoconstrictor. Angiontensin II can also increase ROS production in the body by increasing the activity of NADPH oxidase in producing superoxide ions [17].

The 300 mg/kg BW dose group of the negative control was the same as the liver condition in the positive control group. This is because matoa leaves contain quercetin-3-O rhamnoside compounds which can function as antioxidants. Giving antioxidants can prevent or reduce damage that occurs in the liver because with the presence of antioxidants such as melanoidin. the free radicals that are

formed can be stabilized by donating their electrons or by binding the free radicals so that they do not react with the components of the cells [18].

3. Conclusion

Extracts and fractions of matoa leaves (*Pometia pinnata*) can lower blood pressure in male Wistar rats induced by angiotensin II. Extracts and fractions of matoa leaves (*Pometia pinnata*) can increase SOD levels in male Wistar rats induced by angiotensin II. Extracts and fractions of matoa leaves (*Pometia pinnata*) can increase GPx levels in male Wistar rats induced by angiotensin II.

References

[1] D. Devangi, C. H. Shashirekha, and S. Shruthi, "A study of chronopharmacological relevance of antihypertensive drugs at a tertiary care hospital - A prospective observational study," *National Journal of Physiology, Pharmacy and Pharmacology*, vol. 8, no. 3, pp. 446-452, 2018.

[2] D. Anggraini, M. Kusnadi, and E. Budianto, "Factors Contributing to Hypertension Incidence in Patients Attending the Adult Polyclinic at Bangkinang Health Center: A Retrospective Study," *Journal of Public Health and Clinical Medicine*, vol. 10, no. 2, pp. 121-130, 2009.

[3] A. Sylvestris, "Hypertension and hypertensive retinopathy," *Faculty of Medicine, University of Muhammadiyah Malang*, vol. 10, no. 1, 2014.

[4] S. A. Zia, A. Mansoor, and U. Kaul, "Angiotensin receptor blockers - Advantages of the new Sartans," *Journal of the Association of Physicians of India*, vol. 61, July 2013.

[5] A. Surya, "Toxicity of Matoa leaf extract (Pometia pinnata) against larvae (Artemia salina L) with brine shrimp lethality test method," *Journal of Clinical Science Health Analysis*, Abdurrab University, ISSN 2338-4921, 2018.

[6] N. W. Martiningsih, G. A. B. Widana, and P. L. P. Kristiyanti, "Phytochemical screening and antioxidant activity test of ethanol extract of Matoa leaves (Pometia pinnata) using DPPH method," *J. Pet*, pp. 332–338, 2016.

[7] S. Lenny, "Flavonoid, phenylpropanoid, and alkaloid compounds," Scientific Work, *Department of Chemistry, FMIPA, University of North Sumatra*, Medan, 2006, p. 7.

[8] I. Juranek and S. Bezek, "Controversy of free radical hypothesis: Reactive oxygen species cause or consequence of tissue injury," *General Physiology and Biophysics*, vol. 24, pp. 263-278, 2005.

[9] J. M. C. Connell and E. Davies, "The new biology of aldosterone," *J. Endocrinol.*, vol. 186, pp. 1-20, 2005.

[10] S. Sagar, M. Kaur, and K. P. Minneman, "Antiviral lead compounds from marine sponges," *Mar. Drugs*, vol. 8, no. 10, pp. 2619-2638, 2010.

[11] K. Roy and J. Racine, "Pathogenesis and epidemiology of osteoarthritis," *Rhode Island Medical Journal*, pp. 19-22, 2013.

[12] S. Rajkumar and A. Jebanesan, "Bioactivity of flavonoid compounds from *Poncirus trifoliate* L. (Family: Rutaceae) against the dengue vector, *Aedes aegypti* L. (Diptera: Culicidae)," *Parasitol. Res.*, vol. 104, pp. 19-25, 2008.

[13] M. Heinrich, J. Barnes, S. Gibbons, and E. M. Williamson, *Pharmacognosy and Phytotherapy*, S. W. R. Syarief, A. C. Aisyah, E. Elviana, and E. R. Fidiasari, trans.; A. H. Hadinata, Ed., Jakarta: EGC Medical Book, 2009.

[14] I. Purwidyaningrum and M. Dzakwan, "Diuretic activity test of Matoa leaves (Pometia pinnata) on male Wistar rats," *Indonesian Journal of Pharmacy*, vol. 12, no. 1, pp. 79–84, 2015.

[15] T. Wresdiyati, A. B. Hartanta, and M. Astawan, "Tepung Rumput Laut (*Eucheuma Cottonii*) menaikkan level superoksida dismutase (Sod)," *J. Vet.*, vol. 12, no. 2, pp. 126–135, 2011.

[16] P. Tiwari, B. Kumar, M. Kaur, G. Kaur, and H. Kaur, "Phytochemical screening and extraction," *International Pharmaceutical Science*, vol. 1, no. 1, pp. 98-106, 2011.

[17] BioVision, "Glutathione Peroxidase Activity Assay Kit," [Online]. Available: http://www.biovision.com, Catalog #K762-100, 2017. [Accessed: Jan. 05, 2024].

[18] M. R. Murali, S. B. Raja, and S. N. Devaraj, "Neutralization of radical toxicity by temperature-dependent modulation of extracellular SOD activity in coral bleaching pathogen *Vibrio shiloi* and its role as a virulence factor," *Arch. Microbiol.*, vol. 192, no. 8, pp. 619–623, 2010.