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THE IN VITRO AND IN VIVO EFFECTS OF *Persea Americana* ETHANOL EXTRACT AS AN ANTIHYPERTENSIVE AND ANTIOXIDANT IN PREDNISONE-INDUCED RATS

Efek In Vitro dan In Vivo Ekstrak Etanol *Persea Americana* sebagai Antihipertensi dan Antioksidan pada Tikus yang Diinduksi Prednison

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ABSTRACT

Hypertension impacts the quantity of free radicals within the body. This study aimed to investigate the effect of antihypertension based on free radical levels in the prednisone-induced rats, focusing on evaluating specific anti-hypertensive fraction of avocado leaves. The study's methods conducting GC-MS, anti-hypertensive, and assessing malondialdehyde levels and catalase enzyme. Systolic blood pressure measurements indicated that the positive control (131 ± 3 mmHg) and ethyl acetate fraction (136 ± 4 mmHg) were statistically similar, significantly different from the negative control group. Diastolic blood pressure measurements showed a blood pressure decreasing in positive control (105 ± 7) that similar statistically with ethyl acetate fraction (104 ± 6), and significantly difference with negative control group. Malondialdehyde levels were notably elevated in both the positive control (37.79 ± 5.47) and ethyl acetate fraction (38.01 ± 5.47) compared to the negative control (-9.07 ± 10.10), indicating increased free radical. Catalase activity demonstrated significant differences, with the positive control (44.06 ± 5.44) and ethyl acetate fraction (44.05 ± 5.45) showing similar levels, both substantially higher than the negative control (13.90 ± 21.50). In summary, this study indentificates several flavonoid compounds that promising anti-hypertension effect from ethyl acetate fraction of avocado leaves extract.

Keywords: *Antihypertensive, Antioxidants, Avocado, Cardiovascular, Catalase, Malondialdehyde*

ABSTRAK

Hipertensi berdampak pada jumlah radikal bebas di dalam tubuh. Penelitian ini bertujuan untuk menentukan efek antihipertensi berdasarkan pada tingkat radikal bebas pada tikus yang diinduksi prednison, berfokus pada pencarian fraksi spesifik yang memiliki aktivitas antihipertensi dari daun alpukat. Metode penelitiannya melakukan GC-MS, antihipertensi, dan menilai kadar malondialdehid dan enzim katalase. Pengukuran tekanan darah sistolik mengindikasikan bahwa kontrol positif (131 ± 3) dan fraksi etil asetat (136 ± 4) sebanding secara statistik dan berbeda signifikan dengan kontrol negatif. Pengukuran tekanan darah diastolik menunjukkan bahwa kontrol positif (105 ± 7) sebanding secara statistik dengan fraksi etil asetat (104 ± 6) dan berbeda nyata dengan kontrol negatif. Tingkat malondialdehid menunjukkan kenaikan pada kelompok kontrol positif (37,79 ± 5,47) dan fraksi etil asetat (38,01 ± 5,47) jika dibandingkan dengan kelompok kontrol negatif (-9,07 ± 10,10), hal ini mengindikasikan peningkatan stres oksidatif.

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Aktivitas enzim katalase menunjukkan perbedaan yang signifikan antara kelompok kontrol positif (44,06 ± 5,44) dan fraksi etil asetat (44,05 ± 5,45), dibandingkan dengan kontrol negatif (13,90 ± 21.50). Ringkasnya, penelitian ini mengidentifikasi beberapa senyawa flavonoid yang menjanjikan efek anti-hipertensi dari fraksi etil asetat esktrak daun alpukat.

***Kata kunci**: Antihipertensi, Antioksidan, Alpukat, Kardiovaskular, Katalase, Malondialdehid*

INTRODUCTION

Cardiovascular diseases pose a major public health challenge in Indonesia, with reactive oxygen species (ROS) identified as primary contributors to their development. Free radicals are atoms possessing one or more unpaired electrons, rendering them unstable, highly reactive, and capable of assaulting neighboring molecules. Various forms of free radicals exist, including OH^- (hydroxyl), ROO^- (peroxyl), H_2O_2, O_2^- (singlet oxygen), NO^- (nitrite oxide), $ONOO^-$ (peroxynitrite), HOCl (hypochlorous acid), and O_2^* (superoxide). Among these, O_2^* stands out as the most free-radical species. When free radicals interact with hydrogen atoms from unsaturated fatty acids within cell membranes, it generates lipid peroxides. This compound, inherently unstable, gives rise to various byproducts, including malondialdehyde (MDA), thereby initiating peroxidation (Xu *et al,* 2019).

MDA is the primary and extensively researched compound resulting from lipid peroxidation, recognized for its mutagenic and toxic properties. Moreover, MDA can be enzymatically generated as a byproduct during the synthesis of thromboxane A2. As an end product of lipid peroxidation, MDA serves as a biomarker for measuring free radicals in various biological samples, including blood, urine, and exhaled breath condensate (EBC), particularly in patients affected by a wide array of diseases such as cancer, cardiovascular disorders, pulmonary ailments, and neurodegenerative conditions. The formation of MDA involves the action of free radicals generated through both enzymatic and non-enzymatic pathways (Cordiano *et al*, 2023). Polymorphonuclear cells, monocytes, and macrophages can generate oxygen and H_2O_2 free radicals, which can damage mitochondria and halt the cell cycle (Munawara *et al,* 2021). O_2^* free radicals are converted into H_2O_2 by the SOD and Cu^{2+} enzyme complex within mitochondria. Subsequently, H_2O_2 is converted into H_2O and O_2 by enzymes such as catalase and glutathione peroxidase (GSH-Px). However, when the quantity of free radicals overwhelms the capacity of these enzymes, external antioxidant compounds become necessary (Pratiwi *et al,* 2022).

Given the established role of free radical in cardiovascular diseases, this study aims to explore the antihypertensive and antioxidant properties of various fractions derived from avocado leaves. Widely available in Indonesia, it is renowned for its medicinal properties and traditional medicinal uses (Qin & Sihotang, 2020). Studies have investigated the blood pressure-lowering effects of avocado leaves decoctions (Mar'iyah *et al,* 2022). According to research, steeping avocado leaves can reduce blood pressure due to active substances like flavonoids and quercetin. Flavonoids are beneficial for preventing osteoporosis, improving arterial function and structure, and stabilizing atherosclerotic plaques, thus reducing blood pressure. Quercetin helps relax arterial muscles and normalize arterial narrowing, leading to decreased blood pressure (Lianti, 2014).

This study focuses on evaluating the antihypertensive and antioxidant effects of three different fractions from avocado leaves, employing advanced analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) (Wijayanti, 2023). FTIR is utilized to analyze molecular vibrations and predict compound structures based on functional groups present in avocado leaf compounds (Pratiwi & Cahyanto, 2023).

MATERIALS AND METHOD

Materials

Materials that used in this research were avocado leaves, ethanol 96%, Equipment should include the type of equipment and its manufacturer. For example: CO2 incubator (ICO50, Memmert).

Place And Time of the Research

This research was carried out in 2023-2024 and was carried out at the Stifar Pharmacology Laboratory of the Semarang Pharmasi Foundation. The research agenda for making avocado leaf extracts and fractions is then tested pharmacologically using male Galus Wistar rats as test animals with the parameters MDA, Catalase Enzyme, IR, GC-MS.

Methods

1. Fraction

The process involved in the extraction and fractionation of avocado leaves is as follows (Tobi *et al,* 2022):

1. 20 grams of ethanol extract of avocado leaves was dissolved in a small amount of ethanol.
2. The dissolved extract was then partitioned with 50 ml of water and 50 ml of n-hexane solvent in a separating funnel, with this process repeated three times.
3. The n-hexane fraction, appearing as the top layer, was separated from the water fraction at the bottom.
4. The collected n-hexane fraction was concentrated using a rotary evaporator at a bath temperature of 50 °C.
5. The remaining water fraction from the n-hexane fraction was further partitioned with 50 ml of ethyl acetate solvent in a separating funnel, repeated three times.
6. The ethyl acetate fraction, found as the top layer, was separated from the water fraction at the bottom.
7. The collected ethyl acetate fraction was concentrated using a rotary evaporator at a bath temperature of 50 °C.
8. The remaining filtrate from the ethyl acetate fractionation, which is the water fraction, was dried in a water bath.

This research was carried out at the Biology Laboratory, Semarang Pharmacy Foundation School of Pharmaceutical Sciences.

2. FTIR

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds. All of the different extracts of *Persea americana* Mill. was used for FTIR analysis. The extract is mixed with powder which has been mashed, homogenized and put into the sample container, the powdered sample of each extract was loaded in FTIR Spectrophotometer (Sumantri *et al,* 2020).

3. GC-MS

Identification was carried out to determine the compound profile in the water fraction, ethyl acetate n-hexane from avocado leaves using GC-MS with MDA and catalase parameters in hypertensive rats. The results obtained were in the form of a compound chromatogram showing one graph and several peaks. The sample was injected into a column of 30 m x 0.25 mm i.d with a thin film of 0.25 µm, the carrier gas used was 1 mL/minute helium, the injector was set at a temperature of 200°C and the column temperature was programmed at a temperature of 50-250°C with a speed of 10°C/minute injection. In MS, an ionization voltage of 70 eV, temperature 250°C, mass range 50-600 was used. The obtained chromatogram and mass spectrum of the unknown compound were then compared with the spectrum of the standard (Melati, 2021; Elisa *et al,* 2021).

4. Flavonoid Content

The determination of flavonoid content involved UV-Vis spectrophotometry to identify the types of flavonoids and ascertain the oxidation pattern and position of free phenolic hydroxyl groups within the flavonoid core. A shear reagent was added to the sample solution, resulting in a maximum shift in wavelength, either towards longer wavelengths (bathochromic) or shorter wavelengths (Courtney, 20212). The fractions from avocado leaves (water, ethyl acetate, and n-hexane) were dissolved in methanol. To the sample solution, three drops of 2M NaOH were added, and the spectrum was measured (with readings repeated after 5 minutes). Subsequently, in the new sample solution, the spectrum was

analyzed after adding 6 drops of 5% $AlCl_3$ reagent and 3 drops of HCl, followed by measuring the absorbance. After substituting the sample, absorption spectrum analysis was conducted following the addition of NaOAc powder and shaking, and then H_3BO_3 powder was added and shaken (Nur *et al,* 2019). This research was conducted at the Biology Laboratory, Semarang Pharmacy Foundation School of Pharmaceutical Sciences.

5. Pharmacological Test

The measurement of blood pressure and MDA levels involved 25 male Wistar rats which were divided into 5 groups. The treatment groups consisted of 2 control groups (positive and negative control) and 3 sample treatment groups (water fraction, ethyl acetate, and n-hexane of avocado leaves ethanol extract). Rats in the positive control group were given 1 mg of Vitamin C. Rats in the negative control group were given 2 ml of 2% NaCl and 1.5 mg of prednisone suspension. Rats in the sample treatment group were given 250 mg/kg of each sample fraction.

Before treatment, rats were acclimatization in 7 days. After the adaptation process, blood was collected from the eyes of rats. After that, blood pressure (systolic and diastolic), MDA levels and catalase enzyme activity were measured. The result of these measurements was used as T0 data before induction was given. The rats in all groups were inducted with 2 ml of 2% NaCl and 1.5 mg/kg of prednisone suspension.

The induction was given orally for 21 days. The second measurement of systolic blood pressure, diastolic blood pressure, MDA levels and activity of catalase enzyme was carried out after the rats were given induction. The result was used as T21 data. After the induction process, the test animals were given the test substance orally for 7 days, and then blood pressure (systolic and diastolic), MDA levels and activity of the catalase enzyme was measured. The result was used as T29 data. The method used to measure blood pressure included non-invasive direct tail-cuff using CODA Instrument®.

MDA kit stock solution with concentrations of 0, 1, 2, 3, 4, 5, 6. 7 and 8 µg/mL taken 100 µL, put in different ependrophs, added 550 µL distilled water, 100 µL TCA 10%, 250 µL HCl 1 N, 100 µL Na-Thio 1% and homogenized. After that, centrifuged at a speed of 500 rpm for 10 minutes. The supernatant was taken, heated in a water bath at 100°C for 30 minutes, left at room temperature, and the absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 532 nm. The absorption results were then made into an MDA standard curve and a linear equation was produced.

Rat blood samples were put into a test tube then 500 µl of 0.9% NaCl was added and homogenization was carried out. The homogenate was taken and transferred to Eppendorf tube. Next, centrifugation was carried out at a speed of 8000 rpm for 20 minutes and the supernatant was taken. 100 µL of supernatant was put into a reaction tube, added with 100 µl TCA, 100 µL 1 N HCL and 100 µL 1% Na-Thio then homogenized again. After that, it was centrifuged at 500 rpm for 10 minutes, and heated in a water bath at 100°C for 30 minutes. The absorbance of the sample was then measured with a spectrophotometer at the maximum wavelength (λ max = 532 nm) (Purwanto & Aprilia, 2022).

RESULT AND DISCUSSION

FTIR

Infrared spectrophotometry is an analytical technique that is carried out by measuring the vibrations of molecules excited by infrared radiation in a certain wavelength range. When infrared radiation is passed through a sample, the molecules can absorb energy and a transition occurs between the basic vibrational level and the excited vibrational level. The infrared spectrum is divided into two, namely the functional group frequency area in the 4000-1400 cm-1 area with easy-to-recognize spectrum peaks and the fingerprint area in the 1400-400 cm-1 wave number range. This has a distinctive absorption band so it requires a special method to determine the functional group (Jumardin *et al,* 2023). FTIR spectrophotometric analysis was used to determine the functional groups of certain organic compounds inside the extract (Afqir *et al,* 2024). FTIR characteristic result test of

the water fraction, ethyl acetate fraction and n-hexane fraction of avocado leaves with quercetin as the standard, can be seen in Figure 1.

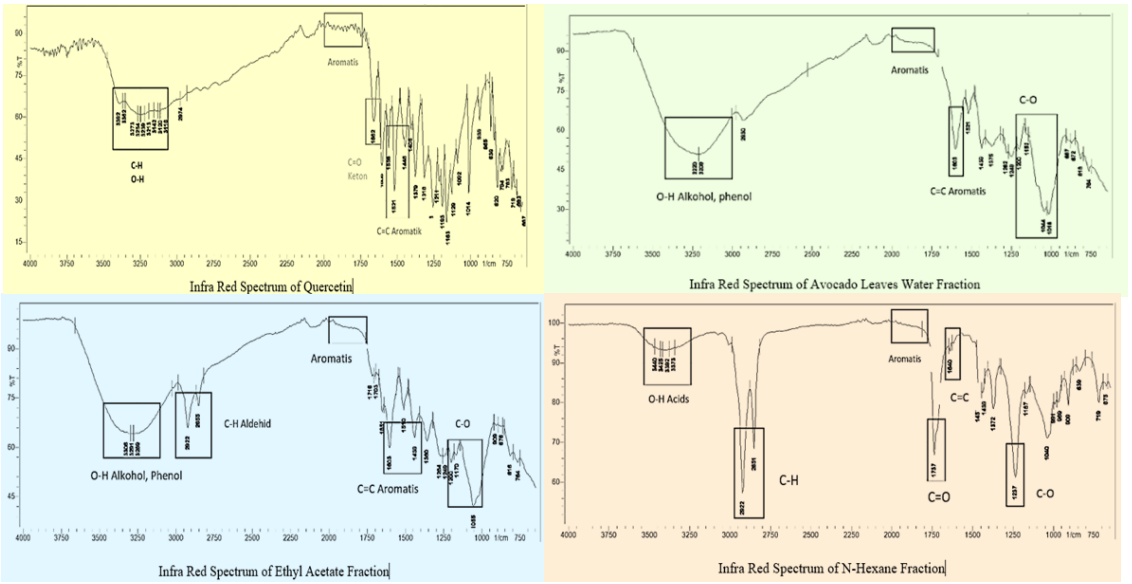


Figure 1. FTIR Spectrophotometry Result of Quercetin and Avocado Leaves Fractions

GC-MS

The result of GC-MS analysis of avocado leaves fractions can be seen in Figure. 2, Figure. 3, Figure. 4, Table. 1, Table. 2, and Table. 3.

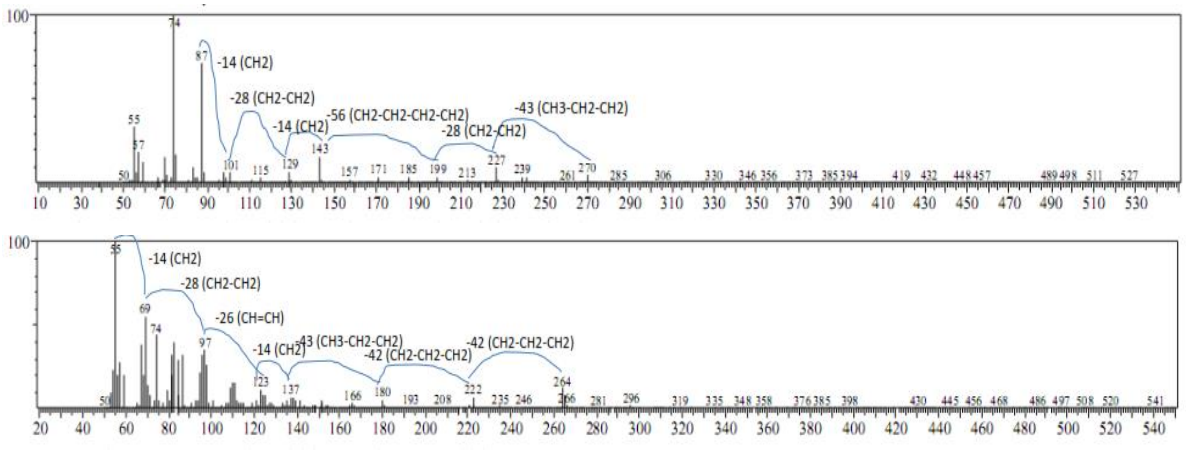
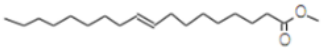
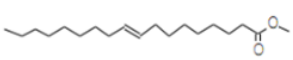


Figure 2. GC-MS Reading Result of Avocado Leaves Water Fraction

Table 1. GC-MS Reading Result of Avocado Leaves Water Fraction

No.	Retention Time (Minutes)	Peak Area (%)	Molecular Weight	Compound Structure	Compound Name
1.	22.08	15,28	3,39		Hexadecanoic acid, methyl ester (CAS)
2.	23.77	24.48	3,99		9-Octadecenoic acid (z)-CAS

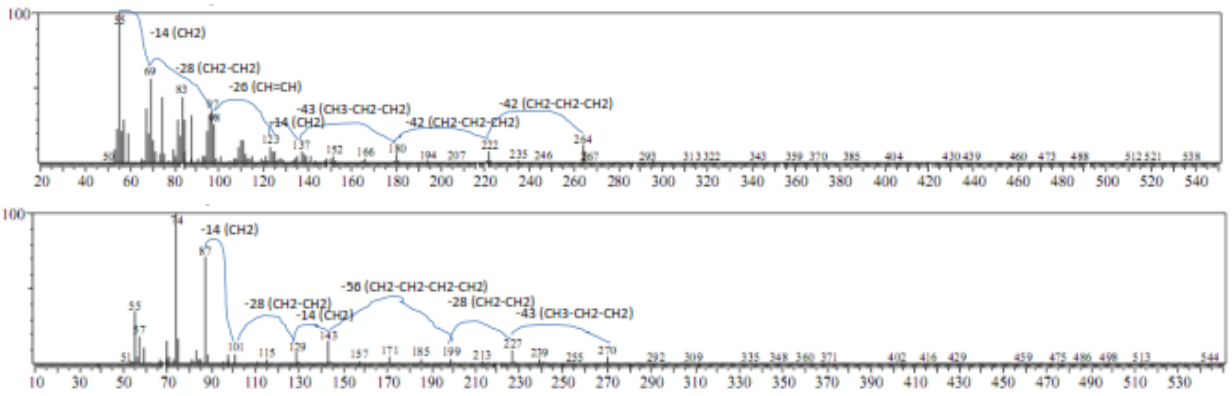
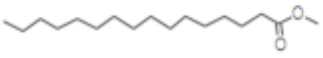


Figure 3. GC-MS Reading Result of Avocado Leaves Ethyl Acetate Fraction

Table 2. GC-MS Reading Result of Avocado Leaves Ethyl Acetate Fraction

No.	Retention Time (Minutes)	Peak Area (%)	Molecular Weight	Compound Structure	Compound Name
1.	22,06	7,80	2,89		Hexadecanoic acid, methyl ester (CAS)
2.	23.76	9,18	2,52		9-Octadecenoic acid (z)- methyl ester (CAS)

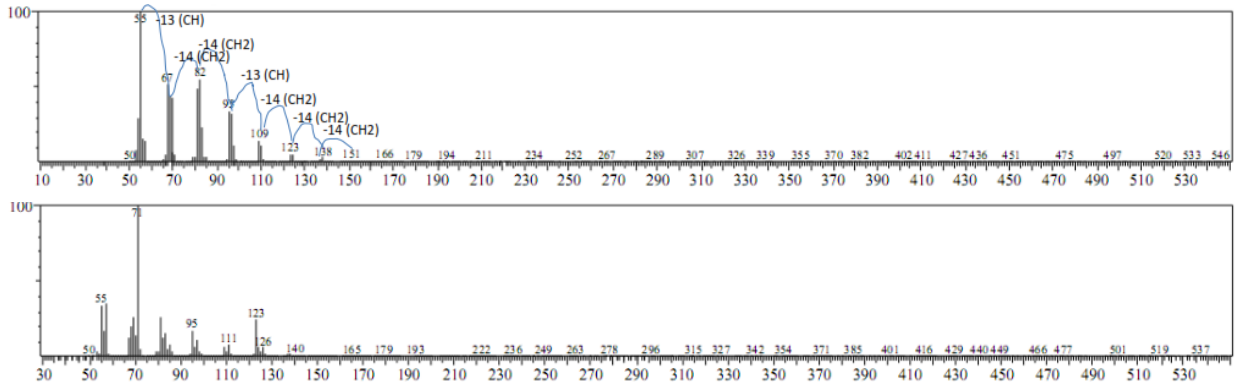
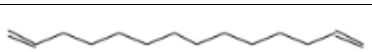
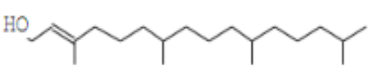


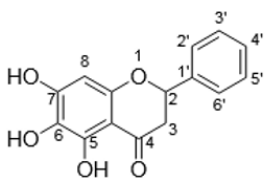
Figure 4. GC-MS Reading Result of Avocado Leaves N-Hexane Fraction

Table 3. GC-MS Reading Result of Avocado Leaves N-Hexane Fraction

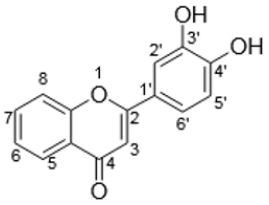
No	Retention time (Minutes)	Peak Area (%)	Molecular Weight	Compound Structure	Compound Name
1.	18,76	19,83	3,38		1,13-TETRADECA-DINE
2.	23,89	10,96	2,53		2-Hexadecen-I-ol,3,7,11,15-tetrame-thyl--,(R-(R*,R*-(E))-(CAS)

Flavonoid Content

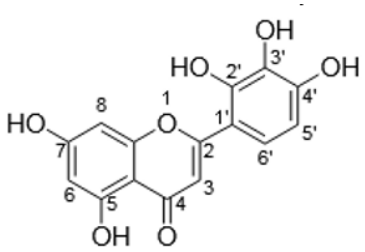
The flavonoid contained in avocado leaves fractions can be seen in **Figure. 5.**



Water Fraction



Ethyl Acetate Fraction



N-Hexane Fraction

Pharmacological Test

Pharmacological test that carried out in this research were systolic and diastolic blood pressure test, malondialdehyde test, and catalase enzyme test. The result can be seen in **Table. 4, Table. 5, Table. 6** and **Table. 7**.

Table 4. Systolic Blood Pressure Result

Groups	Systolic Blood pressure (mmHg)			
	T0	T21	T29	TDS
Positive Control	116±10	149±5	129±3*	131±3[a]
Negative Control	122±5	149±5	143±7*	138±1[b]
Water fraction 250mg/Kg	112±3	157±6	131±5*	133±1[ab]
Ethyl acetate fraction 250mg/Kg	116±10	160±2	130±5*	136±4[a]
n-Hexane fraction 250mg/Kg	111±4	159±4	136±6*	135±1[ab]

a. Different meaning for negative groups < 0.05
b. Different meaning for control groups < 0.05

Table 5. Diastolic Blood Pressure Result

Groups	Diastolic Blood pressure (mmHg)			
	T0	T21	T29	TDD
Positive	97±15	121±5	98±1*	105±7[a]
Negative	102±11	122±2	120±2*	115±5[b]
Water fraction 250mg/Kg BW	91±11	121±2	112±6*	108±4[ab]
Ethyl acetate fraction 250mg/Kg BW	97±14	118±5	98±2*	104±6[a]
n-Hexane fraction 250mg/Kg BW	86±10	121±3	112±6*	106±5[ab]

a. Different meaning for negative groups < 0.05
b. Different meaning for control groups < 0.05

Table 6. Malondialdehyde Level in Wistar Rats

Groups	**T0**	**T21**	**T29**	**Increase Percentage**	**Decrease Percentage**
Positive Control	1.03±0.05	5.81±0.51	3.61±0.05*	467.48±58.94	37.79±5.47[a]
Negative Control	1.00±0.05	5.23±0.08	5.70±0.50*	423.54±26.03	-
Water fraction 250mg/Kg	1.06±0.04	5.91±0.45	4.46±0.17*	457.02±30.87	9.07±10.10[ab]
Ethyl acetate fraction	1.03±0.05	5.83±0.52	3.61±0.06*	467.48±58.94	25.14±6.77[ab]
250mg/Kg	1.04±0.02	5.36±0.32	.24±0.17*	409.99±38.60	38.01±5.47[a]
n-Hexane fraction 250mg/Kg					6.90±3.41[ab]

a. Different meaning for negative groups < 0.05
b. Different meaning for control groups < 0.05

Table 7. Catalase enzyme Level in Wistar Rats

Groups	T0	T21	T29	Decrease Percentage	Increase Percentage
Positive Control	71.01±7.16	22.12±1.00	31.84±0.75*	220.78±27.22	44.06±5.44[a]
Negative Control	70.91±7.22	30.64±3.70	25.90±4.70*	103.60±30.30	13.90±21.50[ab]
Water fraction 250mg/Kg	67.05±7.00	30.30±6.20	33.25±2.00*	132.55±71.45	13.30±21.30[ab]
Ethyl acetate fraction	71.02±7.15	22.12±1.00	31.83±0.74*	219.80±27.21	44.05±5.45[a]
250mg/Kg	70.24±8.05	0.70±4.05	47.01±0.94*	140.70±40.42	70.06±30.50[a]
n-Hexane fraction 250mg/Kg					

a. Different meaning for negative groups < 0.05
b. Different meaning for control groups < 0.05

Infrared spectrum elucidation results of the standard quercetin solution which was a semi-polar compound, were as follows: a peak that appeared in frequency area of 3329 cm-1 showed that this fraction contained O-H group bound to an aromatic ring (phenol). A peak appeared in frequency area of 1662 cm-1 showed that the quercetin solution contained the C=O group that was a characteristic of flavonoids. The presence of a strong sharp peak in frequency area of 1606 cm-1 showed an aromatic C=C group in this standard solution. A peak in frequency area of 1256 cm-1 supported the existence of C-O alcohol group. A sharp peak at the frequency area of 839 cm showed that the quercetin solution had the C-H aldehyde group (Dewi *et al,* 2014; Zhang & Huang, 2014).

The infrared spectrum elucidation results of the water fraction which is a polar compound containing an O-H group bound to an aromatic ring (phenol) was supported by a peak appearance at a frequency area of 3209 cm-1. The presence of an aromatic C=C group which was supported by a peak at a frequency area of 1603 cm-1, and the C-O alcohol group which was supported by the peak appeared at a frequency area of 1249 cm-1(Dewi *et al,* 2014; Zhang & Huang, 2014).

The infrared spectrum elucidation of ethyl acetate fraction which was a semi-polar compound, showed a peak in frequency area of 3291 cm-1 which means the fraction containing an O-H group bound to an aromatic ring (phenol). A peak also appeared at a frequency area of 1718 cm-1 which means this fraction contained the C=O group which was a flavonoid characteristic. The presence of a sharp peak at frequency area of 1603 cm-1 showed that ethyl acetate fraction of avocado leaves contained an aromatic C=C group. A peak that appeared in frequency area of 1249 cm-1 showed that alcohol C-O group was present in this fraction. The sharp peak at frequency area of 816.79 cm-1 showed the presence of aldehyde C-H group (Dewi *et al,* 2014; Zhang & Huang, 2014).

The infrared spectrum elucidation of the n-hexane fraction (a non-polar compound) showed a peak that appeared at a frequency area of 3425 cm-1 which means the n-hexane fraction contained O-H group bound to an aromatic ring (phenol). The appearance of a peak at frequency 1737 cm-1 showed that this fraction contained carbonyl C=O group. The presence of a strong and sharp peak at frequency area of 1457 cm-1 showed the existence of an aromatic C=C group in this fraction. The alcohol C-O group was indicated by appearance of a peak at frequency area of 1237 cm-1. A peak that showed C-H aldehyde group in this fraction appeared at frequency area of 839 cm-1(Dewi *et al,* 2014; Zhang & Huang, 2014).

The function groups contained within the water fraction, ethyl acetate fraction, and n-hexane fraction were also found in the quercetin solution. It can be hypothesized that water fraction, ethyl acetate fraction, and n-hexane fraction contain flavonoid compounds that have the potential for hypertensive activity (Elisa *et al,* 2021). The functional group peaks detected in pure quercetin are also present in the ethyl acetate and n-hexane fractions, but not all of the functional group peaks are present in the water fraction (the C=O and C-H functional group peaks were not detected in the water fraction). However, the functional group

peaks in ethyl acetate are closer to the peaks in pure quercetin than in the n-hexane fraction, so the quercetin compound is likely present in the ethyl acetate fraction. Additional confirmation is needed like combining FTIR with NMR to identify more comprehensive compounds within the avocado leaves extract.

The further test to analyze the compound composition in the sample, GC-MS (Gas Chromatography-Mass Spectrometry) analysis was carried out in all fractions. The results of GC-MS analysis in water fraction showed an area of 15.28% with a retention time of 22.08 minutes. The compound that was identified was hexadecenoic acid, methyl ester (CAS) with a molecular weight of 3.39. Another compound that was identified was 9-octadecenoic acid (z)-CAS, with an area of 24.48% in the retention time of 23.77 minutes and a molecular weight of 3.99.

In the ethyl acetate fraction, compounds that were identified were hexadecenoic acid, methyl ester (CAS) (7.80%) and 9-octadecenoic acid (z)-CAS (23.76%). N-hexane fraction was analyzed and found two compounds consisting of 1,13-tetradecane (18.76%) and 2-hexadecane-I-ol,3,7,11,15-tetramethyl (R-(R*, R*-(E))-(CAS) (23.89%) (Kaban *et al,* 2016).

Previous studies showed that avocado leaves fractions contained many flavonoid compounds through phytochemical screening analyses. This study was useful in determining compounds that have properties in lowering blood pressure, especially in hypertension case study (Elisa *et al,* 2021). In this report, the Chang method was used for flavonoid content determination, in which $AlCl_3$ was involved to create a formation of stable complexes with C-4 keto groups, C-3 or C-5 hydroxyl groups from flavones and flavanols. Quercetin solution was used as a comparison compound since the compound was a flavonoid compound that can form a complex through reaction with $AlCl_3$ (Tobi & Pratiwi, 2023).

The flavonoid content test in the water fraction showed a result of 5,6,7 compounds, which indicated the presence of trihydroxy flavonone compounds. Ethyl acetate fraction contained 3'4' flavonoid compounds, which indicated the presence of dihydroxy flavone compounds. N-Hexane fraction contained 2'3'4'5'7 flavonoid compound, which indicated the presence of pentahydroxy flavanone compound. The results of flavonoid content in the water fraction, ethyl acetate fraction, and n-hexane fraction showed potential compounds, especially in the n-hexane fraction. Compounds contained in the n-hexane fraction played a role in determination of catalase enzyme levels which is related to MDA formation in hypertension conditions. The use of quercetin standard herein was based on its wide distribution in plants. Indeed, 60-70% parts of plants consist of quercetin and its glycosides (Rocha *et al,* 2024).

In this study, NaCl and Prednisone were used as inducing substances. Previous research showed that NaCl and prednisone can increase blood pressure (Courtney, 2012). The results of blood pressure measurement showed that the negative control group was significantly different from the positive control with sig. value < 0.05. The result of systolic blood pressure measurement, the water fraction group (133 ± 4) approached the result of the positive control group (131 ± 3). The result of diastolic blood pressure measurement, the ethyl acetate fraction group (104 ± 6) obtained better results than the positive control group (105 ± 7).

The results of the MDA level measurement in the positive control group (37.79 ± 5.47a) and the ethyl acetate fraction group (38.01 ± 5.47a) were approached (not significantly different), while in the negative control group (-9.07 ± 10.10ab) were significantly different from the positive control group and ethyl acetate fraction with a significant value <0.05. The results indicated that the avocado leaves fraction has the potential to inhibit the formation of excessive MDA levels. MDA is the final product in the lipid peroxidation process which can act as an indicator for cell damage. The higher the ROS production, the more lipid peroxide will result in higher MDA levels (Anggraeny *et al,* 2022). The increased MDA levels due to peroxidation indicate the pathogenesis of several diseases, particularly hypertension (Huang *et al,* 2023). Hypertension is known as a disease where the blood pressure is beyond the normal value of 120/80 mmHg (Courtney, 2012).

Further, the catalase enzyme level analyses showed varied values among the positive control group (44.06 ± 5.44), ethyl acetate fraction (44.05 ± 5.45), n-hexane fraction (70.06 ± 30.50), which was significantly different from the negative control group (13.90 ± 21.50) and the water fraction (13.30 ± 21.30). The activity of the catalase enzyme from rat groups treated with vitamin C, ethyl acetate fraction and n-hexane fractions showed a significant difference > 0.05 (Munawara *et al,* 2021; Astika *et al,* 2020).

Catalase enzyme is a hydroperoxidase enzyme that can protect the body from dangerous peroxide compounds. High peroxide levels can produce free radicals, which can cause damage to the cell membranes and cause several cardiovascular diseases, including hypertension (Astika *et al,* 2020; Griendling *et al,* 2020).

Further study that associated with this research are finding excellent combinations of the three fractions to maximize blood pressure-lowering and antioxidant effects, exploration other flavonoid compounds in the three fractions to find chemical compounds that synergistically have anti-hypertensive effects, and finding specific compounds in avocado leaves that potentially have other therapeutic effects, especially in the cardiovascular system.

CONCLUSION

Three different fractions of avocado leaves fractions (i.e., water fraction, ethyl acetate fraction and n-hexane fraction) have been studied. Several flavonoid compounds from avocado leaves fraction such as trihydroxy flavanone (water fraction), dihydroxy flavone (ethyl acetate fraction) and pentahydroxy flavanone (n-hexane fraction) have the potential to inhibit the formation of excessive MDA levels in hypertensive rat models. The measurement of systolic and diastolic blood pressure showed that the fractions have the potential to lower blood pressure. The water fraction group (133 ± 4) approached the result of the positive control group (131 ± 3) in lowering systolic blood pressure. The result of diastolic blood pressure measurement, the ethyl acetate fraction group (104 ± 6) obtained better results than the positive control group (105 ± 7). The MDA level in the positive control group (37.79 ± 5.47a) and the ethyl acetate fraction group (38.01 ± 5.47a) was not significantly different, while in the negative control group (-9.07 ± 10.10ab), MDA was significantly different from the positive control and the ethyl acetate fraction groups with a sig. value <0.05. The catalase enzyme activity in the positive control group (44.06 ± 5.44), ethyl acetate fraction (44.05 ± 5.45) and n-hexane fraction (70.06 ± 30.50) were significantly different from the negative control group (13.90 ± 21.50) and water fraction (13.30 ± 21.30).

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