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| RESEARCH ARTICLE

Supplementation of *Bouea macrophylla* Fruit Juice Prevent Oxidative Stress in Rats Fed with High-Fat High-Cholesterol Diet through Attenuation of Lipid Peroxidation

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| ABSTRACT

Fruits are rich in fiber and antioxidant compounds that can prevent and treat health disorders related to oxidative stress caused by high-fat high-cholesterol diet (HFHCD). *Bouea macrophylla* is a tropical fruit plant with edible juicy fruits. In this study, we conducted experiments to prove the effectiveness of *B. macrophylla* fruit juice (BMFJ) as an antioxidant agent in rats fed with HFHCD. Male Sprague Dawley rats were fed with HFHCD for 100 days and simultaneously orally supplemented with BMFJ. Atorvastatin was used as a positive control. At the end of the experiment, the blood and hepar were collected and assayed for malondialdehyde (MDA). The in vitro antioxidant activity of BMFJ was also evaluated using the DPPH method. Total phenols and flavonoids contents were determined using Folin Ciocalteu and $AlCl_3$ methods, respectively. Results of the experiments showed that total phenols and flavonoids in BMFJ were 570 mg GAE/g and 31.89 mg QE/g, respectively, and the IC_{50} of radical scavenging activity was 564.271 ppm. The in vivo antioxidant evaluation showed that supplementation of BMFJ significantly prevents the increase of MDA levels, both in serum and liver of rats fed with HFHCD. These findings clearly indicate that supplementation of *B. macrophylla* fruit juice significantly prevents oxidative stress in rats fed with high-fat high-cholesterol diets through attenuation of lipid peroxidation.

| KEYWORDS

Antioxidant; *Bouea macrophylla*; high-fat high-cholesterol diet; malondialdehyde; oxidative stress

| ARTICLE INFORMATION

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1. Introduction

Oxidative stress plays a crucial role in the development of numerous diseases. Various degenerative diseases are caused by excessive production of free radicals. The trigger for the formation of free radicals can be caused by an unhealthy diet, especially high fat high cholesterol diet, which is poor in dietary fibers. An unhealthy diet combined with a lack of regular exercise is the risk factor for various degenerative diseases such as cardiovascular, diabetes, and fatty liver (Di Meo & Venditti, 2020; Lobo et al., 2010).

Fat accumulation in cells is derived from the release of free fatty acids (FFA) in adipose tissue through lipolysis, fatty acid absorption, and de novo lipogenesis (DNL). Adipose tissue that stores excess fat will increase tissue mass, causing adipocyte hypertrophy and hyperplasia. This situation will cause cell death and trigger inflammation. These conditions lead to excessive production of Reactive Oxygen Species (ROS), popularly known as free radicals, causing a condition known as oxidative stress (Buzzetti et al., 2016; Rives et al., 2020; Tsikas, 2017).

The increased free radicals in the body harm the body's cells, including the liver. If the free radicals in the body increase and cannot be overcome by the body's antioxidant system, then the body will experience oxidative stress and induce lipid peroxidation (Cichoz-Lach & Michalak, 2014; Li et al., 2015; Lobo et al., 2010). Lipid peroxidation is a non-enzymatic reaction between free radicals that attack lipids containing double carbon bonds, namely polyunsaturated fatty acids (PUFA). The process begins when reactive oxygen metabolites cause the abstraction of hydrogen from the methylene group from the carbon-carbon double bond of the PUFA molecule, thereby forming fatty acid radicals. These unstable compounds produce lipid peroxyl radicals which then attack other PUFAs. The process continues with an uncontrolled chain reaction; various types of radicals react with each other to form stable products when free radicals react with antioxidants and produce the final product, namely malondialdehyde (MDA) (Petrovic et al., 2020). MDA is a ketoaldehyde, which is a highly reactive and toxic aldehyde; because MDA is more stable than other aldehydes, MDA is used as a widely used biomarker for oxidative stress and lipid peroxidation (Augustine et al., 2021; Ayala et al., 2014).

Oxidative stress can be overcome by modifying lifestyle by implementing healthy eating habits, which aim to control body weight and cardio-metabolic risk factors (Sharifi-Rad et al., 2020). Consumption of functional foods or ingredients that contain lots of fiber and antioxidant compounds such as phenolic compounds is a concept that can be done to prevent or overcome the effects of free radicals and NAFLD (Perdomo et al., 2019; Samtiya et al., 2021).

Function food that is a source of fiber, as well as a source of antioxidant compounds, includes *B. macrophylla* fruit. This fruit contains various active compounds, such as phenolic compounds, flavonoids, flavonols, anthocyanins, and ascorbic acid, which act as antioxidants (Rajan & Bhat, 2016). *B. macrophylla* fruit juice has been shown to have antioxidant activity with an IC_{50} value of 36.4 mg/mL, as well as 121 volatile compounds in ripe *B. macrophylla* fruit identified through GC-MS (Lolaen et al., 2013; Rajan & Bhat, 2017). Currently, there are very few studies related to *B. macrophylla*, including its chemical content, biological activity, and medicinal potential.

This study aimed to determine the effect of *Bouea macrophylla* fruit juice supplementation in preventing and treating oxidative stress induced by a high-fat high-cholesterol diet through malondialdehyde (MDA) levels in the serum and liver of rats.

2. Literature Review

2.1 Bouea macrophylla Griff.

Bouea macrophylla is a tropical fruit plant belonging to the *Anacardiaceae* family and the genus *Bouea*. This plant can be found in Southeast Asia, such as Indonesia, Thailand, Philippines, Vietnam, and Malaysia. In Indonesia, it is cultivated as a fruit tree in humid areas of Sumatra Java, Sumatra, Kalimantan, and Ambon, known by various names such as ramania (Kalimantan), jatake, gandaria (Java), remieu (Gayo), barania (Dayak ngaju), raba-raba (North Sumatra), dandoriah (Minangkabau), wetes (North Sulawesi), Kalawasa, rapo-rapo kebo (Makassar), and buwa melawe (Bugis) (Harsono, 2017; Silalahi et al., 2018).

Bouea macrophylla fruit has an oval-shaped stone type, ± 3-5 cm in diameter; ripe fruit is yellow or orange with a juicy sweet and sour taste (Dechsupa et al., 2019). *Bouea macrophylla* fruit has one seed, with white and reddish-purple color, and has a bitter taste (FAO, 2001). Harsono et al. (2016) stated that purple seeds and opposite leaves can be used as morphological markers of Bouea. The fruit, known as plum mango in English, can be processed into sweets, syrup, and nata. In addition, the unripe fruit can also be processed into a flavoring ingredient in chili sauce and pickles (Rajan & Bhat, 2020; Warella et al., 2016; Warella et al., 2016; Breemer et al., 2021)

Figure 1. *Bouea macrophylla* Griff. fruits

Bouea macrophylla fruit has a carbohydrate content of 88 – 91 NFE, so it can be used as a source of energy and healthy food for people with diabetes. People with diabetes generally find it difficult to regulate a healthy diet to maintain blood sugar levels. *B. macrophylla* fruit also contains fiber that has the potential to slow down the absorption of sugar in the body. Rajan & Bhat (2017) revealed that in unripe fruit, there are 82 volatile compounds, while in ripe fruit, there are 121 volatile compounds.

Lolaen et al. (2013) showed that *B. macrophylla* fruit juice contains saponins and phenolic compounds and has high antioxidant activity with an IC_{50} value of 36.3 mg/mL. These antioxidant compounds, such as phenolic acid, ascorbic acid, tannins, and flavonoids, ward off free radicals, which can cause damage to body cells due to oxidative stress. The ripe fruits also contain anthocyanins. These bioactive compounds are essential regulators of protein expression and activity related to homeostasis and lipid metabolism in the body, especially for fatty liver disease, and can prevent atherosclerosis or blood vessel blockage (Ferramosca et al., 2017).

2.2 Free Radicals and Oxidative Stress

Reactive oxygen species (ROS) is one of the free radicals that can reduce oxygen and has a robust oxidative capacity. ROS also has many physiological activities in intracellular redox signaling and growth regulation. RNS is reactive to various derivatives of nitric oxide metabolites, including nitroxyl anions, nitrosonium cations, higher nitrogen oxides, S-nitrosothiols, and dinitrosyl iron complexes (Ma et al., 2022). ROS and RNS initiate, mediate and modulate intracellular oxidative stress through physiological pathways. Hydroxyl radicals (HO^{-}) are the most reactive among free radicals and contribute significantly to oxidative stress. This condition damages biomolecules induces lipid peroxidation and breaks DNA strands. Under physiological conditions, the balance between ROS, RNS, and antioxidants enables cellular crosstalk, control of intracellular functions, cell-to-cell interactions, proliferation, differentiation, migration, and contraction. ROS-induced oxidative stress and inflammation play a role in the mechanisms leading to liver cell death and tissue injury (Ma et al., 2022; Ore & Akinloye, 2019).

Oxidative stress refers to the imbalance between ROS production and antioxidant defense, leading to impaired signaling, redox control, and molecular damage. Oxidative stress is classified according to its severity as "eustress" (physiological oxidative stress) and "distress" (toxic oxidative load that damages biomolecules). In other words, low OS exposure is helpful for redox signaling, whereas high exposure results in impaired redox signaling and cause damage to essential biomolecules. The most frequently used oxidative stress biomarkers in clinical trials of NAFLD are NO (Nitric Oxide), MDA (malondialdehyde), 8-OH-dG (8-hydroxyguanosine), and CYP2E1 (Cytochrome P450 2E1). The concentration or activity of these biomarkers generally increased in all clinical data reviewed. In all experimental models, MDA is the most widely used biomarker of oxidative stress (Ore & Akinloye, 2019).

Free radicals can come from outside the body (exogenous) or within the body (endogenous). In biological systems, the body can generally produce its antioxidants in the form of enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (endogenous antioxidants) (Ore & Akinloye, 2019).

2.3 Antioxidants: phenols and flavonoids

Antioxidant compounds are compounds that are widely used to counteract oxidative stress-free radicals in cells. These antioxidant compounds are derived from natural and chemical sources. Sources derived from natural ingredients are used because they are much safer to use due to less toxicity and side effects. Plant constituents with antioxidant activity can protect against oxidative stress in biological systems. Other natural antioxidant compounds of plants besides vitamins are phenolic or polyphenolic compounds, which can be flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids (Kurutas, 2016). The flavonoid group with antioxidant activity includes flavones, flavonols, isoflavones, catechins, and chalcones. Meanwhile, cinnamic acid derivatives include caffeic acid, ferulic acid, chlorogenic acid, gallic acid, and others (Grgić et al., 2020).

Phenolic compounds are one of the largest groups of natural compounds and have various biological activities with medicinal potential (e.g., antioxidant and anti-inflammatory), with one aromatic ring (simple phenols) and two or more aromatic rings (polyphenols) with hydroxyl and carboxylic groups (Grgić et al., 2020; Hussain et al., 2019). One group of compounds, including phenolic compounds, is flavonoids. Flavonoid compounds have anti-inflammatory activity by regulating oxidant production and modulation of redox-sensitive signaling pathways, thus inducing immune cell activation. The concentration and form of flavonoid compounds determine the rate of free radical scavenging reactions as antioxidants and their impact on biological systems. Flavonoids are extensively metabolized before reaching the organs where these compounds can exert their bioactivity (Grgić et al., 2020). The bioavailability of phenolic compounds is affected by structural changes and their absorption into the blood (Hussain et al., 2019; Lewandowska et al., 2013).

3. Methodology

3.1 Preparation of Fruit Juice

Fresh ripe fruits of *B. macrophylla* were collected in November 2021 from the wild plant's growth from Paringin Selatan, Balangan, Kalimantan Selatan, Indonesia. The plants and the fruits were identified at Herbarium Bogoriense, Botany, Biology Research – Badan Riset dan Inovasi Nasional (BRIN), Bogor, Indonesia. *B. macrophylla* fruit was washed with water, then processed with a slow-juicer. *B. macrophylla* fruit juice (BMFJ) was collected and immediately frozen, and stored in a freezer. The frozen juice was

freeze-dried at 47◦C, continued running for 174 hours, then stored in a refrigerator until used in the experiment (**Error! Reference source not found.**). During the experimental period, various doses were freshly prepared with 0.5% Na-CMC suspension.

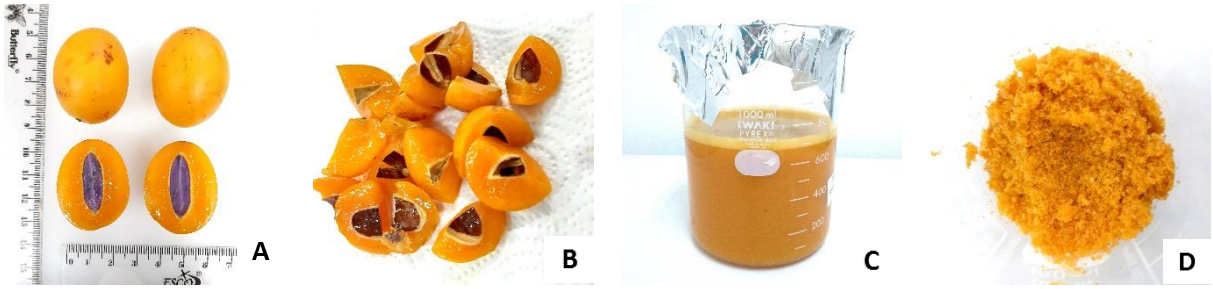

Figure 1. The process of making fresh ripe Bouea macrophylla fruit is processed to obtain fresh juice and freeze-dried results; (a) cross-section of the fruit; (b) seedless fruit; (c) Bouea macrophylla fruit juice (BMFJ); (d) freeze-dried

3.2 Determination of Total Phenolic Content

The total phenolic content of the ethanol extract was assessed using the Folin-Ciocalteu method with slight modifications (Aryal et al., 2019; Nayeem et al., 2022). The number of total phenols was determined based on mg of gallic acid equivalent per gram of sample (mg GAE/g). The concentration range of the standard solution using 0, 10, 30, 50, 70, and 100 ppm. The absorbance was measured at a wavelength of 730 nm.

3.3 Determination of Total Flavonoid Content

The total flavonoid content was determined using the $AlCl_3$ colorimetric method using quercetin standards (Nayeem et al., 2022; Wairata et al., 2022). The total content of flavonoids was measured as mg of quercetin equivalents per 100 grams of sample (mg QE/100 g) using a quercetin calibration curve. The absorption was measured at 434.2 nm using a spectrophotometer.

3.4 DPPH Radical Scavenging Activity Assay

The DPPH assay (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of fruit extracts was determined by Jiratchayamaethasakul et al. (2020) and Sukweenadhi et al. (2020). Reaction mixtures contained 100 µL BMFJ extract and 100 µL DPPH in ethanol. Ascorbic acid was used as a standard. Mixtures were vortexed thoroughly and kept in the dark at room temperature for 30 min. After 30 min of incubation, the absorbances were measured at 517 nm. Antioxidant activity is represented in IC_{50}, which is the concentration of extract that scavenge free radicals by 50%.

3.5 Preparation of High-Fat High-Cholesterol Diet

The high-fat high-cholesterol diet (HFHCD) was prepared by adding 2.5% of cholesterol and 15% of duck egg yolk powder to a standard diet. The duck egg yolk powder was prepared by boiling the eggs for 30 min, and the boiled yolk was mashed and dried at 40-45˚C for 12 h. The composition of the diets (proximate and cholesterol analysis) is shown in **Table 1**.

Table 1. Composition of the diets

Parameter	Standard diet (%)	HFHC[1] diet (%)
Dry matter	90.42	91.45
Ash	5.06	5.29
Crude protein	21.03	24.66
Crude fiber	8.41	0.84
Crude lipid	6.89	17.58
Nitrogen free extract	49.03	43.08
Cholesterol	0.00147	2.5026

[1] HFHC = High-fat high-cholesterol

3.6 Animal and Experimental Design

Thirty Sprague-Dawley rats (6 weeks old, 200 ± 30 g body weight) were purchased from the Indonesian Rat Company (iRATco) and were acclimatized for two weeks with free access to water and standard rodent chow under a 12-hour light-dark cycle. After acclimatization, the rats were randomly segregated into six groups of five, each listed in **Figure 2**. The experiment was conducted for 100 days, and at the end of the experiment, all rats were euthanatized. All experimental procedures were conducted following the animal care guidelines and were approved by The Medical and Health Research Ethics Committee Muhammadiyah University of Prof. Dr. Hamka (Ethical number: 02/22.02/01531).

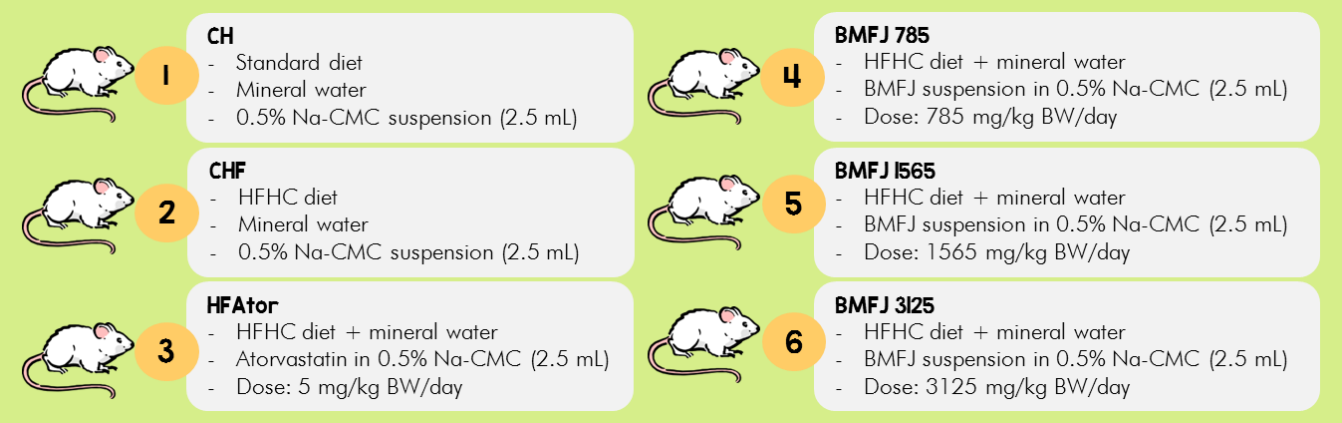

Figure 2. Experimental animal groups, treatment, and dosage.
HFHC: High-Fat High-Cholesterol; BMFJ: *Bouea macrophylla* fruit juice

3.7 Serum and Liver Collection and Processing

On the day of euthanasia, the rats were anesthetized using ketamine hydrochloride (80 mg/kg BW) and xylazine (8 mg/kg BW) after being kept in a fast state for 12 hours, and the blood was collected via intraperitoneal puncture followed by centrifugation to obtain serum (Moorthy et al., 2022). The serum obtained was stored at −20 ◦C, and the liver was isolated for malondialdehyde (MDA) analysis as an indicator of lipid peroxidation.

3.8 Determination of Serum and Hepatic Malondialdehyde (MDA) Concentrations

By the colorimetric method, MDA concentrations were determined using the Elabscience kit (E-BC-K025-S) (Farhood, 2019). Therefore, 100 µL of serum or liver homogenate was added to 100 µL clarificant reagent, 3 mL acid reagent, and 1 mL chromogenic reagent. The mixture was incubated in a water bath for 40 min at 95-100 ◦C, cooled to room temperature, and centrifuged at 3100 rpm for 10 minutes. Subsequently, 3 mL of the supernatant was taken, and the absorbance was read in a spectrophotometer at the wavelength of 532. An analytical calibration curve was prepared using MDA as standard. The results were expressed as nmol of MDA per mL of serum or gram of liver homogenate (nmol/g liver).

3.9 Statistical Analysis

Results are presented as the mean ± SE of the mean. Statistical analyses were performed using IBM SPSS version 16. Group difference was assessed by a one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. A P value < 0.05 was considered statistically significant.

4. Results and Discussion

4.1 Total Phenolic and Flavonoid Content

Quantitative phytochemical analysis revealed a high total phenolic content of *B. macrophylla* fruit juice, 570 mg GAE/g, and flavonoid content of 31.89 mg QE/g. The analysis showed that the fruit juice of *B. macrophylla* contains a large amount of phenolic and flavonoid compounds, which confirms its antioxidant properties. Therefore, according to the results, *B. macrophylla* contains active compounds that have potential as medical drugs.

These results indicate that the fruit juice of *B. macrophylla* contains many phenolic compounds, which are generally associated with its medicinal potential. The levels of phenolic compounds in this *B. macrophylla* fruit juice are high when compared to some variants of tangerine juice (Citrus reticulata) of 208 – 425 mg GAE/L, variants of sweet orange juice (Citrus sinensis) of 78 – 661 mg GAE/L, and cranberry juice (Vaccinium macrocarpon) of 227 – 391 mg GAE/L (Fidelis et al., 2017). Felhi et al. (2016) revealed that the freeze-dried extract of Ecballium elaterium fruit juice had a total phenol content of 78.7 – 106.4 mg GAE/g. Phenolic compounds are one of the largest groups of natural compounds. They have a wide range of biological activities (e.g., antioxidant and anti-inflammatory), with one aromatic ring (simple phenols) and two or more aromatic rings (polyphenols) with hydroxyl and carboxylic groups (Grgić et al., 2020; Hussain et al., 2019).

One group of compounds, including phenolic compounds, is flavonoids. Flavonoids are plants' most abundant group of polyphenolic compounds (Grgić et al., 2020; Hussain et al., 2019). However, from the results of this study, it appears that the flavonoid compounds in the fruit juice of *B. macrophylla* are only about 5.5% of the total phenolic compounds present. However,

the levels of flavonoid compounds in *B. macrophylla* fruit juice are pretty high compared to other fruit juices. Zeghad et al. (2019) examine four freeze-dried edible fruits from Algeria. Grapes (Vitis vinifera) showed a total flavonoid content of 14.37 mg QE/g, pomegranate (Punica granatum) had a concentration of 12.95 mg QE/g, and oranges (Citrus aurantium) had a content of 7.27 mg QE/g. Mahzir et al. (2018) investigated the crown of the god fruit (Phaleria macrocarpa), which had a total flavonoid content of 3.22 mg QE/g. Noni fruit (Morinda citrifolia) with 2.4 mg QE/g (Meilawati et al., 2021). Based on the results of these studies, it appears that the total flavonoid content in various types of fruits is lower than that of *B. macrophylla* fruit juice. Freeze-dried results of several variants of Ecballium elaterium fruit juice with total flavonoid content of 0.6 – 6.5 mg QE/g (Felhi et al., 2016); grapes (Vitis vinifera) 1.3 mg QE/100g(da Silva et al., 2020); starfruit (Averrhoa bilimbi) of 0.29 µg QE/mg; and apple (Malus domestica) 2.06 µg QE/mg (Utami et al., 2019).

Flavonoid compounds have anti-inflammatory activity by regulating oxidant production and modulation of redox-sensitive signaling pathways, thereby inducing immune cell activation. The concentration and form of flavonoid compounds determine the rate of free radical scavenging reactions as antioxidants and their impact on biological systems. Flavonoids are extensively metabolized before reaching the organs where they can exert their bioactivity (Grgić et al., 2020). The bioavailability of phenolic compounds is affected by structural changes and their absorption into the blood (Hussain et al., 2019; Lewandowska et al., 2013).

4.2 Antioxidant Properties (in vitro)

The antioxidant activity of *B. macrophylla* fruit juice was carried out by the DPPH method, using ascorbic acid as the standard. The percentage of inhibition of *B. macrophylla* fruit juice and ascorbic acid at various concentrations is shown in **Figure 3**. Based on the percentage of inhibition, a regression curve was made, and the IC_{50} value was determined (**Table 2**).

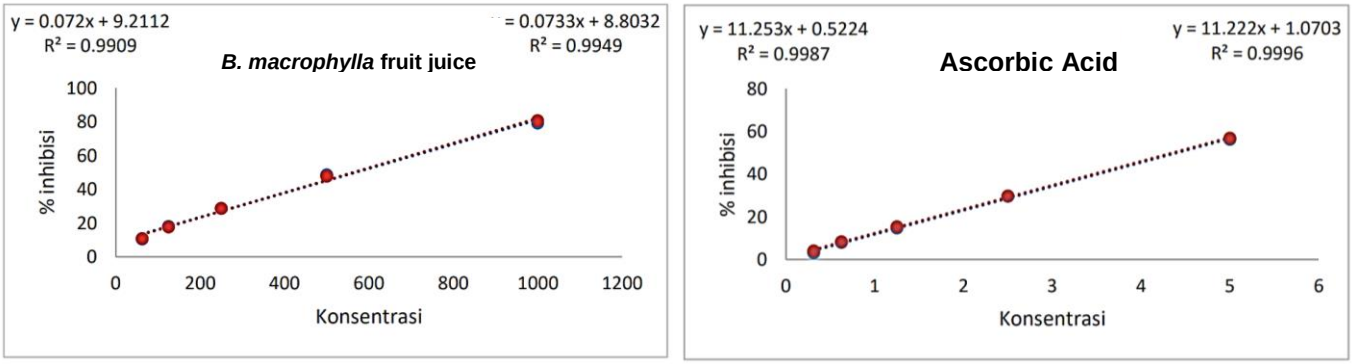

Figure 3. The antioxidant potential is determined by the DPPH radical-scavenging capacity of the BMFJ and the standard; ascorbic acid

Table 2. IC_{50} values of BMFJ and ascorbic acid interpolated from the curve of DPPH radical scavenging ability

Parameter	IC_{50} (ppm)	Comparison to ascorbat acid	Categories
BMFJ	564.271	1/29	Very weak
Ascorbat acid	4.379	-	-

One of the properties of natural ingredients often associated with their medicinal potential is their antioxidant activity. Prevention and cure of degenerative diseases are closely related to antioxidant power; the higher the antioxidant activity, the greater the medicinal potential. The antioxidant activity of *B. macrophylla* fruit juice in this study was analyzed using the DPPH method. This method states the ability of a material to reduce free radicals formed by DPPH (Aykul & Martinez-Hackert, 2016).

From the results of this study, it appears that the fruit juice of *B. macrophylla* has a weak antioxidant power, with an IC_{50} of 564.271 ppm, or a ratio of 1:29 when compared to the standard ascorbic acid (**Table 2)**. *B. macrophylla* fruit juice is made only by squeezing or juicing the flesh of the *B. macrophylla* fruit without extracting it. Other compounds contained in *B. macrophylla* fruit juice that are not antioxidants, such as fiber, cause the concentration of antioxidant compounds in *B. macrophylla* fruit juice to be minor, so the IC_{50} value is considerable, and the antioxidant activity of this fruit juice overall categorized as very weak. However, *B. macrophylla* fruit juice still has medicinal potential due to its high fiber and phenolic content.

4.3 Effect of BMFJ Supplementation on Serum and Hepatic Malondialdehyde (MDA) Concentrations

The high-fat high-cholesterol diet given to male Sprague-Dawley rats for 100 days significantly increased the serum malondialdehyde (MDA) levels, while the effect on the hepatic MDA levels is not significant. BMFJ supplementation, especially the higher dose, significantly prevents the increase of MDA levels in serum caused by the high-fat high-cholesterol diet (**Supplementation** *B. macrophylla* fruit juice has been shown to inhibit the increase in oxidative stress and restore tissue antioxidants in mice fed a high-fat diet. The results showed that significantly, *B. macrophylla* fruit juice with a dose of 1565 mg/kg BW in serum ($p<0.05$) could reduce MDA levels in the serum. Meanwhile, fruit juice at a dose of 3125 mg/kg BW reduced MDA levels better than other doses in the liver. The difference between the two, presumably because the bioactive compounds of *B. macrophylla* fruit juice are distributed more in the serum and to reach the liver, requires a higher dose.

Table 3. Effect of BMFJ supplementation on serum and hepatic MDA

Group	Averages of MDA (nmol/mL)	
	Serum	Hepatic
CH	12,2 ± 4,1^{a}	60,9 ± 15,9^{ab}
CHF	141,0 ± 12,4^{c}	61,4 ± 15,2^{ab}
HFAtor	53,8 ± 7,4^{b}	55,0 ± 3,6^{a}
BMFJ 785	135,4 ± 20,1^{c}	80,8 ± 13,8^{b}
BMFJ 1565	22,2 ± 12,6^{a}	71,0 ± 9,9^{ab}
BMFJ 3125	24,4 ± 11,7^{a}	60,9 ± 15,9^{ab}

, **Error! Reference source not found.**). Supplementation *B. macrophylla* fruit juice has been shown to inhibit the increase in oxidative stress and restore tissue antioxidants in mice fed a high-fat diet. The results showed that significantly, *B. macrophylla* fruit juice with a dose of 1565 mg/kg BW in serum ($p<0.05$) could reduce MDA levels in the serum. Meanwhile, fruit juice at a dose of 3125 mg/kg BW reduced MDA levels better than other doses in the liver. The difference between the two, presumably because the bioactive compounds of *B. macrophylla* fruit juice are distributed more in the serum and to reach the liver, requires a higher dose.

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BMFJ 785	135,4 ± 20,1^{c}	80,8 ± 13,8^{b}
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BMFJ 3125	24,4 ± 11,7^{a}	60,9 ± 15,9^{ab}

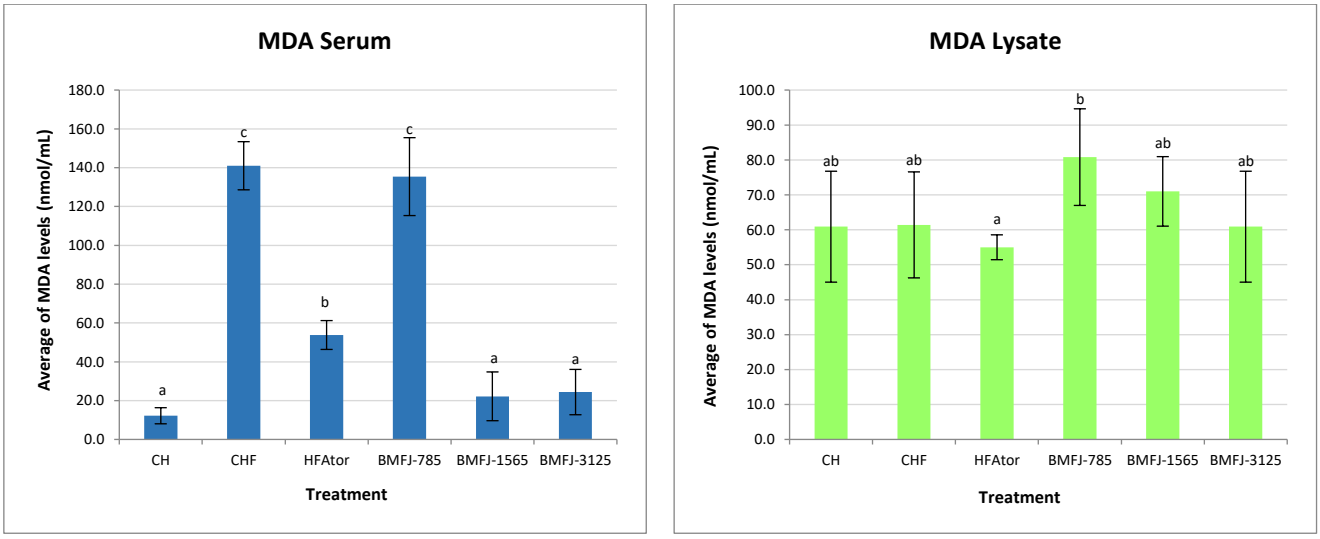

Figure 4. Effect of BMFJ supplementation on serum and hepatic MDA concentrations. MDA concentration serum and hepatic level of all experimental groups. Values were expressed as mean ± SD (standard deviation), n = 5. CH: Healthy control rats, fed on a standard diet; CHF: Untreated control rats, fed with HFHC diet and given 0.5% Na-CMC suspension 2.5 mL/rat; HFAtor: Rats fed with HFHC diet and treated with 5 mg/kg BW atorvastatin; BMFJ 785, BMFJ 1565, and BMFJ 3125: Rats fed with HFHC diet and treated with 0.785, 1.565, and 3.125 g/kg BW/day BMFJ respectively.

Reactive oxygen species (ROS) are endogenous free radicals produced by aerobic organisms through various metabolic reactions. The formation of ROS mainly occurs in oxidative reactions that occur in the mitochondria. ROS can react with various biomolecules and cause disruption of cell function to tissue damage, which occurs in the inflammatory process and degenerative diseases (Ma et al., 2022; Ore & Akinloye, 2019).

Lipids are one of the molecules that are vulnerable and sensitive to free radical oxidation (Phaniendra et al., 2015). The reaction between lipids and free radicals produces lipid peroxide. Lipid peroxidation can occur due to a free radical attack on polyunsaturated fatty acids (PUFA) in cell membranes that contain at least two double bonds. Oxidized lipids are labile and highly reactive, so they tend to react with substances around them. This reaction will cause damage to cellular components and functions and can initiate disorders and diseases (Ayala et al., 2014). The other product of the degradation of the oxidized lipid molecule causes the formation of several specific metabolites, such as malondialdehyde (MDA). MDA is the end product of lipid peroxidase in human and animal tissues. It is also a by-product of the biosynthesis of prostaglandins and thromboxane. Although MDA-modified proteins are mainly located in the mitochondria, MDA is also found in other locations, such as the nucleus, cytosol, and cell membranes, as well as extracellular compartments, such as plasma (Masselli et al., 2020).

Feeding high-fat high-cholesterol (HFHCD) to Sprague-Dawley rats for 100 days in this study resulted in increased levels of malondialdehyde (MDA) in serum and liver. The HFHC diets used contained 17.58% crude fat and 2.5% cholesterol, while the standard diet contained 6.89% crude fat and 0.0014% cholesterol. High-fat foods contribute to fat accumulation, which induces an inflammatory state. Excess fat consumption leads to the expansion of adipose tissue, dysfunction, and metabolic inflammation (O'Brien et al., 2017; Tan & Norhaizan, 2019). As shown in this study, after being fed with HFHC diets and *B. macrophylla* fruit juice supplementation for 100 days, there was an increase in lipid peroxidation via MDA in serum or hepatic, thereby inducing the formation of oxidative stress.

B. macrophylla fruit has been known to contain various groups of active compounds such as phenolic compounds, flavonoids, flavonols, anthocyanins, and ascorbic acid, which act as antioxidants and have potential medicinal effects (Rajan & Bhat, 2016). Flavonoid compounds from the phenol group have been shown to potentially treat diseases associated with high levels of free radicals (Abdo et al., 2022; Bobadoye et al., 2016; Santoso et al., 2018).

Flavonoid compounds exert anti-inflammatory action in experimental animals by regulating oxidant production and modulation of redox-sensitive signaling pathways, thereby inducing immune cell activation (Oteiza et al., 2021). *B. macrophylla* fruit juice contains 570 mg GAE/g total phenol and 31.89 mg QE/g total flavonoid compounds. Flavonoid metabolism as an antioxidant mechanism depends on high concentrations of flavonoids. Briefly, after fruit juice is consumed, it is metabolized into structural metabolites and then transported into plasma, which is metabolized in the liver. However, if the concentration of flavonoids is low, then flavonoid metabolism is only found in plasma and does not reach the liver (Oteiza et al., 2021). This can be seen in the MDA

levels of the research results; *B. macrophylla* supplementation at a dose of 1565 mg/kg BW can significantly reduce serum MDA levels, while a dose of 3125 mg/kg BW can reduce hepatic MDA levels.

5. Conclusion

Bouea macrophylla (BMFJ) fruit juice contains considerable amounts of phenolic and flavonoids phytochemicals, with total phenols and flavonoids content of 570 mg GAE/g and 31.89 mg QE/g respectively, and the IC_{50} of radical scavenging activity was 564.271 ppm. *Bouea macrophylla* fruit juice supplementation inhibited the oxidative stress in rats fed with high-fat high-cholesterol diets through attenuation of lipid peroxidation, showed by the prevention of MDA levels increasing in rats' serum.

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