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Research Article

Comparison of Maceration and Soxhletation Methods on the Antioxidant Activity of the *Bouea macrophylla* Griff Plant.

Tarso Rudiana[1], Siti Nurbayti[2*], Tiara Hadi Ashari[2], Sherina Arestia Zhorif[2], Nani Suryani[1]

[1]*Department of Chemistry, Faculty of Science Pharmacy and Health, Universitas Mathla'ul Anwar, Pandeglang 42273, Indonesia*

[2]*Department of Chemistry, Faculty of Science and Technology, Syarif Hidayatullah Islamic State University, South Tangerang 15412, Indonesia)*

Email: baytikimia@uinjkt.ac.id

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Abstract

The impact of extraction methods on the potency of plant extracts is crucial. *Bouea macrophylla* Griff., a tropical fruit plant rich in phenolic and flavonoid compounds, exhibits strong antioxidant activity. This study aimed to evaluate the effect of maceration and soxhletation extraction techniques on *B. macrophylla* plants on antioxidant activity in vitro using the method of inhibiting DPPH radical compounds. Ethyl acetate and ethanol solvents were utilized to macerate and soxhlet all parts of the plant. Subsequently, each extract was subjected to quantitative analysis for antioxidant activity, total phenolic, and flavonoid content through UV-Vis spectrophotometry. Quantitative analysis revealed substantial antioxidant activity and notable levels of total phenolic and flavonoid content across all plant parts. The result indicated that although both maceration and soxhletation showed a significant impact on yield (p=0.009) and total flavonoids (p=0.038), significant differences in antioxidant activity (p=0.409) or total phenolics (p=0.874) within the plant extracts of *B. macrophylla* were not observed at a significance level of 5%.

Keywords: Antioxidant, *Bouea macrophylla*, DPPH, maceration, soxhlet extraction.

1. INTRODUCTION

Antioxidants play a vital role in inhibiting free radical oxidation within cell membranes by breaking the chain reaction through electron donation in oxidation-reduction reactions. Antioxidants stabilize free radical compounds in cell membranes through three mechanisms, namely hydrogen atom transfer, electron transfer, and metal chelation. Phenolic compounds and flavonoids are widely used as sources of antioxidants [1]. Antioxidant properties of phenolic and flavonoid compounds, namely by transferring hydrogen atoms to free radicals [2].

Plants are a source of phenolic and flavonoid compounds which act as antioxidants. Extraction is the first and important step to obtain antioxidant compounds from plants. Conventional extraction methods of maceration and soxhletation are widely used because the equipment is simple. Apart from the extraction method, solvent selection is an important factor in obtaining antioxidant compounds. Phenolics and flavonoids dissolve in polar and semipolar solvents. Differences in extraction methods and the use of solvents in extracting plant tissue can have an influence on the antioxidant activity of the resulting plant tissue extract, where it is important to choose the appropriate extraction method [3].

The Anacardiaceae family, prevalent in Indonesia, encompasses various fruit-bearing plants like Gandaria (*Bouea macrophylla* Griff), kedondong (*Spondias dulcis*), Dahu (*Dracontomelon dao*), cashew (*Anacardium occidentale*) and mango (*Mangifera indica*) cherished for their culinary and medicinal uses. Among them, *B. macrophylla* stands out due to its exceptional antioxidant potency derived from its high phenolic content [4–8]. *B. macrophylla* plant is one of the plants that is used as herbal medicine because every part of the plant has high antioxidant

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activity derived from phenolic compounds [4,9]. *B. macrophylla* grows in hot and humid tropical regions within lowland forests [10]. *B. macrophylla* yields significant antioxidant extracts from its stems and seeds, displaying varying IC_{50} values. Notably, the ethyl acetate and ethanol extracts from *B. macrophylla* stems exhibit IC_{50} values of 4.89 µg/mL and 4.95 µg/mL, respectively, while the ethyl acetate extract from its seeds reveals an IC_{50} value of 2.95 µg/mL.

Apart from the extraction method, selecting plant parts as a source of antioxidants is also the most important thing to obtain the best antioxidant compounds. Therefore, this study evaluated the extraction and antioxidant activity tests on all parts of the *B. macrophylla* plant to study the effect of different extraction methods on *in vitro* antioxidant activity, total phenolic content, and total flavonoid content.

2. RESEARCH METHODS

Materials and Tools

Laboratory glassware, macerator, soxhletizer, vacuum rotary evaporator, UV-Vis Spectrophotometer (Genesys 10s). Samples of *B. macrophylla* plants which include all parts of the plant include roots (BMR), stems (BMST), bark (BMB), leaves (BML), fruit (BMF), fruit skin (BMFS), and *B. macrophylla* seeds (BMSD) collected from Pandeglang Regency-Indonesia, re-distilled grade organic solvents (ethyl acetate, acetone and ethanol), methanol p.a (Merck), distilled water, aluminum chloride (Merck), sodium acetate (Merck), 2,2'-diphenyl picrylhydrazyl (Himedia), quercetin (Sigma Aldrich), gallic acid (Sigma Aldrich), and Folin-Ciocalteu reagent (Merck).

Plant Materials

Each part of the B. macrophylla plant including roots (BMR), stems (BMST), bark (BMB), leaves (BML), fruit (BMF), fruit skin (BMFS), and seeds (BMSD) was cleaned using water. Each sample was dried, chopped and ground using a grinder or blender until it became powder.

Maceration

The sample maceration process refers to research by Hilma et al. (2020), with modifications to the solvent used. 100 g of each ground sample was macerated with ethyl acetate (-EA) in a dark vessel until the entire sample was submerged. The maceration process was carried out for 3x24 hours. The solvent was changed every 24 hours. The ethyl acetate extract was filtered and evaporated to obtain a concentrated extract using a rotary evaporator at 40 °C. Maceration in the same way for each sample was carried out using 96% ethanol solvent (-E). The percentage yield of the concentrated extract obtained was calculated.

Soxhletation

Soxhletation was carried out using the Puspitasari and Proyogo (2016) method, 10 g of each sample was soxhleted with 300 mL of solvent. The soxhletation process was carried out at 77 °C for ethyl acetate (-EA) and 78 °C for ethanol (-E). The soxhletation process was stopped when the solvent droplets were colorless or for 6 hours. Each extract was concentrated using a vacuum rotary evaporator at a temperature of 40 °C and the percentage yield was calculated.

Analysis of Antioxidant Activity

Antioxidant activity was analyzed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [14] with some modifications. Quercetin was used as a positive control. Initially, a 10 mg sample extract was dissolved in 10 mL of methanol, resulting in an extract solution with a concentration of 1000 ppm. Subsequently, this extract solution was prepared across multiple concentration series. Both the positive control solution and the sample solution were then combined with 2 mL of 0.002% DPPH. The mixture was homogenized and incubated for 20 minutes in the dark and at room temperature. The test solution was analyzed with a UV-Vis spectrophotometer. The absorbance obtained was used to determine the percent inhibition and IC_{50} value of each extract.

Total Phenolic Analysis

The quantification of total phenolics followed the Folin-Ciocalteu method, with gallic acid used as the standard [15]. 25 mg of the extract was dissolved in 25 mL of distilled water to achieve a concentration of 1000 ppm. From this solution, 0.5 mL was pipetted and further diluted with 1 mL of distilled water. Extract (0.5 mL) was pipetted and 0.3 mL of Folin Ciocalteu reagent was added. The mixture was reacted with 2 mL of 15% Na_2CO_3 to increase the pH of the test solution to make it alkaline. Subsequently, 2.2 mL of distilled water was introduced, and the mixture was incubated for 120 minutes at room temperature. Absorbance was measured at a wavelength of 753 nm with a UV-Vis spectrophotometer.

Total Flavonoid Analysis

A sample of 50 mg was dissolved in 50 mL of methanol (1000 ppm). The sample solution was taken 1 mL, diluted with 2 mL of distilled water. 1

mL of the diluted extract solution was pipetted then 0.5 mL of 10% $AlCl_3$ reagent, 0.5 mL of 1 M CH_3COONa and 0.5 mL of distilled water were added. The mixture was incubated for 30 minutes. The absorbance value of the test solution was measured at a wavelength of 432 nm using a UV-Vis spectrophotometer. The quercetin solution was used as a standard solution.

Statistical Analysis

The data obtained were analyzed statistically to determine the influence of the extraction method and two types of solvent on the percentage yield, antioxidant activity, total phenolics and total flavonoids from *B. macrophylla* plant extracts. The Kruskal Wallis method was used for data on percentage yield and total phenolics, while Two-Way Analysis of Variance was used to examine data on antioxidant activity and total flavonoids. The Least Significant Different (LSD) test was used on flavonoid data to determine significantly different flavonoid values in the samples. The p value <0.05 indicates there is a significant difference between treatments.

The obtained data were statistically analyzed to determine the influence of extraction methods and two types of solvents on the percentage yield, antioxidant activity, total phenolic, and total flavonoid content of *B. macrophylla* plant extracts. Kruskal-Wallis method was used for percentage yield and total phenolic data, while Two-Way Analysis of Variance was employed to assess antioxidant activity and total flavonoid data. Least Significant Different (LSD) analysis was used for flavonoid data to determine significantly different flavonoid values among the samples. A value of $p<0.05$ indicates a significant difference between treatments.

3. RESULTS AND DISCUSSION

Yield analysis

The roots, bark, stems, seeds, fruit, rind and leaves of B. macrophylla plants were extracted using the maceration and soxhletation method with ethanol (-E) and ethyl acetate (-EA) solvents. The soxhletation method was carried out on each sample in 6-7 cycles for 6 hours and maceration extraction for 3 days with solvent changes every 24 hours. Each extract from both methods was concentrated until a concentrated extract in the form of a paste was obtained. The yield obtained from the concentrated extract of each sample is shown in Table 1.

The soxhletation method was the best method for obtaining extract yields based on the mean rank value in the non-parametric Kruskal-Wallis test (Table 1). Based on this test, the extraction method had a significant influence on the overall yield of the extract in the sample with a significance value of $0.009<0.05$ ($p<\alpha$). The results shown in Table 1, the soxhletation method with ethanol solvent provided large extract yields for the bark, roots and leaves of B. macrophylla. The soxhletation method with ethyl acetate solvent provides large extract yields for the roots, stems and skin fruit of B. macrophylla, while for the fruit, the maceration method with ethanol solvent.

Table 1. % yield of *B. macrophylla* plant extract

Extract	Maceration			Soxhlet		
	Sample Weight (g)	Extract Weight (g)	Yields (% (w/w))	Sample Weight (g)	Extract Weight (g)	Yield (% (w/w))
BMR-E	100.00	9.70	9.70	10.00	1.00	10.00
BMR-EA	100.00	3.40	3.40	10.00	2.20	22.00
BMB-E	100.00	15.60	15.60	10.00	3.00	30.00
BMB-EA	100.00	1.80	1.80	10.00	1.10	11.00
BMST-E	100.00	3.40	3.40	10.00	1.20	11.88
BMST-EA	100.00	0.70	0.70	10.00	2.00	19.80
BMSD-E	50.00	11.30	22.60	10.50	2.40	22.86
BMSD-EA	50.00	9.30	18.60	10.60	2.10	19.81
BMF-E	100.00	12.10	12.10	20.80	2.00	9.62
BMF-EA	100.35	2.60	2.59	21.00	2.20	10.48
BMFS-E	40.91	9.50	23.22	10.07	2.10	20.85
BMFS-EA	40.03	4.60	11.50	10.04	1.40	30.43
BML-E	100.10	19.80	19.78	10.03	4.40	43.87
BML-EA	100.00	5.40	5.40	10.00	4.00	40.00
Mean Rank		**21.7**			**35.93**	
Asymp. Sig			**0.009**			

Table 2. Antioxidant activity of B. macrophylla extracts

Extract		IC_{50} (µg/mL)	Antioxidant Activity Category
Standard	Quercetin	3.03 ± 0.03	Very strong
Maceration	BMR-E	24.78 ± 0.23	Very strong
	BMR-EA	14.43 ± 0.09	Very strong
	BMB-E	5.83 ± 0.01	Very strong
	BMB-EA	17.07 ± 0.12	Very strong
	BMST-E	22.16 ± 0.90	Very strong
	BMST-EA	56.83 ± 0.52	Strong
	BMSD-E	4.02 ± 0.07	Very strong
	BMSD-EA	4.23 ± 0.03	Very strong
	BMF-E	289.95 ± 5.84	Not active
	BMF-EA	50.00 ± 0.00	Very strong
	BMFS-E	222.53 ± 10.88	Not active
	BMFS-EA	11.34 ± 0.71	Very strong
	BML-E	11.81 ± 0.04	Very strong
	BML-EA	20.36 ± 0.44	Very strong
Soxhlet Extraction	BMR-E	71.18 ± 1.50	Strong
	BMR-EA	162.10 ± 2.52	Weak
	BMB-E	7.37 ± 0.01	Very strong
	BMB-EA	12.01 ± 0.01	Very strong
	BMST-E	33.01 ± 0.12	Very strong
	BMST-EA	27.59 ± 0.71	Very strong
	BMSD-E	5.59 ± 0.05	Very strong
	BMSD-EA	4.03 ± 0.21	Very strong
	BMF-E	44.63 ± 0.33	Very strong
	BMF-EA	63.69 ± 1.07	Strong
	BMFS-E	7.37 ± 0.01	Very Strong
	BMFS-EA	12.01 ± 0.01	Very strong
	BML-E	12.73 ± 0.18	Very strong
	BML-EA	19.66 ± 0.17	Very strong

The extraction process carried out on plant tissue is expected to open the matrix bonds to obtain target compounds. Extraction efficiency for plant tissue samples depends on the nature of the sample matrix, the analyte to be extracted, and the location of the analyte in the matrix. [16]. According to Golmakani and Moayyedi (2015), The yield of extract obtained in plant tissue extraction can also be influenced by the extraction time and heating treatment during extraction. Temperature is the main influence in increasing the solubility of target compounds in plant tissue and solvents. The kinetic energy provided in the soxhletation method causes more and more interparticle collisions of the target compound with the solvent to create new intermolecular interactions. Agitation treatment that is not too frequent in the maceration method is not optimal enough to increase the solubility between the target compound and the solvent and break the intermolecular interactions of the target compound with the compounds that make up plant tissue.Sahirman et al. (2008) states, the speed of stirring or agitation is related to the energy required for the collision process so that the reaction can take place perfectly.

Obtaining a relatively greater extract yield in ethanol solvent compared to ethyl acetate indicates that B. macrophylla plants contain many polar compounds, such as phenolics, flavonoids and saponins which have –OH groups in their compounds [19]. Hydrogen bonds occur between the -OH group of polar secondary metabolite compounds and ethanol. Ethyl acetate solvent can extract semipolar and non-polar compounds, such as several triterpenoid compounds, tannins, coumarins, sterols and polymethoxy aglycone flavonoids [20]. Semipolar solvents or those with lower polarity can cause cell walls in plants containing semipolar compounds, such as sterols, to be easily degraded, so that bioactive compounds will easily come out (like dissolve like) [4,21].

Antioxidant Activity

The antioxidant activity of each B. macrophylla plant extract that was obtained was tested using the DPPH Free Radical Scavenging

method. DPPH free radicals will be reduced or receive hydrogen atom donors from antioxidant compounds, which will then produce the reduced form, namely DPPH-H with the purple color losing to faded yellow. Tests using the DPPH method can obtain antioxidant activity as an IC_{50} value which is defined as the substrate concentration that causes a loss of 50% of DPPH activity [22].

The antioxidant activity measured in each sample extract (Table 2) was compared with the quercetin compound as a standard. Quercetin was chosen as the standard because quercetin is a flavonoid aglycone from the flavonol subclass which has very strong antioxidant activity and is widely synthesized in all plant tissues [23]. The IC_{50} values that have been obtained are processed statistically to see the effect of the extraction method on antioxidant activity on the sample extract as a whole. Data on antioxidant activity and % yield were subjected to two-way analysis of variance (ANOVA), where the extraction method did not have a significant effect on the antioxidant activity of B. macrophylla plant extracts at the 5% significance level with a significance value of 0.409>0.05 ($p>\alpha$).

Data on the IC_{50} value of B. macrophylla plant extracts against DPPH shown in Table 2 shows that in the maceration method there are two extracts that are not active as antioxidants, namely fruit ethanol extract (IC_{50} 289.95 ± 5.84 µg/mL) and fruit peel (IC_{50} 222.53 ± 10.88 µg /mL). The soxhletation method using the same solvent in both samples had a greater IC_{50} value so that the antioxidant activity was very strong, namely 44.63 ± 0.33 µg/mL for fruit ethanol extract and 7.37 ± 0.01 µg/mL for fruit peel ethanol extract. The heating process during soxhletation can increase the effectiveness of the solvent in breaking down cell walls in fruit tissue and fruit skin, as well as increasing the solubility of secondary metabolite compounds that have antioxidant activity with the solvent during the extraction process. Strong matrix bonds between the target antioxidant compound and the compounds that make up the fruit tissue and fruit skin can cause the maceration extraction method to be ineffective for extracting antioxidant compounds in the fruit tissue. It is also possible that semipolar antioxidant compounds are found in many fruit tissues and fruit skins in B. macrophylla.

The extract that had the strongest antioxidant activity belonged to seed samples extracted using the maceration method with ethanol solvent. The IC_{50} value for this extract is 4.02 ± 0.07 µg/mL, which has a very slight difference with the quercetin standard which has an IC_{50} value of 3.03 ± 0.03 µg/mL. It can be assumed that in B. macrophylla seeds, many antioxidant compounds come from the phenolic group, especially flavonoids in the flavonol subclass, and the maceration method can be the optimum method for the B. macrophylla seeds in extracting antioxidant compounds. Each part of the B. macrophylla plant has a different optimum extraction method to obtain extracts with the strongest antioxidant activity. The relative soxhletation extraction method produces extracts with very strong and powerful antioxidants in B. macrophylla plants.Murugan & Parimelazhagan (2014) has also previously reported that the soxhletation method produces extracts with the strongest antioxidant activity (IC_{50} 10.6 µg/mL) against the inhibition of DPPH radicals in the methanol extract of the Osbeckia parvifolia Arn plant and can be used as an optimum extraction method compared to the maceration method (IC_{50} 13.6 µg/mL).

Differences in antioxidant activity in plant parts can occur because each part of the plant has a different abundance and type of compounds contained. This can be caused because each plant organ has its own function, where the chemical compounds that will be synthesized in the cells must be in accordance with the needs in carrying out the functional function of the plant organ. Phenolic compounds and flavonoids are the compounds that are thought to play the most important role as antioxidants in B. macrophylla plants. Haminiuk et al. (2012) said phenolic compounds, especially flavonoids, are widely distributed in plants and are known as natural antioxidants. Flavonoid compounds have a real role in plant stress defense such as in protection against damage caused by pathogens, wounds or excess UV light.

Tabel 3. Antioxidant activity categories are based on IC_{50} values

IC_{50} (µg/mL)	**Antioxidant Activity Category**
<50	Very strong
51 – 100	Strong
101 – 150	Moderate
151 – 200	Weak
> 200	Not active

Source: [22]

Total Phenolic and Flavonoid Compound

Phenolics are secondary metabolite compounds found in B. macrophylla and act as free radical scavengers. Phenolics are able to provide hydrogen atoms from their hydroxyl groups to free radical compounds so that their oxidation activity can be reduced. Determination of phenolic content was carried out using the Folin-Ciocalteu method which gives a blue color due to the reduction process of Mo(VI) to Mo(V) by phenolic compounds [26]. Gallic acid is used as a standard solution because it is a simple phenolic compound and a stable compound. The phenolic content data obtained (Table 4) was analyzed using the Kruskal Wallis method, producing a p value of 0.874 (>0.05) which indicates that there is no significant influence between the extraction method and the total phenolics of B. macrophylla plants.

Table 4 shows that the phenolic levels between treatments of each sample did not differ much, but the highest levels were obtained by macerated ethyl acetate seed extract (BMSD-EA), namely 407.808 ± 8,253 mg GAE/g extract among other plant parts. Previous research also stated that B. macrophylla seeds have the highest phenolic content compared to other parts. Some of the phenolic compounds contained in the seed extract are gallic acid, tannic acid, ellagic acid, catechin, caffeine, and several other compounds. [27].

Flavonoids are also included in the group of phenolic compounds which have a role as antioxidants because they are able to provide protons to radical compounds. Flavonoids have an important role not only as antioxidants, but also as pigments in plant cell vacuoles [28]. Flavonoid testing of B. macrophylla extract was carried out using the colorimetric method using aluminum chloride reagent. The reaction between aluminum chloride and flavonoids can produce a yellow complex due to the bond between Al and the hydroxyl group [29]. Quercetin is used as a standard solution because of its high abundance in plants. Based on the results of the Two-way ANOVA test, it is known that the extraction method has a significant influence on the total flavonoid content of B. macrophylla extract ($p<0.05$).

Tabel 4. Total phenolic and flavonoid levels of *B. macrophylla* plant extracts

Sample	**Phenolic (mg GAE/ g extract)**		**Flavonoid (mg EQ/ g extract)***	
	Maceration	**Soxhletation**	**Maceration**	**Soxhletation**
BMR-E	68.748 ± 1.411	97.690 ± 8.468	5.082 ± 0.349^{abdeghiklopv}	3.096 ± 0.348^{abdehiklopuv}
BMR-EA	84.690 ± 2.012	18.233 ± 1.071	11.842 ± 0.279$^{cghjnqrsuwyz\ aa}$	10.954 ± 0.837$^{agnu\ aa}$
BMST-E	216.171 ± 13.738	23.601 ± 1.004	1.609 ± 0.069$^{cfgijmnqrstuwxz\ aa}$	3.413 ± 0.276$^{cgjnqrstuvwxyz\ aa}$
BMST-EA	98.343 ± 1.212	92.616 ± 0.201	30.543 ± 0.628^{abehijlpv}	6.020 ± 0.419^{abehilpvw}
BML-E	250.374 ± 19.188	281.418 ± 1.240	20.864 ± 0.971$^{cegjnqrsuwy\ aa}$	9.124 ± 0.296^{abehilpsv}
BML-EA	97.904 ± 12.674	165.145 ± 5.240	76.903 ± 0.984^{bfi}	4.067 ± 0.075^{abitv}
BMB-E	320.286 ± 6.734	190.674 ± 2.016	8.502 ± 0.348 $^{gknu\ aa}$	5.848 ± 0.487^{abilpvx}
BMB-EA	295.524 ± 7.408	255.657 ± 0.335	10.763 ± 1.460 $^{cgjlnqrsuvwxyz\ aa}$	10.141 ± 1.655^{abehilpvy}
BMF-E	55.575 ± 11.129	87.943 ± 14.193	1.866 ± 0.417 ^{abcehilpv}	1.556 ± 0.138 $^{dgnu\ aa}$
BMF-EA	46.458 ± 8.126	270.244 ± 6.459	1.556 ± 0.138 $^{dgnu\ aa}$	2.505 ± 0.069 ^{abehilprv}
BMFS-E	161.172 ± 0.202	174.269 ± 6.83	31.159 ± 0.086^{bim}	22.176 ± 0.198 ^{abhilopvz}
BMFS-EA	88.709 ± 2.621	30.286 ± 3.37	32.521 ± 0.822 ^{abdehiklnp}	24.799 ± 0.281 $^{abdehiklopv\ aa}$
BMSD-E	299.850 ± 0.688	372.073 ± 1.389	1.174 ± 0.415$^{acgjnqrstuwxyz\ aa}$	3.945 ± 0.139 $^{gou\ aa}$
BMSD-EA	407.808 ± 8.253	367.674 ± 12.083	8.644 ± 0.139 $^{bcfgjmnqrstuwxyz\ aa}$	1.904 ± 0.069 $^{cgjpqrsuwxyz\ aa}$
Asymp. Sig (*p*<0,05)	**0.874**		**0.038**	

*Values with different letters (a-aa) indicate a statistically significant difference ($p<0.05$)

B. macrophylla leaf extract which was macerated using ethyl acetate had the highest flavonoid content (76.903 ± 0.984 mg EQ/g extract) with a value that was very far from the other sample extracts (Table 4). This is in accordance with the opinion of previous research which stated that the maceration method was able to provide a higher flavonoid content than soxhletation [30]. This is because maceration can increase the contact time between sample particles and the solvent. Using the maceration method is also able to protect flavonoid compounds which are susceptible to high temperatures. Ethyl acetate solvent can attract flavonoid compounds because some flavonoids are semipolar compounds [31]. The flavonoid compounds that were successfully detected and isolated from the B. macrophylla plant were quercetin, kaempferol, and apigenin [27], naringenin and luteolin [32], as well as fustin dan garbanzol [33].

4. CONCLUSIONS

The maceration and soxhletation methods in extracting *B. macrophylla* plants had a significant influence on the percentage yield (p=0.009) and total flavonoids (p=0.038), however, did not have a significant influence on antioxidant activity (p=0.409) and total phenolics (p=0.874) *B. macrophylla* plant extract at a significance level of 5%.

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