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Original Article

Total Flavonoid Content and Antioxidant Activity of Different Polarity Extracts from *Pereskia bleo* Leaves

Kandungan Flavonoid Total dan Aktivitas Antioksidan dari Ekstrak Polaritas yang Berbeda dari Daun Pereskia bleo

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Abstract

Antioxidant agents are essential for the body due to its ability to scavenge free radicals. Medicinal plants contain phytochemicals that act as antioxidants. The current research aimed to determine the total flavonoid content (TFC) and antioxidant activity of *Pereskia bleo* leaves extracts from various solvents with different polarities. The nonpolar solvent (n-hexane) was used as the first step of extraction and its residues were then macerated using semi-polar (ethyl acetate) and polar (ethanol 96%) solvents consecutively. The TFC was determined using the colorimetric method while antioxidant activity was examined through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Antioxidant activity was presented as Inhibitory Concentration 50 (IC_{50}) and Antioxidant Activity Index (AAI). It is noticeable that among analyzed extracts, the ethyl acetate extract of *P. bleo* leaves contained the highest flavonoid content (15.052 ± 0.172 g quercetin equivalent/100 g extract). Furthermore, the greatest antioxidant activity was obtained from n-hexane extract with the value of IC_{50} and AAI being 217.307 ppm and 0.230, respectively. Pearson coefficient correlation (r) between TFC and AAI was -0.106. The current study concluded that *P. bleo* leaves extracts using solvents with different polarities showed variation in TFC values and antioxidant activity. Moreover, TFC was not the main contributor to the antioxidant activity of *P. bleo* leaves extracts.

Abstrak

Agen antioksidan sangat penting bagi tubuh karena kemampuannya untuk menangkal radikal bebas. Tanaman obat mengandung fitokimia yang berperan sebagai antioksidan. Penelitian ini bertujuan untuk mengetahui kandungan flavonoid total (TFC) dan aktivitas antioksidan dari ekstrak daun *Pereskia bleo* dari berbagai pelarut dengan kepolaran yang berbeda. Pelarut nonpolar (n-heksana) digunakan sebagai langkah pertama ekstraksi dan residunya kemudian dimaserasi menggunakan pelarut semi polar (etil asetat) dan polar (etanol 96%) secara berurutan. TFC ditentukan dengan menggunakan metode kolorimetri sementara aktivitas antioksidan diperiksa melalui metode 2,2-difenil-1-pikrilhidrazil (DPPH). Aktivitas antioksidan disajikan sebagai Inhibitory Concentration 50 (IC50) dan Indeks Aktivitas Antioksidan (IAA). Terlihat bahwa di antara ekstrak yang dianalisis, ekstrak etil asetat daun *P. bleo* memiliki kandungan flavonoid tertinggi (15,052 ± 0,172 g setara kuersetin/100 g ekstrak). Selanjutnya, aktivitas antioksidan terbesar diperoleh dari ekstrak n-heksana dengan nilai IC50 dan AAI masing-masing sebesar 217,307 ppm dan 0,230. Korelasi koefisien Pearson (r) antara TFC dan AAI adalah -0,106. Penelitian ini menyimpulkan bahwa ekstrak daun *P. bleo* yang menggunakan pelarut dengan kepolaran yang berbeda menunjukkan variasi nilai TFC dan aktivitas antioksidan. Selain itu, TFC bukan merupakan kontributor utama dari aktivitas antioksidan ekstrak daun *P. bleo*.

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INTRODUCTION

Antioxidant agents are essential for the body to prevent the damaging effect of free radicals, such as the development of degenerative diseases.[1] Medicinal plants are one of the antioxidant sources. Phytochemicals in medicinal plants possess an important role to present biological activity including antioxidant activity. Variation of phytochemical content in medicinal plants is due to many factors such as botanical origin, climate, harvest time, and plant genotype.[2,3]

Pereskia bleo or *Jarum Tujuh Bilah* is a medicinal plant from Cactaceae that could be found in tropical and subtropical countries including Malaysia, Singapore, and Indonesia. Leaves are the most utilized part to maintain well-being and treat many diseases traditionally.[1] Literature study revealed that most of the studies using *P. bleo* were originally

from Malaysia. Those studies were using extracts and fractions from many kinds of solvents such as methanol, ethanol, n-hexane, ethyl acetate, and dichloromethane.[1,4–7] Moreover, the concentration of phenolic, flavonoids, and tannin compounds in *P. bleo* has been analyzed in an experiment.[8]

In the current study, the sample of *P. bleo* leaves were originally from Indonesia and used a different extraction method from other existing studies. The dried samples were extracted using the maceration method with different polarity solvents which were n-hexane (non-polar), ethyl acetate (semipolar), and ethanol 96% (polar) consecutively. This method would be classified the phytochemicals based on their polarity. The total flavonoid content (TFC) and antioxidant activity of extracts were analyzed. In addition, the relation between TFC and antioxidant activity was determined using statistical analysis. The results obtained from this study will contribute to improving the understanding of the role of phytochemicals in presenting biological activity.

RESEARCH METHOD

Tools and Materials.

Tools. UV-Vis Spectrophotometer (Shimadzu, UV 1800 Type) and Rotary Evaporator (Buchi, Swiss).

Materials. The fresh leaves of *P. bleo* were collected from Desa Seririt, Buleleng, Bali Province, Indonesia from November 2019 until March 2020. Plant determination was conducted at the Research Centre for Plant Conservation and Bali Botanical Garden, National Research and Innovation Agency (Badan Riset dan Inovasi Nasional). DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, and quercetin (CAS 117-39-5) were bought from Sigma-Aldrich. Other chemical reagents were analytical standards.

Sample preparation

The fresh leaves were sorted, cleaned, and cut into smaller pieces before going through the drying process. The dried samples then were turned into powder and went through 60 Mesh sieves.

Extract preparation

Plant powder was extracted using the maceration method in different types of solvents, which were n-hexane, ethyl acetate, and ethanol 96% consecutively. The solvent was then evaporated using a rotary evaporator to obtain the crude extracts.

Phytochemicals screening of dried samples and extracts

Phytochemical screening was conducted to detect secondary metabolites, which were alkaloid, flavonoid, phenolic, quinone, saponin, and steroid/triterpenoid in the dried sample and extracts using available procedures[9].

Total Flavonoid Content (TFC)

The TFCs of extracts were determined using standard procedures[10]. Quercetin was used as a reference compound to obtain a calibration curve. Extracts and quercetin were diluted in methanol to certain concentrations, then to 0.5 mL of the solution were added as much as 0.1 mL of aluminum (III) chloride 10%, 0.1 mL of sodium acetate 1 M, and 2.8 mL of aquadest. The mixtures were incubated at room temperature for 30 minutes and analyzed with Spectrophotometer UV-Vis at λ 415 nm. TFC was calculated as gram quercetin equivalent/100 g extract.

IC_{50} and Antioxidant Activity Index (AAI) calculations

The analysis methods to determine the antioxidant activity of extracts (IC_{50} and AAI) were adopted from another experiment[2]. A series of concentrations were made for each extract and then mixed with DPPH solution (50 μg/mL) in a 1:1 ratio. The mixtures were incubated for 30 minutes in the dark and their absorbances were observed at λ 516 nm. Methanol, DPPH solution (50 μg/mL), and ascorbic acid (2-6 μg/mL) were used as blank, control, and reference solution.

IC_{50} is the concentration of extract that can scavenge 50% of the free radical (DPPH). Further, IC_{50} was calculated through the equation of linear regression where the y-axis was the percentage of DPPH scavenging activity, and the x-axis was antioxidant concentration. The number of 50 was interpolated to the equation as the value of y, then the x was considered as the IC_{50}.

AAI was classified into weak (AAI < 0.5), moderate (AAI 0.5 – 1.0), strong (AAI 1.0 – 2.0), and very strong (AAI > 2.0)[11]. The equation (1) was used to calculate the AAI.

$$\text{AAI (DPPH)} = \frac{\text{final concentration of DPPH (μg/mL)}}{\text{Inhibitory Concentration 50 (μg/mL)}} \quad \text{......... (1).}$$

Statistical Analysis

All experiments were conducted in triplicates and stated as mean ± SD. Variance analysis was conducted using one-way ANOVA-post hoc Tukey ($p<0.05$). Pearson coefficient of correlation (r) was analyzed to determine the correlation between the value of TFC and AAI. The statistical analysis was conducted using SPSS 22.0 for Windows.

RESULT AND DISCUSSION

Phytochemical analysis was conducted to determine the existence of secondary metabolites in dried powder and extracts (**Table 1**). The dried powder of *P. bleo* leaves contained alkaloids, flavonoids, phenolic compounds, tannin, and steroid/ triterpenoid. The dried leaves were extracted using different polarities of solvents to separate the phytochemicals according to their polarity. n-Hexane extract of *P. bleo* leaves had flavonoid, phenolic, and steroid/triterpenoid while alkaloid, flavonoid, phenolic, and tannin were detected in the ethyl acetate and ethanol extracts. Another study also revealed that *P. bleo* leaves contained various secondary metabolites which were alkaloid, flavonoid, phytosterol glycoside, lactone, phenolic compounds, sterols, and terpenoids.[1] The existence of secondary metabolites in botanical extracts might contribute to their antioxidant activity.[2,12]

Figure 1 showed the TFC of *P. bleo* extracts which the ethyl acetate extract had the highest TFC (7.03±0.04 g QE/100 g extract). Another study also gave a similar result that the ethyl acetate could extract flavonoids from plants.[13] A research from Malaysia showed a different result where the highest TFC was given by n-hexane fraction of *P. bleo* leaves (39.86 mg catechin/gram dried sample), followed by methanol extract (27.62 mg catechin/gram dried sample) and ethyl acetate fraction (20.38 mg catechin/gram dried sample).[8] Different results of phytochemical content might be due to many reasons such as the geographical origin of plants, different harvest times, and climate factors.[2,3]

Table 1. Phytochemical screening of dried leaves and extracts from *Pereskia bleo*

Phytochemical group	Samples			
	Dried leaves	n-Hexane extract	Ethyl acetate extract	Ethanol extract
Alkaloid	+	-	+	+
Flavonoid	+	+	+	+
Saponin	-	-	-	-
Phenolic	+	+	+	+
Tannin	+	-	+	+
Quinone	-	-	-	-
Steroid/ triterpenoid	+	+	-	-

+ : detected in sample; - : not detected in sample

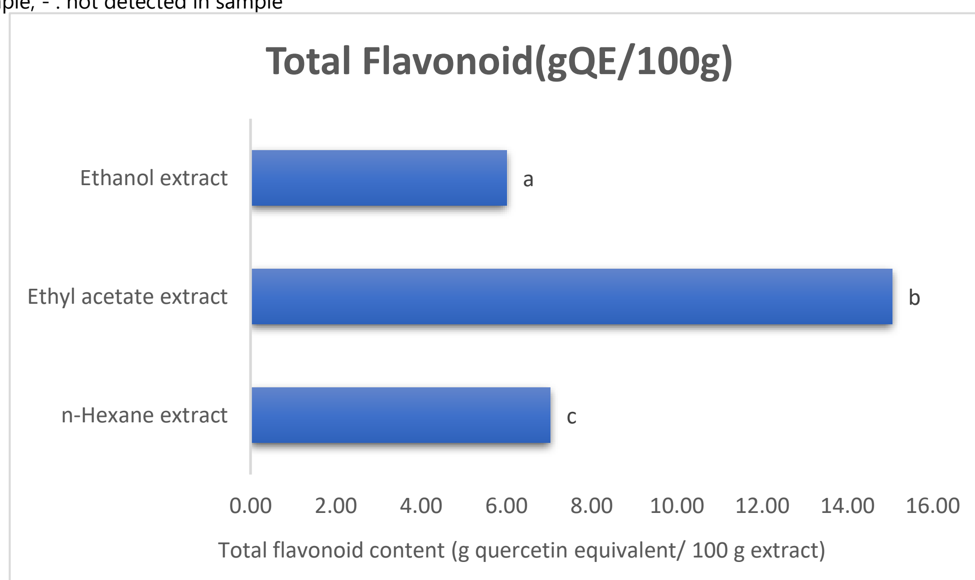

Figure 1. Total flavonoid content of *Pereskia bleo* extracts. The different alphabets show a significant difference (p<0,05).

The calculation of IC_{50} and AAI could be seen in **Table 2**. IC_{50} is a concentration in which the sample could scavenge 50% of the free radical activity or reduce 50% of free radical absorbance. Its value was greatly influenced by the analysis method, especially by the concentrations of free radicals being used in experiments. On the other hand, the value of AAI would remain the same although different concentrations of free radicals are applied.[11] Therefore, it is better to express the antioxidant activity as Antioxidant Activity Index (AAI).

Table 2. IC_{50} and Antioxidant Activity Index (AAI) of extracts from *Pereskia bleo* leaves

Extract	IC_{50} (µg/ mL)	AAI
n-Hexane	217.31[a]	0.23[a]
Ethyl acetate	366.33[b]	0.14[b]
Ethanol	533.14[c]	0.09[c]

a-c = the different alphabets in the column show a significant difference ($p<0.05$)

The current study showed that the n-hexane extract of *P. bleo* leaves had the strongest ability to scavenge the free radical of DPPH with an AAI value was 0.23. According to the AAI values, all extracts were classified as weak antioxidant agents ($AAI < 0.5$). Another experiment also showed a similar result that n-hexane extract of *P. bleo* leaves (EC_{50} 210 µg/mL) had higher antioxidant activity than ethyl acetate extract (EC_{50} 225 µg/mL).[1] Previous research also revealed that the n-hexane fraction of fresh leaves of *P. bleo* had a stronger ability to scavenge the free radical of DPPH than its ethyl acetate fraction and methanol extract.[4] These findings indicated that nonpolar compounds might also possess a high potency of antioxidant activity compared to semi-polar and polar compounds.

The relationship between TFC and antioxidant activity was examined using statistical analysis. The value of Pearson correlation (r) in the range of 0.61 to 0.97 is considered a positive correlation between TFC and AAI.[14] In the current experiment, the statistical analysis revealed that there is no significant correlation between TFC and AAI with the value of Pearson coefficient correlation being -0.106. This result indicated that TFC was not a major contributor to antioxidant activity by DPPH. Another study found that other than flavonoids, there were other classes of secondary metabolites such as phenolics that acted as antioxidant agents.[7,15,16] Furthermore, the chemical structure of phytochemicals also shows great impacts on antioxidant activity. The small number of flavonoids with hydroxyl groups at C4′ and C5′ could give a very strong antioxidant activity.[17,18]

CONCLUSION

The n-hexane, ethyl acetate, and ethanol extracts of *P. bleo* leaves showed variations in the value of total flavonoid contents and antioxidant activity. The n-hexane extract showed the greatest antioxidant activity while the highest total flavonoid content was found in the ethyl acetate extract. The concentration of flavonoids was not considered the main contributor to the antioxidant activity of *P. bleo* leaves.

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CONFLICT OF INTEREST

No conflict of interest.

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