

Potensi Aktivitas Antioksidan Ekstrak Etanol Dan Fraksi Ciplukan (*Physalis angulata*) Pada DPPH (1,1-difenil-2-pikrihidrazil)

Potential Antioxidant Activity Of Ethanol Extract And Fraction Of Ciplukan (*Physalis angulata*) On DPPH (1,1-diphenyl-2-picrylhydrazyl)

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(tanggal diterima: 31-01-2022 , tanggal disetujui:19-04-2022)

INTISARI

Antioksidan eksogen pada tanaman diketahui memiliki efek samping yang kecil, murah dan digunakan dalam mencegah penyakit. Dengan berkembangnya penggunaan tanaman dan sayuran sebagai sumber antioksidan alami, banyak peneliti tertarik untuk menginvestigasi dan mempelajari hal ini. Dalam penelitian ini, ekstrak etanol dan fraksi tanaman ciplukan (*Physalis angulata*) dilakukan investigasi dan evaluasi potensi aktivitas antioksidannya. Fokus dari penelitian ini adalah menetapkan kadar dan mengevaluasi aktivitas antioksidan dari ekstrak etanol dan fraksi ciplukan.

Akar, batang, dan daun ciplukan diekstraksi dengan menggunakan metode maserasi kemudian dipartisi dengan fraksi polar (air), non polar (n-heksana), dan semipolar (etil asetat). Hasil ekstraksi kemudian dianalisis kadar flavonoid total dengan menggunakan kuersetin sebagai standar dan aktivitas antioksidan pada DPPH. Hubungan antara kadar total flavonoid dan aktivitas antioksidan ditentukan dengan metode regresi linier.

Ekstrak etanol daun ciplukan memiliki nilai total flavonoid tertinggi yaitu 38.04 ± 0.8 mg/g kemudian diikuti oleh ekstrak akar (9 ± 0.2 mg/g), dan ekstrak batang (7.1 ± 0.1 mg/g). Fraksi etil asetat dari ekstrak etanol daun ciplukan memiliki aktivitas antioksidan yang tinggi yaitu 32.10 ± 0.2 µg/ml yang dihitung sebagai IC50 kemudian diikuti oleh fraksi air (38.20 ± 0.8 µg/ml), dan fraksi heksana (38.20 ± 0.8 µg/ml). Hasil analisis regresi linier menunjukkan aktivitas antioksidan berkorelasi dengan nilai total flavonoid dan disimpulkan bahwa flavonoid merupakan komponen antioksidan utama pada tanaman ciplukan.

Kata kunci : ciplukan; DPPH; aktivitas antioksidan; flavonoid total

ABSTRACT

Exogeneous antioxidant from plant had been known have less side effect, less expensive and usually available as source to prevention disease. With rising interest and widespread of used plants and vegetables that play role as natural antioxidant many researcher interest to investigate and study these parts. In this study, ethanol extract and fraction of ciplukan (*Physalis angulata*) was investigated and evaluated the potential antioxidant activity. The focus in this work was to determinate of total flavonoid and evaluate antioxidant activity of ethanol extract and fraction of ciplukan.

Stem, leaves, and roots of ciplukan was extracted by using maceration method then it was partitioned with polar fraction (water), non polar (n-hexane), and semipolar (ethyl acetate). Quercetin used as the reference in the study for determinate total flavonoid content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to evaluate antioxidant activity. The correlation between total flavonoid and antioxidant activity was analyzed by using linier regression method.

The leaves ethanol extract of ciplukan was containing the highest (13.7 ± 0.9 mg/g) and followed by roots (9 ± 0.2 mg/g) and stem extract (7.1 ± 0.1 mg/g). From the fraction of leaves ethanol extract of ciplukan, the ethyl acetate fraction showed the highest flavonoid content (38.04 ± 0.8 mg/g) followed by the water fraction (15.36 ± 0.6 mg/g) and the hexane fraction (15.36 ± 0.6 mg/g).



mg/g). The ethyl acetate fraction from leaves ethanol extract showed the highest antioxidant activity calculated as IC_{50} ($32.10 \pm 0.2 \mu\text{g/ml}$) then water fraction ($38.20 \pm 0.8 \mu\text{g/ml}$), and hexane fraction ($38.20 \pm 0.8 \mu\text{g/ml}$). The results from regression linier analysis were showed antioxidant activity was correlated to the total flavonoid content and exhibited flavonoid compound play major as antioxidant component in this plant.

Keyword: ciplukan; DPPH; antioxidant activity; total flavonoid

1. INTRODUCTION

Antioxidants are the compound that delay or inhibit free radical with giving an electron mechanism (1). Free radical can cause an aging, cancer, cardiac and central nervous system disorders (2). Exogenous antioxidant can be found in the plants and vegetables that play role as human diet that prevention disease (2). With rising interest and widespread of dietary antioxidant, many researcher have study to investigate exogenous antioxidant from plants source due to they are less expensive, available, and have less side effect compared to synthetic counterparts (3). The antioxidant activity has been reported from Mengkudu (*Morinda Citrifolia*) and Wangon (*Olox psittacorum*) (4,5).

Ciplukan (*Physalis angulata*) has been known as medicinal plant is belong to the *Solanaceae* family and commonly used as antibacterial, anti-inflammatory, anti-analgesic, and antioxidant due to its functional properties (6). From the previous study, Kusumaningtyas *et al* reported fruit and leaves ciplukan contains phenolic in plant extract and it has antioxidant activity both of ethanol and water extract (7). Juice drinks that made from fruit and bud ciplukan also were confirmed have antioxidant activity and several components are considered as antioxidant (phenolic, flavonoid and alkaloid) (6). However, from the best our knowledge there are no reported about major compound that play role as antioxidant component and their correlation between them in ciplukan plant. The focus in this work was to determinate of total flavonoid and evaluate antioxidant activity of ethanol extract and fraction of ciplukan.

2. MATERIAL AND METHODS

2.1. MATERIAL

Leaves, stem and roots of ciplukan plant (*Physalis angulata*) was collected from Tulungagung, Jawa Timur and identified at LIPI Cibinong, Indonesia where a herbarium specimen was deposited. Spectrophotometer UV-Vis (PG Instrument T80+), rotary evaporator (IKA RV 05), DPPH (1,1-diphenyl-2-picrylhydrazyl) (MERCK), quercetin (MERCK), ethanol 96 % (MERCK), n-hexane (MERCK), ethyl acetate (MERCK), aquadest (MERCK).

2.2. METHODS

Preparation Samples

Leaves, stem and roots of ciplukan plant were taken it washed with distilled water and dried at room temperature (30 oC) to a constant weight. The dried materials were grounded into powder and 1 kg of each material powder was mixed



with ethanol for 24 h at room temperature. The crude of leaves extracts were fractionated using different solvents n-hexane, ethyl acetate and water. The supernatant of crude extract and fraction then filtered with Whatman no. 41. Each filtrate of crude extract and fraction was concentrated using rotary evaporator under reduced pressure and temperature at 40 oC. The extraction flow chart of sample can be seen at Figure 1.

Phytochemical screening of plant materials

Phytochemical screening of the plant material was assessed by using several method and summarized in the Table 1.

Table 1. Phytochemical screening test performed on ciplukan (*Physalis angulata*) plant material

Phytochemical	Test performed	Reference
Alkaloid	Mayer's test	(8)
Polyphenol	Ferric chloride test	(9)
Flavonoid	Shinoda's test	(10)
Saponin	Foaming/Frothing test	(11)
Tanin	Ferric chloride test	(12)
Triterpenoid	Liebermann test	(13)

Determination of total flavonoid

Total flavonoid was determined using the method of Ordonez et al (14). Quercetin was used as standard solution and measured at 420 nm with spectrophotometer UV-Vis. Total flavonoid content was calculated as quercetin using a calibration curve, where x is the absorbance and y is the concentration of quercetin (mg/g).

Antioxidant activity

This test has been measured by several research methods that use DPPH to determine antioxidant activity (2,14–18). The plant extract and fraction with different concentration were mixed with the DPPH solution. After 30 minutes at room temperature in the dark, the absorbance of the mixture was measured at 517 nm. The antioxidant activity was calculated using the following formula :

$$\text{Antioxidant activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the control absorbance and A_1 is the sample absorbance

Statistical analysis

Experimental data were analyzed with one way analysis of variance (ANOVA) with 95 % confidence intervals and linear regression analysis by using Microsoft Excel 2019 and SPSS Statistic Software 24.00 for Windows. ANOVA is used to measure standard deviation (SD) and linear regression analysis is used to determine the relationship between total flavonoid content and antioxidant activity from each extract source and fraction.



3. RESULTS AND DISCUSSION

Phytochemical screening

The extraction yields of leaves, stem, and roots by using ethanol of ciplukan (*Physalis angulata*) were 61.37 g, 18.73 g, and 2.60 g respectively. From the fraction of leaves ethanol extract, the extraction yields for water fraction, hexane fraction and ethyl acetate fraction were 21.0 g, 5.10 g, and 10.1 g respectively. The phytochemical screening (Table 2) analysis of ciplukan plant showed the presence of alkaloid, polyphenol, flavonoid, saponin, tannin, and triterpenoid. On the other hand, each of stem extract, roots, leaves ethanol extract and fraction of leaves ethanol extract exhibited the absence of anthocyanin.

Table 2. Preliminary phytochemical screening of ciplukan (*Physalis angulata*) and its fractions (+ = presence ; - = absence)

Phytoconstituens	Ethanol extract			Ethanol leaves extract fraction		
	Leaves	Roots	Stem	Hexane	Ethyl acetate	Water
Alkaloid	+	+	+	-	+	+
Polyphenol	+	+	+	-	-	+
Flavonoid	+	+	+	+	+	+
Saponin	+	+	+	-	-	+
Tannin	+	+	+	-	-	-
Triterpenoid	+	+	+	+	+	+
Anthocyanin	-	-	-	-	-	-

Total flavonoid content

Plants have different groups of phenolic components, such as flavonoids, anthocyanins, and simple phenolics. These groups had been attracted because of their function including free radical scavenging (2). The total of total flavonoid content of the leaves, stem, roots and fraction of leaves ethanol extract, as determined by Ordonez method, was expressed as quercetin equivalents (14). As shown in Table 3, The leaves ethanol extract of ciplukan was containing the highest (13.7 ± 0.9 mg/g) and followed by roots (9 ± 0.2 mg/g) and stem extract (7.1 ± 0.1 mg/g). From the fraction of leaves ethanol extract of ciplukan, the ethyl acetate fraction showed the highest flavonoid content (38.04 ± 0.8 mg/g) followed by the water fraction (15.36 ± 0.6 mg/g) and the hexane fraction (15.36 ± 0.6 mg/g). From this study, high level of flavonoid contents, especially in leaves extract was showed and this may explain the widespread folklore to use this plant for medicine.

DPPH radical scavenging activity

As shown in Table 3, the ethanol extract from leaves, roots, stem, and fraction of the leaves ethanol extract were active against DPPH. In this work, there was increased DPPH radical with increasing concentration of ciplukan plant extracts. It can be happen because components of plant extract donate hydrogen ions into DPPH radicals (19). The ethyl acetate fraction from leaves ethanol extract showed the highest antioxidant activity when compared with quercetin and



calculated as IC₅₀ (32.10 ± 0.2 µg/ml). The highest activity of ethyl acetate fraction from leaves ethanol extract can be due to the presence of different type of functional group because using different type of solvent for extraction (20). High content of flavonoids also serve as antioxidant compound in ciplukan plant and can remove free radical (21).

Table 3. Quantification of phytoconstituents in ciplukan (*Physalis angulata*) and its fractions

Contents	Ethanol extract			Ethanol leaves extract fraction		
	Leaves	Roots	Stem	Hexane	Ethyl acetate	Water
Total flavonoid (quercetin mg/g)	13.7 ± 0.9	9 ± 0.2	7.1 ± 0.1	14.11 ± 0.2	38.04 ± 0.8	15.36 ± 0.6
Antioxidant activity (IC ₅₀ µg/ml)	190 ± 4.7	520 ± 6.0	1120 ± 14.5	44.18 ± 0.5	32.10 ± 0.2	38.20 ± 0.8

Relationship between total flavonoid content and antioxidant activity

In this study, linear regression analysis was used to examine the correlation between total flavonoid and antioxidant activity (IC₅₀) in the six samples. In Figure 2, the correlation coefficient between total flavonoid and DPPH scavenging activity (R² are 0.8438 and 0.7937) was found to be very significant, more than 0.5. These positive linear correlation values between total flavonoid and the antioxidant activity indicate that total flavonoid play an important role as antioxidant compound in ciplukan.

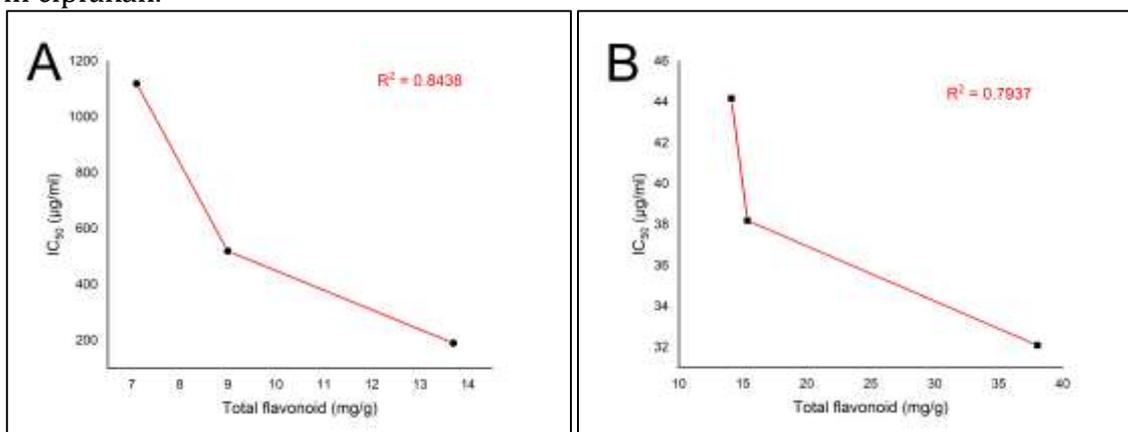


Figure 2. Correlation between total flavonoid content and IC₅₀ by using DPPH from different extract (A) and fraction (B)

4. CONCLUSION

In this work, the ethyl acetate fraction from leaves ethanol extract of ciplukan showed the highest content of total flavonoid compound and antioxidant activity in dpph assay. The results showed that part of ciplukan, especially in leaves could be a potential source as antioxidant to prevent oxidative stress.



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