Pharmaceutical and Clinical Journal of Nusantara (PCJN)
Volume 03, No 01, July 2023, Page 01 - 12
E-ISSN 2985 – 4040 (Online Media)
https://nusantarascientificjournal.com/index.php/pcjn/index
https://doi.org/10.58549/pcjn.v3i01.71

Antioxidant Activity of Ethanol Extract of Chinese Petai Peel (*Leucaena leucocephala* (Lam.) de Wit) Using DPPH (1,1-diphenil-2-picrylhydrazyl) Method

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Abstract

The Chinese petai plant (Leucaena leucocephala (Lam.) de Wit) has been used in various systems of traditional medicine for the treatment of various diseases in humans. The Chinese petai plant (Leucaena leucocephala (Lam.) de Wit) contains antioxidant secondary metabolites that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. The purpose of this study was to determine the class of chemical compounds contained in the ethanol extract of Chinese petai peel and the antioxidant activity of the ethanol extract of Chinese petai peel (EEKPC). The stages of this research included: collection and processing of simplicia; preparation of the extract by maceration with 70% ethanol; characterization test of simplicia powder; phytochemical screening of ethanol extract of petai cina; antioxidant activity test of EEKPC using the DPPH (1,1-diphenyl-2-pikrilhidrazyl) free radical scavenging method using UV-Vis spectrophotometry at a wavelength of 516 nm. The results of the phytochemical screening examination of Chinese Petai Peel Ethanol Extract (EEKPC) contained alkaloids, flavonoids, saponins, tannins, and triterpenoids. The results of measuring the antioxidant activity of EEKPC showed strength in the "strong" category with an IC_{50} value of 91.0189 µg/ml.

Keywords: *Antioxidant, Chinese Petai Peel, DPPH*

Abstrak

Tanaman Petai Cina (Leucaena leucocephala (Lam.) de Wit) telah digunakan dalam berbagai sistem pengobatan tradisional untuk pengobatan berbagai penyakit pada manusia. Tanaman Petai Cina (Leucaena leucocephala (Lam.) de Wit) mengandung metabolit sekunder bersifat antioksidan yang dapat menghambat reaksi oksidasi, dengan mengikat radikal bebas dan molekul yang sangat reaktif. Tujuan dari penelitian ini adalah untuk mengetahui golongan senyawa kimia yang terkandung pada ekstrak etanol kulit petai cina serta untuk mengetahui aktivitas antioksidan Ekstrak Etanol Kulit Petai Cina (EEKPC).Tahapan penelitian ini meliputi: pengumpulan dan pengolahan simplisia, pembuatan ekstrak secara maserasi dengan pelarut etanol 70%, uji karakterisasi serbuk simplisia, skrining fitokimia ekstrak etanol kulit petai cina, uji aktivitas antioksidan EEKPC dengan metode peredaman radikal bebas DPPH (1,1-difenil-2-pikrilhidrazil) dengan menggunakan spektrofotometri UV-Vis pada panjang gelombang 516 nm. Hasil pemeriksaan skrining fitokimia Ekstrak Etanol Kulit Petai Cina (EEKPC) mengandung senyawa alkaloid, flavonoid, saponin, tanin dan triterpenoid. Hasil pengukuran aktivitas antioksidan EEKPC menunjukan kekuatan dengan kategori "kuat" dengan nilai IC_{50} sebesar 91,0189 µg/ml.

Kata kunci: Antioksidan, Kulit Petai Cina, DPPH

Received: *May 2023* ***Accepted:*** *July 2023*

INTRODUCTION

The human body needs important substances, namely antioxidants, which can help protect the body from free radical attacks by reducing the negative effects of these compounds. Antioxidants are compounds that can counteract or

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reduce the negative effects of oxidants. Antioxidants work by donating an electron to compounds that are oxidants so that the activity of these oxidant compounds can be inhibited[1].

Antioxidants are needed by the body to protect it from free radical attacks[2]. Free radicals are unstable compounds or molecules in cells that have one or more unpaired electrons in their outer orbitals[3]. The presence of unpaired electrons causes electrons to act reactively, so that the compound attacks and binds electrons to the molecules around it. The impact of the reactivity of free radical compounds can be in the form of cell or tissue damage, degenerative diseases, or cancer[4]. Therefore, the body needs antioxidants to fight the effects of exposure to these free radicals.

Some substances that have antioxidant properties are flavonoids, polyphenols, beta-carotene, lutein, lycopene, selenium, zinc, anthocyanins (dyes in fruits and vegetables), as well as vitamins A, C, and E[5]. Flavonoids are the largest group of phenolic compounds found in nature. Flavonoids are found in various plants and are distributed in two parts, such as fruit, leaves, seeds, roots, bark, stems, and flowers. Flavonoid compounds are substances that give plants yellow, red, blue, and purple colors[6].

Chinese petai contain flavonoids and alkaloids, which are often found in leaves, pods, and seeds. Compounds contained in Chinese petai plants are secondary metabolites with antioxidant properties that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. The ethanol extract of Chinese petai leaves (*Leucaena glauca* (L.) Benth) has an IC_{50} value of 86.309 µg/ml which is categorized as a strong antioxidant [7]. In addition, Chinese petai seeds (Leucaena leucocephala (Lam) de Wit) have very strong antioxidant activity. with an IC_{50} value of 11.80 µg/ml[8]. There are many methods of reducing free radicals including the DPPH (1,1-diphenyl-2-pikrilhidrazyl) method, FRAP (Ferric Reducing Antioxidant Power), and CUPRAC (Cupric Ion Reducing Antioxidant Capacity) . In this study, the DPPH radical scavenging method was used. Testing antioxidant activity for certain compounds or plant extracts using the DPPH method is the best because this method is easy to do, fast, and sensitive[9]. Based on this and the compounds contained therein, the authors are interested in conducting research on the antioxidant activity of the ethanol extract of Chinese petai skin (*Leucaena leucocephala* (Lam) de Wit). by the DPPH (1,1-diphenyl-2-

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picrylhydrazyl) method using UV-Vis Spectrophotometry.

METHODOLOGY

The method of research employed is descriptive research. In this study, parts of the simplicia plant were collected, an ethanol extract was made, the simplicia powder was analyzed, phytochemicals in the ethanolic extract of Chinese petai skin were looked for, and the DPPH free radical scavenging method and a visible spectrophotometer were used to measure antioxidant activity.This study utilized the skin of the Chinese Petai (*Leucaena leucocephala* (Lam.) de Wit) as its sample. District of Batu Bara, North Sumatra province. The Chinese Petai skin sampling procedure was conducted on purpose, i.e., without comparison to similar plants from other regions.

Apparatus

The apparatus used is laboratory equipment, including an analytical balance, furnace, oven, porcelain crucible, desiccator, test tube, dropper pipette, evaporating cup, watch glass, stir bar, sieve, blender, Erlenmayer, beaker glass, measuring cup, measuring flask, volume pipette, suction ball, rotary evaporator, funnel, filter paper, aluminum foil, micropipette, and UV-Vis Spectrophotometry.

Materials

The materials used in this study were Chinese Petai Peel (*Leucaena leucocephala* (Lam.) de Wit), 70% ethanol, methanol, distilled water, concentrated hydrochloric acid, 2 N hydrochloric acid, iodine, potassium iodide, mercury (II) chloride, bismuth (III) nitrate, amyl alcohol, magnesium powder, iron (III) chloride, n-hexane, acetic anhydrous acid, concentrated sulfuric acid, DPPH (1,1-diphenyl-2-picryhydrazyl)

Sample Preparation

Chinese petai skin is cleaned of impurities, washed thoroughly with running water, drained, weighed wet, and dried at 40-60ºC. Weigh the dry weight of the simplicia, then the dried Chinese petai skin is blended and the powder obtained is weighed and then extracted with 70% ethanol solvent[10].

Ethanol Extract Preparation

Chinese Petai Peel The ethanol extract of Chinese petai peel is carried out by maceration using 70% ethanol solvent. Weigh 500g of the simplicia powder into a vessel, then pour 75 parts (3750 ml) of 70% ethanol extract and leave it for 5 days and stir occasionally. After 5 days the mixture is filtered and the dregs are squeezed out. Wash the

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dregs with 70% ethanol as much as 25 parts (1250 ml) and then squeezed. Then Maserate 1 and 2 were combined to obtain 100 parts (5000 ml) of macerate and transferred into a closed vessel, left in a cool place protected from light for 2 days and filtered 9. Maserate was concentrated with a rotary evaporator at a temperature not exceeding 60ºC and a thick extract was obtained[11].

Characterization of Simplicia

Simplicia examination includes; determination of water content, determination of water-soluble essence content, determination of ethanol-soluble essence content, determination of total ash content, determination of acid-insoluble ash content[12].

Phytochemical Screening

Phytochemical screening was carried out to identify secondary metabolites found in simplicia and Chinese petai skin extract. The phytochemical screening of Chinese petai peel extract includes examination of alkaloids, flavonoids, saponins, tannins, and steroid/triterpenoid compounds[13].

Antioxidant Activity Test

Antioxidant activity was tested using the DPPH (1,1-diphenyl-2-pikrylhidrazyl) method as a free radical, and its absorbance was measured using visible spectrophotometry at a wavelength of around 516 nm. The principle is to measure the absorbance of DPPH as a free radical in methanol solution before and after adding the test material, so that the decrease in absorbance (% inhibition) is obtained as the ability of the test sample to absorb DPPH, by calculating the IC_{50} value (the concentration of the test sample that captures free radicals is 50 %) is a parameter in determining the antioxidant activity of the test sample [14].

Preparation of DPPH Blanc Solution (o.5 mM)

Weigh 10 mg of DPPH into a 50 ml volumetric flask. Dissolved with methanol up to the boundary line, then a mother liquor was obtained with a concentration of 200 µg/ml[15].

Preparation of DPPH Blanc Solution (C=40 µg/ml)

Pipette 1 ml of DPPH solution (200 µg/ml), put it into a 5 ml volumetric flask, make up the volume with methanol up to the boundary line (obtain a concentration of 40 µg/ml)[15].

Maximum Absorption Wavelength Measurement

The absorption of DPPH solution with a concentration of 40

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µg/ml was measured at a wavelength of 400-800 nm[15].

Determination of Operating Time

The absorbance of the DPPH solution (concentration 40 µg/ml) was measured at a wavelength of 516 nm every 1 minute for 20 minutes and it was observed that the solution began to produce a stable absorbance which would be used as the operating time in the next procedure[2].

Preparation of Chinese Petai Peel Ethanol Extract Raw Solution (EEKPC)

Weigh 1 g of EEKPC and then put it into a 100 ml volumetric flask. Dissolved with methanol and then made up to volume up to the boundary line (concentration of 10.000 µg/ml) this is called LIB I. Pipette 5 ml of LIB I then put into a 50 ml volumetric flask, then dissolved with methanol to the boundary mark (concentration of 1000 µg/ ml) is called LIB II[2].

Antioxidant Testing of Chinese Petai Peel Ethanol Extract (EEKPC)

Concentration is determined after several orientations. Pipette from LIB II as much as 0.25 ml; 0.375 ml; 0.5 ml; 0.625 ml; and 0.750 ml using a micropipette each put into a 5ml volumetric flask, with (concentrations of 50 µg/ml, 75 µg/ml, 100 µg/ml, 125 µg/ml, 150 µg/ml, respectively). Then into each volumetric flask put 1 ml of DPPH solution. The volume was made up with methanol, then allowed to stand for 5-10 minutes, then the absorption was measured using a UV-Vis spectrophotometer at a wavelength of 516 nm.

Determination of Percent Attenuation (% inhibition)

Antioxidant ability is calculated from the decrease in absorption of DPPH solution (attenuation/decrease in DPPH purple color) due to the addition of extract solution as a test material as a comparison material. The difference in the absorption value of the DPPH solution before and after the addition of the test solution is calculated as the percent damping.

Determination of IC_{50} Value

The IC_{50} value is a number indicating the concentration of the test sample (µg/mL) which provides DPPH reduction of 50% (able to inhibit or reduce the oxidation process by 50%). A value of 0% means that it has no antioxidant activity, while a value of 100% means total absorption and the test needs to be continued with dilution of the test solution to see the limit of its activity concentration. The calculation results are entered into the regression equation with extract concentration

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(µg/mL) as the abscissa (X axis) and the % attenuation (antioxidant) value as the ordinate (Y).

RESULT AND DISCUSSION

Extraction of Chinese Petai Peel from 500 g of Chinese petai peel simplicia powder using 5 L of 70% ethanol solvent, using the maceration method and then evaporated with a rotary evaporator at a temperature of 40-50℃ to obtain a thick extract of 20.5 g.

Characterization of Simplicia

Chinese Petai Skin The results of the examination of the simplicia characterization of Chinese petai peel obtained can be seen in Table 1.

Table 1. Characterization of Chinese Petai Peel Simplicia

No.	Characterization of Simplicia	Examination Results (%)	MMI
1.	Moisture Content	4.66%	<10%
2.	Water Soluble Extract Content	16%	>30%
3.	Concentration of Soluble Juice in Ethanol	18.7%	>6%
4.	Total Ash Content	3.77%	<4%
5.	Acid Soluble Ash Content	0.08%	<1%

Based on the results in Table 1, the simplicia characteristics of Chinese petai skin meet the general requirements of the Indonesian Materia Medika (MMI)

Examination of water-soluble extracts and ethanol-soluble extracts was carried out to determine the content of polar compounds in the simplicia. Examination of the ash content was carried out to determine the mineral content from the initial process to the formation of the extract. The characterization results produced meet the requirements according to the test parameters for the characterization of simplicia which has the same tribe as Chinese petai skin, namely the Mimosaceae tribe which is listed in the Materia Medika Indonesia (MMI), except for the determination of water-soluble extracts, this is influenced by the part of the plant used in the study.

Phytochemical Screening

The phytochemical screening test was carried out to determine the class of chemical compounds contained in the ethanol extract of petai cina. The results of the phytochemical screening can be seen in Table 2.

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Table 2. Phytochemical screening of Chinese Petai peel ethanol extract

No	Phytochemical Compound	Results
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Saponin	Positive
4	Tannin	Positive
5	Triterpenoids	Positive

The results of the phytochemical screening showed that the ethanol extract of petai cina contains the same secondary metabolites, namely the alkaloid group, characterized by the presence of a white or yellow precipitate when added with mayer reagent, the formation of brown or orange brown precipitate when added with dragendroff reagent, and the formation of brown precipitate. until black when added bouchardat reagent. Flavonoids are characterized by the formation of an orange color on the amyl alcohol layer with the addition of magnesium metal and concentrated hydrochloric acid. Saponins are characterized by the formation of foam and are not lost by the addition of 2N hydrochloric acid. Tannins are characterized by the appearance of a blue-black or green-black color with the addition of 1% iron (III) chloride reagent. Triterpenoids are characterized by the appearance of a pink or purple color with the addition of acetic anhydrous and concentrated sulfuric acid.

Antioxidant Activity

The results of an analysis of the antioxidant activity of an ethanol extract of Chinese petai peel carried out with the 1,1-diphenyl-2-pikrilhidrazyl (1,1-diphenyl-2-pikril) entrapment method by UV-Visible spectrophotometry are presented here.

Determination of Maximum Absorption Wavelength

Results Using a UV-Visible Spectrophotometer, a measurement of the maximum absorption of a 40 g/ml DPPH solution in methanol was carried out. According to the findings of the measurements, the DPPH dissolved in methanol generates a maximum absorption of 0.947 at a wavelength of 516 nm. This wavelength falls within the visible light wavelength range, which spans from 400 to 800 nm. Figure 1 displays the results of the measurements taken [11].

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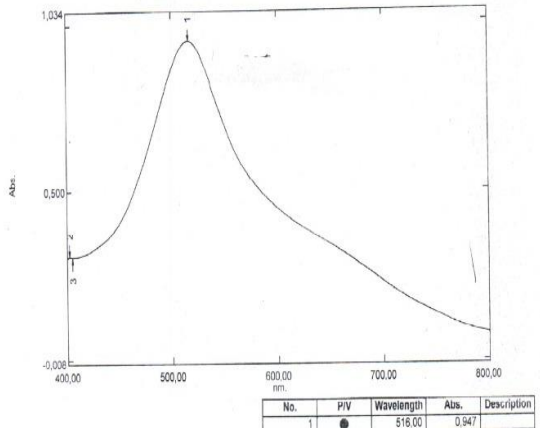

Figure 1. Maximum absorption curve of 40 µg/ml DPPH solution in methanol

Determination of Operating Time

Operating time determination aims to determine the stable measurement time. The measurement time is determined by measuring the relationship between the measurement time and the absorbance of the solution. Determination of the operating time of 40 µg/ml DPPH solution in methanol obtained a stable working time of 5-10 minutes. Data from the results of operating time measurements can be seen in Figure 2.

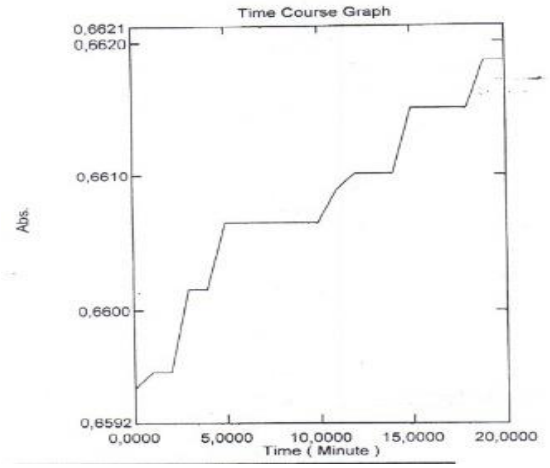

Figure 2. Graph of Operating Time

Antioxidant Activity Test of Chinese Petai Peel Ethanol Extract (EEKPC)

Antioxidant activity test of ethanol extract of petai cina with concentrations of 50 µg/ml, 75 µg/ml, 100 µg/ml, 125 µg/ml, 150 µg/ml with DPPH control solution can be seen in table 3.

Table 3. EEKPC Antioxidant Activity Test

Concentration	Absorbance Measurement		
	1	2	3
0	0.996	0.996	0.996
50	0.781	0.779	0.777
75	0.677	0.677	0.671
100	0.475	0.478	0.478
125	0.373	0.373	0.374
150	0.231	0.230	0.229

The table above shows that with increasing EEKPC concentration, the absorbance of the sample will decrease and the inhibition level will increase. The absorbance of the sample decreases because the electrons in the DPPH

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become paired with the sample electrons which causes the color of the solution to change from deep purple to clear yellow. This condition indicates that the value of the level of inhibition increases with increasing sample concentration because more and more antioxidant compounds in the sample can counteract free radicals[16].

Percent Analysis of DPPH Free Radical Squeezing EEKPC Test Solution

The results obtained from various concentrations of the EEKPC test solution can be seen in table 4.

Table 4. Percent Analysis of Free Radical Squeezing of DPPH EEKPC Test Solution

No	Concentration (µg/ml)	% Dumping
1	0	0
2	50	21.7871
3	75	32.2289
4	100	52.1084
5	125	62.5167
6	150	76.9076

The relationship between the concentration and percent scavenging of DPPH free radicals and the ethanol extract of petai cina can be seen in Figure 3. The data obtained was a linear regression equation Y = 0.5215 X - 2.5336 with a correlation coefficient of r = 0.9954. Based on these data it can be concluded that there is a linear relationship between concentration and the percent attenuation of the ethanol extract of Chinese petai peel (EEKPC).

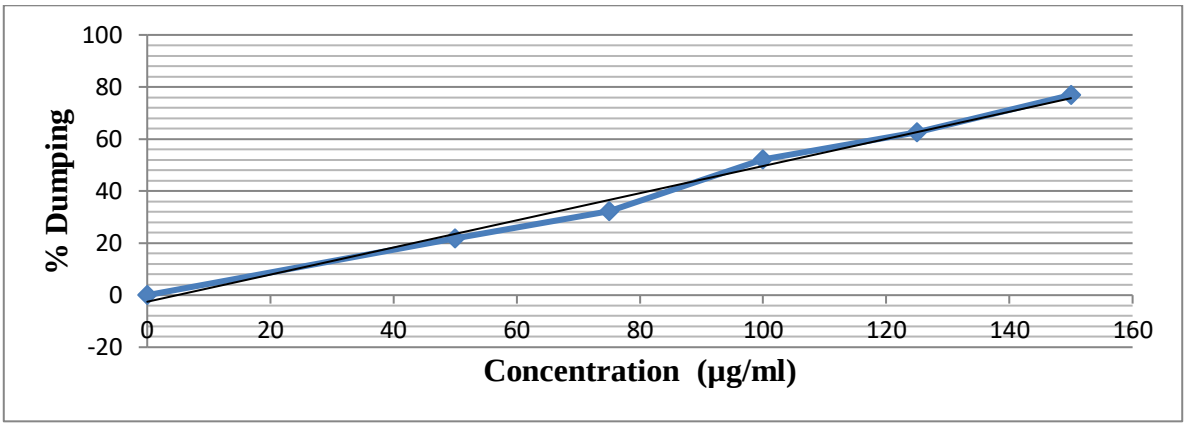

Figure 3. Graph of EEKPC's Percent Attenuation Analysis

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IC50 Value Analysis (Inhibitory Concentration)

The IC50 value was obtained based on the calculation of the regression equation by plotting the concentration of the test solution and the percent absorption of DPPH as a parameter of antioxidant activity, the sample concentration (µg/ml) as the abscissa (X axis) and the value of % inhibition as the ordinate (Y axis)[17]. The results of the linear regression equation and the results of the analysis of the IC50 value obtained from the antioxidant activity test of the ethanol extract of Chinese petai bark can be seen in table 5.

Table 5. Linear regression equation and IC50 analysis obtained from Chinese Petai Peel Ethanol Extract (EEKPC)

Solution test	Regression equation	Regression	IC50 (µg/ml)
EEKPC	Y= 0.5215X-2.5336	0.9954	91.0189

Table 5 shows that EEKPC shows that the antioxidant activity is in the strong category with an IC50 value of 91.0189 µg/ml. Antioxidants play a role in preventing tissue damage caused by free radicals by eliminating the formation of radicals, reducing or increasing their breakdown[18].

CONCLUSION

The results of the phytochemical screening of the ethanol extract of Chinese petai peel (Leucaena leucocephala (Lam) de Wit) contain alkaloids, flavonoids, saponins, tannins and triterpenoids. And the antioxidant activity of Chinese petai peel (Leucaena leucocephala (Lam) de Wit) shows strength in the "strong" category with an IC50 value of 91.0189 µg/ml.

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