Tropical Health and Medical Research
Vol. 6, No. 2, August 2024, pp. 36-44
ISSN (Online): 2684-740X
Journal homepage: https://tropicalhealthandmedicalresearch.com

Antioxidant Activity and Marker Compound Levels of Cempedak Leaf (*Artocarpus integer*) Extract in Different Solvent Variations

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Abstract: Cempedak (*Artocarpus integer*) is known to contain phenolic compounds. Phenolic compounds are secondary metabolites of plants included in marker compounds that act as natural antioxidants because they have hydroxyl groups that can reduce free radicals. Plants containing phenolic compounds are known to have strong antioxidant activity. This study aimed to determine the antioxidant activity and levels of marker compounds from ethanol extracts of cempedak leaves in various solvent variations. Cempedak leaves were dried in an oven and then powdered. Cempedak leaf powder was extracted using methanol, 70% ethanol, 96% ethanol using the maceration method, and distilled water was extracted using the boiling method. The extract was tested for antioxidant activity using the DPPH (1,1-diphenyl-2-picryl-hydrazyl) method based on the IC50 value. Determination of marker compound levels using the Folin-Ciocalteu reagent with a gallic acid comparator using a UV-Vis spectrometer instrument. The total phenolic content determination test results were expressed as gallic acid equivalents per gram of extract (GAE/g extract). The results of the antioxidant activity test showed the IC50 value of the methanol extract was 84.48 ppm (strong), the distilled water extract was 54.74 ppm (strong), the 70% ethanol extract was 72.21 ppm (strong), and the 96% ethanol extract was 24.55 ppm (very strong). The results of the marker compound determination showed the total phenolic content of the methanol extract was 28.22% w/w, the distilled water extract was 22.59% w/w, the 70% ethanol extract was 29.55%, and the 96% ethanol extract was 31.14% w/w. It can be concluded that the cempedak leaf extract extracted with 96% ethanol has very strong antioxidant properties and the highest marker compound content among other solvents. For further analysis, 96% ethanol solvent can be used for the cempedak leaf extraction.
Keywords: Antioxidant; *Artocarpus integer*; cempedak; solvent

INTRODUCTION

Cempedak (*Artocarpus integer*) is a plant widely found in Kalimantan. Cempedak produces phenolic compounds, including stilbenoids, terpenoids, flavonoids, and aryl-benzofuranone[1]. Cempedak leaves are empirically used as anthelmintics, bacteriocides, anti-inflammatory, analgesics, anticancer, antimicrobials, and degenerative diseases[2].

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Most herbal plants in Indonesia still need more chemical content, one of which is cempedak, which has not been widely studied for its potential as a medicinal ingredient[3].

Solvents affect the chemical content (qualitative) and chemical compounds (quantitative) levels in an extract and the production process of herbal products. Solvents dissolve compounds based on the principle of like dissolves like, namely dissolving compounds according to the polarity of the solvent. Different solvents will produce different extract characteristics, so it is important to conduct research on using them[4].

Medicinal plants are a source of natural exogenous antioxidants. Polyphenols, carotenoids, and vitamins are sources of natural antioxidants found in plants[5]. Antioxidants are substances that can prevent damage and oxidative stress in cells because they can neutralize free radicals[6]. Phenolics are a group of marker compounds that have aromatic rings that act as antioxidants that function as protection from DNA damage[4]. The antioxidant activity of phenolics is related to overcoming degenerative diseases[7]. The determination of marker compound levels is included in specific parameters to ensure the quality of an extract. The specific parameter aspects of the extract are focused on the active compounds responsible for providing pharmacological effects[8].

Previous studies have shown the flavonoid content and activity of cempedak leaves in protecting the skin from UV rays[6]. Other studies have shown that the fraction of cempedak leaf extract has an SPF value of 4.78 - 8.51[2]. Cempedak leaves can also increase the immune system, especially as nutraceuticals[9]. Cempedak leaves are known to have antibacterial activity with the ability to inhibit the growth of *S. aureus* and *E. coli* bacteria[10]. Cempedak leaves can lower blood glucose levels in mice with a hyperglycemia model[11]. More research is still needed to determine the best solvent for extracting cempedak leaves. Cempedak leaves contain phenolic groups that are effective as antioxidants, so they can be a parameter in determining the best solvent in the extraction process. This study aimed to determine the antioxidant activity and levels of marker compounds from ethanol extracts of cempedak leaves in various solvent variations.

MATERIALS AND METHODS

Research Design

This type of research is experimental research. This research was conducted quantitatively to determine the total phenol content and antioxidant activity in dry extracts of cempedak leaves (*Artocarpus integer*) extracted using distilled water, methanol, 70% ethanol, and 96% ethanol, respectively.

Materials

The materials used in this study include cempedak leaves (*Artocarpus integer*), gallic acid (Sigma), distilled water (Onelab), methanol, 70% ethanol, 96% ethanol (Merck), DPPH (Sigma), Folin-Ciocalteu reagent, Silica Gel F254 TLC Plate (Merck).

Plant Determination

Determining chempedak plants (*Artocarpus integer*) was conducted at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung University of Banjarbaru, South Kalimantan Province.

Sample Preparation

Cempedak leaves were collected from Banjar Regency, Pengaron District, South Kalimantan. They are cleaned with running water, then cut into small pieces and dried in

an oven at 50-60°C. The dried cempedak leaves are ground again into smaller particles using a blender to make powder. The leaf powder is then sieved using a 20-mesh sieve[12].

Extract Preparation

Cempedak leaves (*Artocarpus integer*) are extracted using the maceration and boiling methods. The maceration method is used for methanol, 70% ethanol, and 96% ethanol solvents, while the boiling method is used for distilled water solvents. The simplicia is put into a pan containing 1:30 distilled water and boiled for 15 minutes while stirring occasionally. The boiling results are filtered hot to obtain a liquid extract[13]. Extracts using the maceration method were made by weighing 75 grams of *Artocarpus integer* leaf simplicia powder and then putting it into a macerator with a ratio of 1:10; after all the powder was submerged, stirring was carried out. The solvent in the maceration process was replaced every 1 x 24 hours; the maceration was filtered using filter paper, and the maceration was carried out for 3 x 24 hours. The liquid extract was then dried using a drying method using a drying cabinet at a temperature of 50°C for 4 x 24 hours until a dry extract was obtained.

Phytochemical Screening

The four extracts obtained underwent phytochemical screening, which used specific reagents to determine the presence of phenolics, flavonoids, alkaloids, steroids, saponins, and tannin compounds. Positive results were indicated by a change in color or sediment formation after the specific reagent was added[3].

Marker Compound Levels

The gallic acid content series was made with 10, 20, 30, 40, and 50 ppm concentrations. Pipette each concentration series of as much as 1 mL into a test tube, add 2.5 mL of 5% Folin-Ciocalteau reagent, and let the solution stand for 3 minutes. Then, add each solution with 2 mL of 1 M Na_2CO_3 and let it stand for 45 minutes. The absorbance is read using UV-Vis spectrophotometry at a maximum wavelength of 744 nm. A calibration curve of the relationship between absorbance and gallic acid concentration (µg/mL) is made to obtain the standard curve equation $y = bx + a$[14]. The four extracts were weighed at 10 mg, and then ethanol was added to the boundary mark in a 10 mL measuring flask. Take as much as 0.5 mL and put it into a test tube plus 2.5 mL of 5% Folin-Ciocalteu reagent, let it stand for 3 minutes, then add 2 mL of 1 M Na2CO3 and let it stand for 45 minutes. Absorbance was read using UV-Vis spectrophotometry at a maximum wavelength of 744 nm. The blank used was a reagent that did not contain gallic acid[15].

Antioxidant Activity Test

The four extracts were weighed, each 10 mg, then put into a 10 mL measuring flask, and ethanol was added to the limit mark. The solution was diluted to 100 ppm, 80 ppm, 60 ppm, 40 ppm, and 20 ppm. 0.4 mM DPPH solution was put into a test tube as much as 0.5 mL, and various concentrations of the extract solution were added to each tube. The operating time was 28 minutes, and the absorbance was read at a maximum wavelength of 516 nm[15]. The strength of antioxidant activity is known based on the IC50 value. The lower the IC50 value, the stronger the antioxidant power; this is due to the sample concentration required to produce free radical-reducing activity by 50% smaller. The antioxidant power categories are as follows, namely < 50 ppm (very strong), 50-100 ppm (strong), 100-250 ppm (moderate), 250-500 ppm (weak), and > 500 ppm (inactive)[14].

RESULTS AND DISCUSSION

Results of Plant Determination

The chempedak plant sample used in this study, which has the Latin name *Artocarpus integer*, was taken from Maniapun Village, RW 02 RT 02, Pengaron District, Banjar Regency, South Kalimantan Province. The cempedak plant determination was carried out at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru, South Kalimantan Province, obtaining a Test Result Certificate number 024c/LB.LABDASAR/II/2022.

Results of Extraction

The extraction results were weighed, and then the percent yield was calculated by comparing the weight of the extract with the weight of the extracted powder. The results obtained were then presented. The percent yield results are presented in Table 1.

Table 1. Percentage Yield from Extract Leaf Cempedak

Sample	Replication	Weight extract (g)	Weight fraction (g)	Yield (%)	Average
Extract Aquadest	1	75	6.49	8.65	8.35%
	2	75	7.44	9.92	
	3	75	4.87	6.49	
Extract Methanol	1	75	20.31	27.08	25.99%
	2	75	19.95	26.60	
	3	75	18.22	24.29	
Extract Ethanol 70%	1	75	16.89	16.89	24.64%
	2	75	20.74	20.74	
	3	75	17.83	17.83	
Extract Ethanol 96%	1	75	13.53	18.04	18.64%
	2	75	14.06	18.74	
	3	75	14.36	19.15	

The yield percentage indicates the active compounds extracted from cempedak leaf powder. Based on the results of the study, the average yield percentage from the highest to the lowest was methanol extract (25.99%), 70% ethanol extract (24.64%), 96% ethanol extract (18.64%), and water extract (8.35%). Sari et al. (2021) research showed the yield of distilled water extract of jackfruit leaves of 6.668% from an extract weight of 20 grams and jackfruit leaf simplicia powder of 300 grams[14]. Rizki et al. (2021) research yielded a 96% ethanol extract from cempedak leaves of 26.29%. The difference in yield values is influenced by the effectiveness of the extraction process, such as time, temperature, stirring, and selection of solvent types. The higher the yield, the more weight of the extract obtained, so the better[4]. However, the extract yield that is considered optimum is more than 10%. This study showed that methanol extract, 70% ethanol extract, and 96% ethanol extract had a yield above 10%.

Results of Phytochemical Screening

Phytochemical screening was carried out to determine the group of compounds contained in the extract. If there is a difference in the content of chemical compounds, it

can be seen in the phytochemical screening results. The results of phytochemical screening are presented in Table 2.

Table 2. Screening Results Phytochemicals Extract Leaf Cempedak

Group Compound	Extract Aquadest	Extract Methanol	Extract Ethanol 70%	Extract Ethanol 96%
Phenolic	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Alkaloid	+	+	+	+
Saponins	+	+	+	+
Steroid	-	-	-	-

Phytochemical screening results showed that the four extracts contained the same group of compounds, namely phenolics, flavonoids, tannins, alkaloids, and saponins. The four extracts do not contain steroids. The phenolic test showed positive results marked by a change to a blackish color due to the nature of the -OH group in phenolic compounds, which quickly releases itself to form a chelate compound with metal to form a complex polymer that causes a dark color. The complex formed is iron (III) hexaphenolate[16]. The flavonoid test showed positive results marked by a change in color from orange to red[17]. This red color is formed due to reduced Mg and concentrated HCl in flavonols, flavanones, xanthones, and flavonols. Concentrated HCl dripped on the sample hydrolyzes flavonoids into their aglycones (O-Glycosyl)[18].

Results of Marker Compound Level Determination

The total phenolic levels in the four extracts were determined to determine marker compound levels. The colorimetric method with Folin Ciocelteau reagent was used. Gallic acid was used as a comparator. The results of the determination of marker compound levels are presented in Table 3.

Table 3. Marker Compound Levels of Extract Leaf Cempedak

Sample	Total Phenolic Content (µ gEK /mg)	Phenolic Content (% w/w)	Average Total Phenolic Content (% w/w)
Extract Aquadest	228.094	22.809	22.59%
	223.163	22.316	
	228.830	22.652	
Extract Methanol	285.493	28.493	28.22%
	278.866	27.886	
	282.258	22.652	
Extract Ethanol 70%	296.712	29.671	29.55%
	294.390	29.493	
	292.825	29.282	
Extract Ethanol 96%	307.357	30.735	31.14%
	306.783	30.678	
	320.350	32.035	

The results of the determination of the content showed the total phenolic content from the highest to the lowest, namely 96% ethanol extract (31.14%), 70% ethanol extract (29.55%), methanol extract (28.22%), and aquadest extract (22.59%). The difference in total phenolic content values between extracts with different solvents is estimated due to differences in the chemical properties of the solvents used. Phenolic compounds are semi-polar and can be completely dissolved in ethanol solvents, especially 96% ethanol, which has a low polarity compared to distilled water[18]. Phenolic compounds in cempedak leaves are semi-polar, so they are easily extracted in solvents with a broad polarity range, namely 96% ethanol solvent. 96% ethanol is an extraction solvent with a more comprehensive extraction range than distilled water, methanol, and 70% ethanol. 96% ethanol has a semi-polar polarity level, which can attract polar and non-polar compounds in the sample[20].

In contrast, other solvents can only attract compounds that tend to be polar. Based on the principle of like dissolves like, the compound will dissolve in a solvent that matches its polarity[15]. Bioactive compounds in plants have different affinities based on the nature of the solvent used. Phenolic compounds have several groups, both polar and non-polar compounds. Ethanol solvents are expected to extract more phenolic compounds because of their broader reach.

Phenolics are essential medicinal compounds that are natural antioxidants[19]. Antioxidants from phenolics and their derivatives effectively prevent and cure diseases such as cancer and cardiovascular disease. Several phenolic compounds, such as phenol, cinnamic acid, rosmarinic acid, gallic acid, and flavonoids, and their derivatives are chemical compounds widely found in plants[20].

Previous research by Rizki et al. (2021) showed that the total phenol content of ethanol extract of chempedak leaves was 3.7% w/w (3). Research by Sikarwar et al. (2015), which has the genus *Artocarpus* and used the leaf part, namely Artocarpus atlitis, stated that the total phenolic content was 2.6% w/w (1). Research by Leng et al. (2018) showed that the total phenolic content in *Artocarpus* was 14.4% w/w[21]. A comparison between several similar studies shows that the total phenolic content is quite diverse. This is likely due to differences in the solvents used, the extraction process carried out, and differences in the places where the plants grow, and the plant species used.

Antioxidant Activity Test Results

Antioxidant activity is a pharmacological activity parameter used to determine the ability of the extract to fight free radicals. Free radicals are triggers for degenerative diseases, cancer, and premature aging. The results of the antioxidant activity test on cempedak leaf extract are presented in Table 4.

The results of the antioxidant activity test showed the highest to lowest antioxidant abilities, namely 96% ethanol extract (24.55 ppm), distilled extract (54.74 ppm), 70% ethanol extract (72.16 ppm), and methanol extract (84.48 ppm). 96% ethanol extract with a concentration of 24.55 ppm has absorbed 50% of free radicals. The ability of 96% ethanol extract is twice as strong as distilled water extract and three times stronger than methanol extract. The difference in IC50 values between extracts is due to differences in the selection of solvents during extraction and extraction methods. Differences in polarity between solvents affect the compounds dissolved in an extract. 96% ethanol solvent is a solvent that has a wide range of attracting compounds during the extraction process because it has semi-polar properties[22].

Table 4. Results of Antioxidant Activity Test of Cempedak Leaf Extract

Sample	Concentration (ppm)	Percent Inhibition	IC50 value	Activity
Extract Aquadest	20	36.112	54.74 ppm	Strong
	40	44.355		
	60	52.257		
	80	59.829		
	100	67.806		
Extract Methanol	20	20.022	84.48 ppm	Strong
	40	29.078		
	60	38.933		
	80	48.180		
	100	56.922		
Extract Ethanol 70%	20	23.697	72.16 ppm	Strong
	40	32.993		
	60	43.979		
	80	53.857		
	100	64.309		
Extract Ethanol 96%	20	47.913	24.55 ppm	Very Strong
	40	56.898		
	60	65.952		
	80	75,420		
	100	83.820		

The selection of solvents to be used in the extraction process must consider the nature of the compound content; essential properties are the polarity and polar groups of a compound. In principle, a material will easily dissolve in a solvent of the same polarity. The distilled water extract is estimated to have fewer bioactive compounds that function as antioxidants because it is influenced by the solvent used. Rizki's research (2021) showed that the results of the antioxidant activity of the ethanol extract of cempedak leaves (*Artocarpus integer*) were included in the strong category with an IC50 value of 52.7706 ppm[3]. Halimatussa'diah's research (2014) showed that the antioxidant activity of cempedak leaves showed very strong results[23]. Taufiqurrahman's research (2021) showed that the results of the antioxidant activity of cempedak leaves were very strong[14].

This study also showed a correlation between marker compound levels and antioxidant activity. High marker compound levels are directly proportional to the antioxidant ability of cempedak leaves. Similar studies also show that total phenol content is directly proportional to antioxidant activity. The research of Rizki et al. (2021), Anwar & Triyasmono (2018), and Kusumowati (2011) showed that antioxidant activity and total phenolic content are directly proportional. The greater the phenol content, the greater the antioxidant activity[6,24,25].

This research's limitation is that solvent optimization was only measured by looking at the parameters of the highest levels of phenolic compounds and antioxidant power. According to the research objectives, further research can be carried out using other parameters.

CONCLUSION

The results of the antioxidant activity test showed the IC50 value of the methanol extract was 84.48 ppm (strong), the distilled water extract was 54.74 ppm (strong), the 70% ethanol extract was 72.16 ppm (strong), and the 96% ethanol extract was 24.55 ppm (very strong). The results of the marker compound determination showed the total phenolic content of the methanol extract was 28.22% w/w, the distilled water extract was 22.59% w/w, the 70% ethanol extract was 29.55%, and the 96% ethanol extract was 31.14 % w/w. It can be concluded that the cempedak leaf extract extracted with 96% ethanol has very strong antioxidant properties and the highest marker compound content among other solvents. For further analysis, 96% ethanol solvent can be used for the cempedak leaf extraction.

CONFLICT OF INTEREST

In this study there is no conflict of interest

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