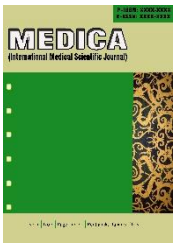
MEDICA

(International Medical Scientific Journal)
Vol.6, No.3, October 2024, pp.107 – 114
ISSN 2622-660X (Online), ISSN 2622-6596 (Print)
https://journal.ahmareduc.or.id/index.php/medica



Phytochemical Identification and Antioxidant Activity Test of Acetone Extract of Celery Leaves (Apium graveolens L.) Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) Method

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Info Article

Article History:
Received:
10 August 2024
Accepted:
14 September 2024
Published:
30 October 2024

Keywords:
Antioxidant Activity Test
Acetone Extract
Celery Leaves
DPPH

Abstrak

Free radicals are formed in the body through oxidation processes and cell combustion during breathing, metabolism, excessive physical activity, as well as exposure to external pollution. Antioxidants play an important role in protecting the body from oxidative damage. Celery leaves (Apium graveolens L.), which are rich in flavonoid compounds, have the potential to be a natural source of antioxidants. This study aims to identify the secondary metabolite content and measure the antioxidant activity of acetone extract of celery leaves based on IC50 values using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The study uses a quasi-experimental design with purposive sampling techniques. The antioxidant activity of acetone extract of celery leaves at concentrations of 30 ppm, 50 ppm, 70 ppm, and 90 ppm was tested using a UV-VIS spectrophotometer at a wavelength of 516 nm. The results showed the presence of secondary metabolite compounds such as alkaloids, flavonoids, phenols, triterpenoids, saponins, and tannins in the acetone extract of celery leaves. The IC50 value of the acetone extract of celery leaves was 115.09490 ppm, indicating moderate antioxidant activity (IC50 value between 100–250 ppm). These findings conclude that acetone extract of celery leaves has potential to be used as a natural antioxidant source.

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1. INTRODUCTION

Free radicals are molecules with unpaired electrons that are unstable and can cause damage to surrounding molecules (Maharani et al., 2021). Free radicals that form covalent bonds with enzymes or receptors can damage the targeted compounds and create new free radical compounds from molecules that have lost electrons. Free radicals can be present in the human body as byproducts of oxidation and cell combustion processes that occur during breathing, cellular metabolism, excessive physical activity, and exposure to external pollutants such as vehicle emissions, cigarette smoke, and solar radiation (Maharani et al., 2021).

An excess of free radicals in the body causes an imbalance between free radicals and antioxidants (oxidative stress), which can lead to oxidative damage at the cellular, tissue, and organ levels, accelerating the aging process and the development of diseases. Oxidative stress can cause biochemical damage to tissues (necrosis), and it underlies nearly all pathophysiological conditions caused by free radicals, such as heart attacks, cancer, cataracts, and decreased kidney function (Yuslianti, 2018).

The human body needs intake of compounds known as antioxidants, which can capture and neutralize free radicals to prevent or inhibit further reactions that cause damage. The formation of free radicals can be inhibited by antioxidants. Antioxidants are any substances that can delay or prevent oxidation damage to target molecules. Chemically, antioxidants are compounds that donate electrons; biologically, they are compounds that can suppress the negative effects of oxidants, including enzymes and metal-binding proteins (Handito et al., 2022).

Antioxidants are classified into two types: synthetic antioxidants and natural antioxidants. Synthetic antioxidants are produced through chemical synthesis, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Natural antioxidants are extracted from natural materials. The use of synthetic antioxidants tends to have negative health effects, as some are harmful and carcinogenic. Natural antioxidants receive greater attention because they are easier to obtain and safer for human consumption (Rahmi, 2017). Antioxidants are obtained from fruits and vegetables that contain vitamins A, C, E, folic acid, anthocyanins, phenolic compounds, and flavonoids.

One plant that has potential as a natural antioxidant is celery. Celery (Apium graveolens L.), which belongs to the Apiaceae family, is known as a culinary herb and can also be used as an antioxidant (Setyaningrum et al., 2021). Celery is widely available and commonly used, but many people are still unaware of its potential as a natural antioxidant. According to research conducted by Shalsyabillah and Sari (2023), phytochemical screening of celery leaves showed that ethanol extract of celery leaves tested positive for flavonoids, steroids, saponins, and tannins. Setyaningrum et al. (2021) stated that oven-dried celery leaves at 40°C resulted in the highest antioxidant activity, with an IC50 value of 33.43 ppm.

Another study by Prayoga et al. (2019) identified phytochemical compounds and antioxidant activity of crude pepe leaf extracts using five different solvents: distilled water, 95% methanol, 70% ethanol, 70% acetone, and 96% ethyl acetate. The study found that 70% acetone was effective in extracting phytochemicals such as alkaloids, saponins, phenols, flavonoids, and steroids in sterol form, with the highest antioxidant activity based on free radical inhibition percentage at 62.06%, an IC50 value of 200.775 mg/L, extract yield of 32.35%, total phenols of 36.14 mg GAE/g extract, and total flavonoids of 50.37 mg QE/g extract.

The extraction used the maceration method with 70% acetone as a solvent. Cold extraction (maceration) was chosen to prevent degradation of compounds due to heating. Acetone was selected as the solvent because it can dissolve both hydrophilic and lipophilic

plant components. The advantages of acetone include its ability to mix with water, volatility, and low toxicity

One of the methods used to test antioxidant activity is the DPPH method. DPPH is a commonly used method for evaluating the antioxidant activity of various compounds or natural extracts. This method is conventional and has long been used for determining antioxidant activity. The DPPH method is easy to use, fast, fairly accurate, and well-suited for use in organic solvents (Sastrawan et al., 2013). A substance is considered a very strong antioxidant if it has an IC50 value below 50 ppm, strong if between 50–100 ppm, moderate if between 100–150 ppm, weak if between 150–200 ppm, and very weak if above 200 ppm (Leksono et al., 2018). This study aims to identify the secondary metabolite content and measure the antioxidant activity of acetone extract of celery leaves based on IC50 values using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

2. METHOD

This study employed a quasi-experimental research design. A 70% acetone extract of celery leaves (Apium graveolens L.) at concentrations of 30, 50, 70, and 90 ppm was prepared, and each concentration was controlled and then measured for its antioxidant content. The study on Phytochemical Identification and Antioxidant Activity Testing of Acetone Extract of Celery Leaves (Apium graveolens L.) Using the DPPH Method was conducted from May 2024 to July 2024. The extraction, phytochemical screening, and antioxidant activity measurements were carried out at the Faculty of Agriculture, Politeknik Negeri Pontianak. The sampling technique used in this study was purposive sampling, where each member of the population could be selected as a sample, provided that it met the predetermined characteristics.

Primary data in this study consisted of the DPPH absorbance values after the addition of celery leaf extract (Apium graveolens L.), which were then used to calculate the IC50 value. Secondary data included IC50 values for celery leaves obtained from other sources. Data collection was performed through laboratory examination by systematically recording the results shown on a UV-VIS spectrophotometer. The instruments used in this research included volumetric flasks, stopwatches, and a UV-VIS spectrophotometer.

The data obtained was analyzed using Microsoft Excel 2021 to determine the IC50 (Inhibitor Concentration 50%) value. The steps used in data analysis were:

1. Percentage inhibition was calculated using the following formula:

$$\text{inhibition } \% = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\%$$

2. The percentage inhibition values obtained were plotted on a linear regression curve, with the inhibition percentage on the y and the extract concentration on the x.
3. The IC50 value was obtained by finding the linear regression equation $y = ax + b$, where y is substituted with 50, and the resulting x is the IC_{50} value.
4. The values of a and b from the equation $y = ax + b$ were derived from the regression curve, and then the equation was solved to find the value of x, which represents the IC_{50}.

Explanation**:**

y = Value of 50
x = IC50 value
a = Slope or gradient of the line
b = Intercept on the y

3. RESULTS AND DISCUSSION

The research sample consisted of celery leaves obtained from Flamboyan Market, located on Jalan Gajah Mada, Benua Melayu Darat, South Pontianak District, Pontianak City, West Kalimantan**.** The extraction process was carried out in the Biological Chemistry Laboratory, Department of Agricultural Technology, Politeknik Negeri Pontianak. The extraction yielded 17.3 grams of thick acetone extract of celery leaves (*Apium graveolens* L.). The moisture content test of the acetone extract of celery leaves showed a result of 5.111%, while the drying shrinkage test showed a result of 6.446%.

Table 1. Phytochemical Screening Results of Acetone Extract of Celery Leaves (*Apium graveolens* L.)

No.	Test Parameter	Method/Reagent	Observation Result	Description
1	Flavonoids	Lead (II) acetate	Yellow color	Positive
2	Alkaloids	Dragendorff's reagent	Orange precipitate	Positive
3	Phenols	2% $FeCl_3$	Dark green color	Positive
4	Steroids	Liebermann–Burchard	Brown ring	Positive
5	Triterpenoids	Liebermann–Burchard	Brown ring	Positive
6	Saponins	2M HCl	Stable foam	Positive
7	Tannins	1% $FeCl_3$	Dark greenish-black color	Positive

Table 1 shows that the phytochemical screening was conducted to identify the secondary metabolite compounds present in the acetone extract of celery leaves (*Apium graveolens* L.). These secondary metabolites are potential antioxidant compounds.

Table 2. Antioxidant Activity Test Results of Acetone Extract of Celery Leaves (*Apium graveolens* L.)

Concentration (ppm)	Absorbance	% Inhibition	Avg % Inhibitior	IC50 (ppm)
30 ppm (U1)	0.607	20.34%		
30 ppm (U2)	0.607	20.34%		
30 ppm (U3)	0.608	20.21%		
30 ppm (U4)	0.608	20.21%		
30 ppm (U5)	0.608	20.21%		20.23%
30 ppm (U6)	0.609	20.08%		
50 ppm (U1)	0.581	23.75%		
50 ppm (U2)	0.576	24.41%		
50 ppm (U3)	0.576	24.41%		
50 ppm (U4)	0.575	24.54%		
50 ppm (U5)	0.575	24.54%		24.39%
50 ppm (U6)	0.574	24.67%		C50:115.09 ppm
70 ppm (U1)	0.522	31.50%		
70 ppm (U2)	0.500	34.38%		
70 ppm (U3)	0.497	34.78%		
70 ppm (U4)	0.491	35.56%		
70 ppm (U5)	0.489	35.83%		34.71%
70 ppm (U6)	0.486	36.22%		
90 ppm (U1)	0.508	33.33%		
90 ppm (U2)	0.491	35.56%		

90 ppm (U3)	0.490	35.70%	
90 ppm (U4)	0.486	36.22%	
90 ppm (U5)	0.478	37.27%	35.98%
90 ppm (U6)	0.474	37.80%	

Table 2 shows that the antioxidant activity test of the acetone extract of celery leaves (*Apium graveolens* L.) using the DPPH method at a wavelength of 516 nm yielded an IC50 value of 115.09490 ppm.

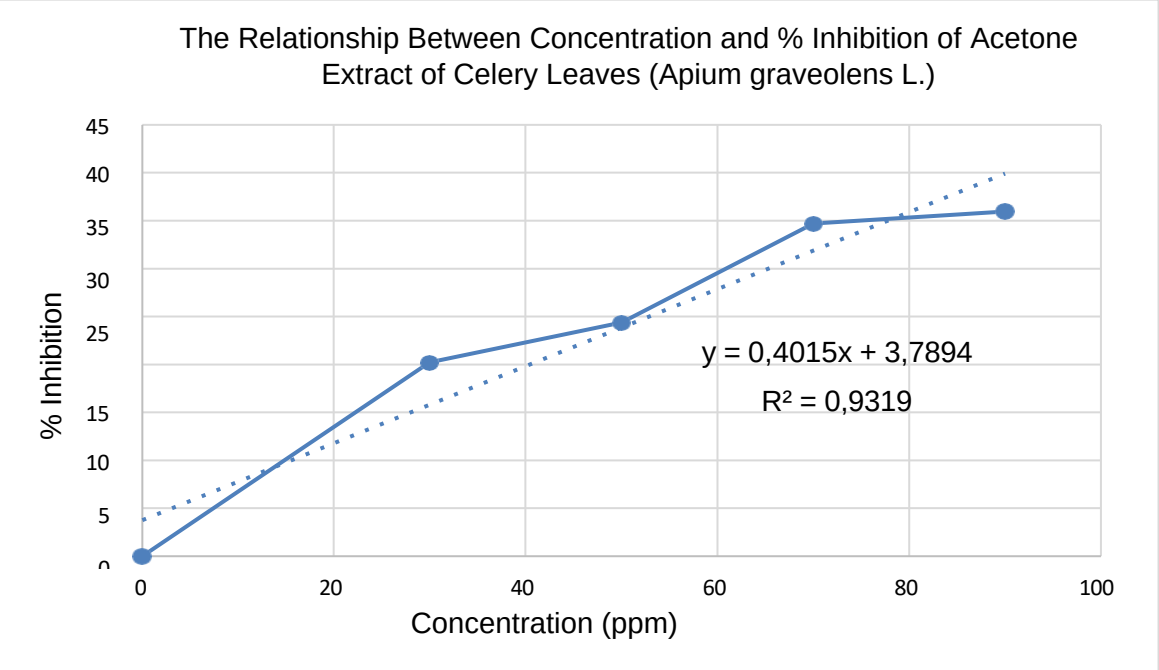

Figure 1. Curve Showing the Relationship Between Concentration and % Inhibition of Acetone Extract of Celery Leaves (Apium graveolens L.)

Figure 1 shows that the curve was obtained using linear regression in the data processing application Microsoft Excel 2021. The values of a and b in the equation $y = ax + b$ appear on the curve, which is then used to determine the value of x from the equation, where the y coefficient in the linear equation is set to 50. Meanwhile, the x coefficient—representing the IC50 value in the linear equation—indicates the concentration of the extract to be determined. Based on the above equation, the IC50 value of the acetone extract of celery leaves is as follows:

$y = ax + b$
$y = 0.4015\ x + 3.7894$
$50 = 0.4015\ x + 3.7894$
$50 - 3.7894 = 0.4015\ x$
$46.2106 = 0.4015\ x$

$$x = \frac{46.2106}{0.4015}$$

$x = 115.09490$

Based on the x value obtained from the linear equation, the extract concentration was found to be 115.09490 ppm, which is classified as a moderate antioxidant.

DISCUSSION

The plant used in this study is celery leaves (Apium graveolens L.). The celery leaves were purchased on May 30, 2024, at Flamboyan Market, Gajah Mada Street, Benua Melayu Darat, Pontianak South District, Pontianak City, West Kalimantan. The obtained sample was sorted wet, meaning fresh plants were selected according to the sample criteria for the study and to avoid contamination by dirt and other unnecessary parts. A total of 2 kilograms of sorted celery leaves were washed slowly with running water and then drained. The next step was drying for 16 hours to reduce the moisture content in the sample to prevent spoilage and mold growth. Drying was done using a cabinet dryer at 40°C to control the temperature and minimize damage to the simplicia caused by excessive heat, which could affect or damage the active compounds in the simplicia. The dried simplicia was then ground using a blender and sieved to obtain 117.8 grams of simplicia powder.

The extraction was carried out using the maceration method with 70% acetone as the solvent, because acetone is a semi-polar organic solvent that can attract both polar and non-polar compounds, allowing the compounds to be extracted into the acetone solvent. The maceration process lasted 3 days, with the solvent being replaced every 24 hours. During the maceration process, the mixture was stirred several times to ensure the active compound extraction was maximized. The filtrate, which was the extract solvent, was collected and evaporated using a rotary evaporator at 40°C to obtain a thick acetone extract of celery leaves.

Based on the phytochemical identification performed, the thick extract of celery leaves tested positive for alkaloids, indicated by the formation of an orange precipitate. Flavonoids tested positive with a yellow color formation. Phenols tested positive with a green color formation. Steroid/triterpenoids tested positive with the formation of a brownish ring. Saponins tested positive with the formation of stable foam for 10 minutes. Tannins tested positive with the formation of a dark green color.

Antioxidant activity was tested using a UV-VIS spectrophotometer. The antioxidant test was conducted quantitatively to determine the remaining DPPH absorbance after adding the acetone extract of celery leaves. If a compound has antioxidant activity, there will be a decrease in absorbance caused by the increased presence of antioxidant particles that inhibit free radicals in the solution, thus reducing light absorption. The decrease in absorbance is indicated by the color change in the sample solution from dark purple to pale yellow. This corresponds to the Lambert-Beer law, which states that colored solutions have maximum absorption; the more intense the color in the sample, the higher the absorbance. Conversely, if the solution does not have a strong color, the resulting absorbance will be low.

The color change is due to the reduction in conjugated double bonds on DPPH as it captures an electron from the radical-scavenging substance, preventing that electron from resonating. This change can be measured and recorded with a spectrophotometer. DPPH absorbance is measured against a blank, which is the DPPH absorbance in methanol without the addition of the test substance. The degradation of DPPH color is directly proportional to the concentration of the extract added. Based on the obtained DPPH absorbance values, the percentage of radical inhibition (% inhibition) can be determined, and from this, the IC50 (inhibitory concentration) value can be calculated.

The antioxidant activity test results show that the acetone extract of celery leaves has a moderate antioxidant activity, meaning its antioxidant strength is neither too strong nor too weak, but it is quite effective in capturing and neutralizing free radicals, with an IC50 value between 100-250 ppm, specifically 115.09490 ppm.

The acetone extract of celery leaves exhibits moderate antioxidant activity. This is related to the secondary metabolite compounds present in celery leaves. Flavonoids play a role as antioxidants by capturing DPPH free radicals. DPPH free radicals oxidize flavonoids, forming a radical with low reactivity. Flavonoids donate hydrogen radicals from the aromatic ring and produce non-toxic flavonoid radicals. Tannin compounds can also function as biological antioxidants. A higher tannin content increases antioxidant activity because tannins are composed of polyphenolic compounds that have free radical-scavenging activity (Purwaningsih & Deskawati, 2021).

These compounds have antioxidant activity, but the content of these compounds in the plant is likely very low, resulting in a less-than-optimal antioxidant activity. Therefore, quantitative tests are needed to determine the concentration of secondary metabolites in the test plant. The phytochemical content in a plant is influenced by both internal and external factors. Internal factors such as genetics and external factors such as light, temperature, humidity, pH, nutrient content in the soil, and altitude affect the plant's growth. Differences in growing locations impact plant growth and development, which, in turn, disrupts the metabolism, leading to variations in the quantity of compounds produced.

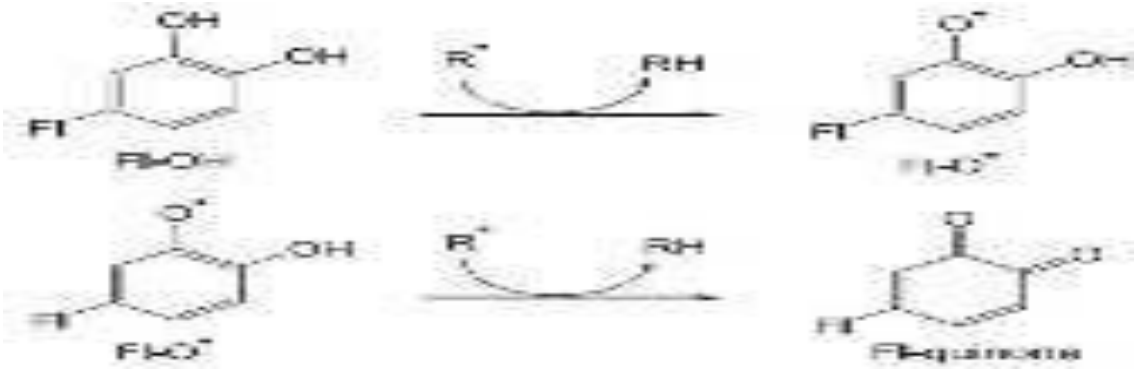

Figure 3. Reaction Between Flavonoids and DPPH Radicals.

Flavonoids have antioxidant properties because they are capable of transferring an electron to free radical compounds, where R• represents a free radical compound, FI-OH represents the flavonoid compound, and FI-OH• represents the flavonoid radical (Hassanpour, & Doroudi, 2023).

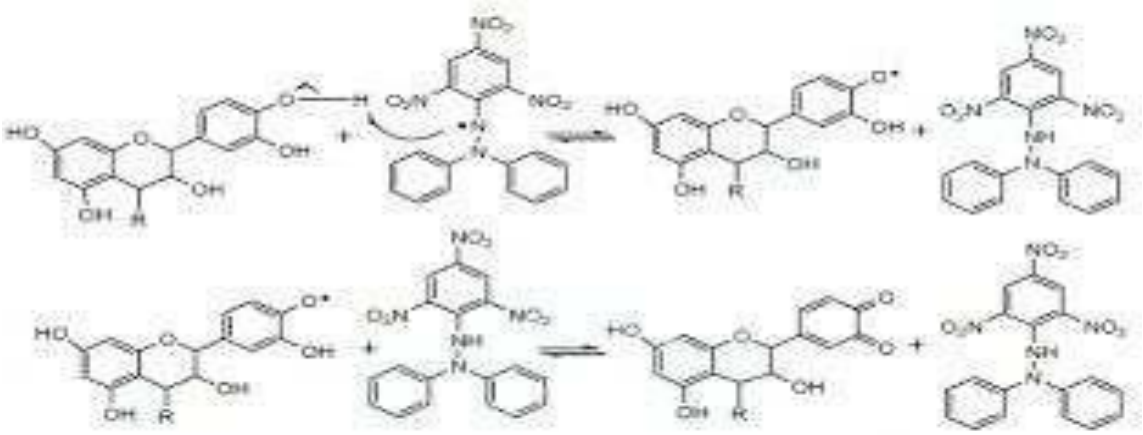

Figure 4. Reaction Between Tannins and DPPH Radicals.

The image above shows that all three isolates belong to the flavonol group, which contains more than one hydroxyl (OH) group. Tannins are classified as flavonoids, which are good reducing compounds and inhibit many oxidation reactions. Tannins contain antioxidants because they have phenolic groups, where phenols are known for their ability to combat free radicals. Phenols also have a benzene ring structure that binds to hydroxyl groups (Mabruroh, 2015; Hassanpour, & Doroudi, 2023).

4. CONCLUSION

Based on the antioxidant activity study of the acetone extract of celery leaves (Apium graveolens L.) using the DPPH method, it can be concluded that this extract contains a variety of secondary metabolite compounds such as alkaloids, flavonoids, phenols, terpenoids/triterpenoids, saponins, and tannins. The IC50 value of the acetone extract of celery leaves, which is 115.09490 ppm, indicates that its antioxidant activity potential is categorized as moderate. Therefore, it is recommended to conduct further research to quantitatively test the content of secondary metabolite compounds in the acetone extract of celery leaves (Apium graveolens L.).

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