Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. 9 No. 4, October - December 2024
https:/ojs.ummada.ac.id/index.php/iojs

ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT *Areca catechu* L. Stalk USING THE DPPH METHOD

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Submitted: November 15, 2024 Revised: December 13, 202 Accepted: December 26, 2024

ABSTRACT

A monocotyledonous plant from the wild palm group, the areca nut (*Areca actechu* L.), has numerous health advantages. Most people in Sorong City empirically cure illnesses with areca nut medication. Using the DPPH (2,2 diphenyl-1-picrylhydrazyl) technique, the secondary metabolite chemicals and antioxidant activity were determined. The areca palm stalks were extracted using a maceration method with a 96% ethanol filter. The IC_{50} value was used to test for antioxidants using UV-Vis spectrophotometry. Phytochemical analysis revealed that the areca stem extract contained tannins, alkaloids, steroids, and flavonoids.The results of the antioxidant activity test of areca nut stalk extract showed that the IC_{50} value in replication 1 was 32.66µg/mL, replication 2 was 32.88µg/mL, and replication 3 was 32.84 µg/mL. Conclusion: Ethanol extract of areca stalk (*Areca catechu* L.) has secondary metabolite compounds and antioxidant activity with an average IC_{50} value of mean ± SD = 32,79 ± 0,114.

Keywords: antioxidants, dpph method, *Areca catechu* L.

INTRODUCTION

Figure 1. *Areca catechu* L.

Free radicals are a popular topic in the medical world. Free radicals have been shown to play a role in various diseases (Fitria *et al*., 2013). Antioxidants are one of the compounds that can play a role in fighting free radicals (Labola & Puspita, 2017). Antioxidant molecules are naturally present in plants, specifically as byproducts of their secondary metabolites. Plant secondary metabolites include alkaloids, flavonoids, terpenoids, tannins, and saponins. As bioactive substances are utilized in medicine, chemicals derived from plants are significant (Anisa *et al*., 2023; Muthmainnah, 2017).

Areca nut (*Areca cathecu* L.) is one of several plants with potential as an antioxidant. Areca nut (*Areca cathecu* L.) is a plant often used as medicine by the community to cure

various diseases. In the results of previous research, areca nut (*Areca cathecu* L.) has been proven to have many benefits in terms of treatment for various diseases. The benefits of areca nut include anti-microbial, anti-schizophrenic, anti-inflammatory, anti-migraine, and improving memory (Marina,2020).*Areca cathecu* L. also contains phenols, flavonoids, alkaloids, saponins, tannins, and terpenoids which are also secondary metabolite compounds(Yani & Suwendar, 2022).

In previous research, the betel nut plant was reported to have alkaloids, flavonoids, saponins, and tannins which are secondary metabolite compounds, and areca nut skin contains tannins, saponins, flavonoids, and alkaloids (Cahyani & Yulianis, 2020).Tannin and flavonoid compounds are also found in areca nut seeds, apart from that, carbohydrates, fats, fiber, alkaloids, and minerals are also found (Petrina *et al*., 2017).

This study used the DPPH (2,2 diphenyl-1-picrylhydrazyl) technique to screen for phytochemicals and determine whether areca palm stalk extract has antioxidant activity. The goal was to identify the secondary metabolite molecules. Areca nut stalks have the potential to be a very helpful source of information for the community, demonstrating that they are not simply organic waste but also have health benefits.

MATERIALS AND METHODS

Tools and materials

This research uses UV-Vis spectrophotometry (Termo Genesis 150). The materials used include areca stem (*Areca catechu* L.), 2,2 diphenyl-1-picrylhydrazyl/DPPH (PT. HiMedia), and quercetin (PT. Merck Indonesia), Ethanol 96% (PT. Merck Indonesia), Ethanol 96% p.a (PT. Merck Indonesia), Asam sulfat (PT. Satona), Asam klorida 2N (PT. Satona), dan Besi (III) klorida (PT. Aneka Kimia Inti).

Research Procedures

Extraction of*Areca catechu* L.

Areca catechu L. stems were obtained from Sorong City, Southwest Papua Province by plucking them directly from the tree during harvesting. Making a thick extract of areca nut stalks is carried out using the maceration method, namely soaking dried simplicia of areca nut stalks in 96% ethanol solvent. The maceration process was carried out for three days, and then re-macerated again for two days. During maceration, stir 3 times a day and use a water bath to thicken the areca stem extract.

Phytochemical Screening of *Areca catechu* L. Stalks.

a. Flavonoid Compound Test

A test tube was filled with 2 mL of the extract and 2–3 Pb II acetate was added. If a yellow precipitate appears, flavonoid chemicals are present (Erawati *et al*., 2024; Yuda, 2017).

b. Alkaloid Compound Test

The extract (2 mL) was placed in a test tube, 5 mL of HCN 2N was added, and the mixture was heated and cooled. One milliliter of the resulting solution was placed in three different test tubes, and the respective reagents were added. If in Meyer's reagent, there is a white or yellow precipitate, in Dragendrof's reagent there is an orange precipitate, and in Bouchardat's reagent there is a black-brown precipitate, it means it is positive for alkaloids (Erawati *et al*., 2024; Muthmainnah, 2017).

c. Tannin Compound Test

Put 1gram of thick extract into a test tube, add 10 mL of hot water then boil for five minutes. Then add 3-4 drops of $FeCl_3$ were added to the filtrate. The presence of catechol tannins occurs because the color changes to blue-green and pyrogallol tannins because the color changes to black-blue (Erawati *et al*., 2024; Muthmainnah, 2017).

d. Saponin Compound Test

Put 1 gram of the sample extract was placed in a test tube, 10 mL of hot water was added, and the mixture was allowed to sit until it cooled and shaken for 10 seconds. If foam

is formed as high as 1-10 cm, no less than 10 minutes and on the rise. If foam is formed as high as 1-10 cm <10 minutes and persists when 2N HCl is added, it indicates that it is positive for saponin. (Muthmainnah, 2017).

e. Steroid and Terpenoid Compound Test

The sample extract (2 mL) was placed in a test tube, and two drops of anhydrous sulfuric acid reagent and one drop of sulfuric acid were added. Steroids are present if the color changes to green or blue and terpenoids are present if the color changes to red or purple (Ningsih, 2017).

Antioxidant Activity Test of *Areca catechu* L. stalks

a. Preparation of 0.4 mM DPPH Solution and Determination of Maximum Wavelength (λ max) of DPPH

DPPH powder was weighed as much as 0.0157 grams dissolved in ethanol p.a in a 100 mL volumetric flask. The DPPH solution was then pipetted to a volume of 1 mL into a 5 mL volumetric flask and the volume was made up to the mark with ethanol p.a.. The absorbance of the DPPH solution was measured at a maximum wavelength of 400-600 nm to obtain an absorbance at a wavelength of 517 nm.

b. Preparation of Ethanol Extract Stock Solution and Test of Ethanol Extract of *Areca catechu* L. Stalks 1000ppm

A total of 10 mg of *Areca catechu* L. stalk extract was weighed and dissolved with ethanol p.a until homogeneous in a beaker then transferred into a 100mL volumetric flask and the volume was filled with ethanol p.a to the mark. The stock solution was then made in five concentrations, namely 2 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm, by pipetting the 0.01 stock solution; 0.03; 0.04; 0.05; and 0.06mL into a 5 mL flask and adding ethanol p.a to the mark.

c. Measurement of antioxidant activity of the ethanol extract of *Areca catechu* L. Stalks.

Take 2mL of test solution from each concentration and add 1mL of 0.4mM DPPH then stored for 30 minutes in the dark. The absorbance was measured at a wavelength of 517nm, and the absorbance results were calculated to obtain the percent inhibition value.

d. Preparation and Measurement of Antioxidant Activity of quercetin standard solution

Quercetin (1 mg) was placed in a beaker and dissolved in ethanol until homogeneous, then transferred to a 10mL flask and the volume was increased to the mark. Quercetin was then divided into five concentrations, namely 0.1ppm, 0.2ppm, 0.3ppm, 0.4ppm, and 0.5ppm by pipetting 0.005 quercetin stock solution; 0.001; 0.015; 0.02; and 0.025mL into a 5mL volumetric flask, the volume added with ethanol p.a to the mark.

The comparison solution for each concentration was 2 mL, and 1 mL of 0.4 mM DPPH. The absorbance was measured at a wavelength of 517nm after storage for 30 minutes in the dark.

Data Analysis

Antioxidant activity was calculated using the following equation:

$$\%\ \text{Inhibitory activity} = \frac{(\text{blank absorbance} - \text{sample absorbance})}{\text{blank absorbance}} \times 100$$

After obtaining the percentage inhibition results, the IC_{50} value was calculated by entering the calculation results in the form of the regression equation y= ax+b. The x-axis is areca nut stalk extract and the y-axis is the IC_{50}value (Suyatmi *et al*., 2019).

RESULTS AND DISCUSSION

The part of *Areca catechu* L. used in this research was the stalk obtained from Sorong City, Southwest Papua Province. A 300 g sample of *Areca catechu* L. stalk was extracted using the maceration method to produce a thick extract with a yield value of 19.7%. The aim of choosing a maceration method is to make it safe for compounds that are not heat resistant

so they do not decompose easily and this method is simpler (Dwi Puspitasari & Proyogo, 2017). The choice of 96% ethanol as a filter is due to its universal nature, good absorbance, high filtering ability, and non-toxicity (Wendersteyt *et al*., 2021). The yield results obtained are under the provisions in the Indonesian Herbal Pharmacopoeia, namely the yield requirement is not <10% (Badriyah & Farihah, 2023).

Table I. ***Phytochemical screening results for Areca catechu L. Stalks.***

Compound class	Reagent	Observation result	Test results
Flavonoids	Lead (Pb II acetate)	Yellow precipitate	+
Alkaloids	Dragendrof	Light brown precipitate	+
	Bouchardat	Dark chocolate deposits	+
	Mayer	No yellow precipitate	-
Terpenoids	Liberman-Burchard	Not red	-
Steroids		Green	+
Saponin	HCl 2N	Doesn't foam	-
Tannin	$FeCl_3$	Dark blue or dark green	+

Information: (+) The presence of secondary metabolite compounds
(−) Absence of secondary metabolite compounds

Phytochemical screening is a preliminary step in the identification of secondary metabolites in *Areca catechu* L. stalk extracts. The method used for this purpose is the tube test, which looks for color changes in a test sample. This investigation revealed several secondary metabolites including flavonoids, alkaloids, tannins, steroids, terpenoids, and saponins. Yellow precipitates were observed in the stalk extract of *Areca catechu* L., indicating positive flavonoid results. This is because flavonoid molecules have a hydroxy group in their benzene ring (Saputera, 2019). Three different reagents, meyer, dragendrof, and bouchardat, were used to identify alkaloids. The presence of light brown precipitates in the dragendrof reagent and dark brown precipitates in the bouchardat reagent was identified, indicating the existence of positive alkaloids. The precipitate is created when the potassium-alkaloid complex attaches to the alkaloid reagent to form K^+ ions, which then link with the free electrons of the nitrogen atom. The unbound electrons of the nitrogen atom and K^+ ions will form bonds with one another in alkaloid reagents (Oktavia & Sutoyo, 2021). Identified steroids in areca nut stalk extract are characterized by a change in color to bluish green which is caused by the steroid compound being oxidized by the conjugated double bonds formed (Sulistyarini *et al*., 2016). The color change to blackish green indicates that there is tannin in the extract, a result of the reaction between F^{3+} ions and tannin (Ergina, 2014).

Testing of the antioxidant activity extract of *Areca catechu* L. stalks and the quercetin reference solution was carried out using UV-Vis spectrophotometry using the DPPH method. *Areca catechu* L. stalk extract and a comparison solution of quercetin were made in five different concentrations and the *Areca catechu* L. stalk extract was replicated three times. The concentrations of *Areca catechu* L. stalk extract were 2, 6, 8, 10, and 12 ppm, whereas the quercetin concentrations were 0.1, 0.2, 0.3, 0.4; and 0.5 ppm. Quantitative tests using DPPH were performed by determining the IC_{50} value of the ethanol extract of *Areca catechu* L. stalks. Quercetin was used as a positive control. This compound has biological activity with the ability to ward off strong free radicals and is a flavonoid (Hasanah *et al*., 2023).

The standard curve created based on the "Lambert-Beer" law shows a linear relationship between concentration and absorbance at a certain wavelength (Prasetyo *et al*., 2021). The wavelength was determined to be 517 nm by measuring the maximum λ of DPPH in the 400–600 nm range. According to opinion Cahyaningsih *et al*., (2019), The wavelength range for standard DPPH solutions that can be measured is 400-800nm. The antioxidant activity of the extract was expressed as the percentage of inhibition of DPPH free radicals by the extract. The antioxidant power was measured using the IC_{50} value, where a sample concentration of 50% inhibited DPPH free radicals.

Table II. ***Test Results of Antioxidant activity of Areca catechu L. Stalks.***

Replication	Concentration (µg/mL)	Absorbance	% inhibition	IC_{50}
1	12	0.477	16.02	32.66
	10	0.500	11.97	
	8	0.550	3.17	
	6	0.552	2.82	
	2	0.556	2.11	
2	12	0.478	15.85	32.88
	10	0.500	11.97	
	8	0.552	2.82	
	6	0.553	2.64	
	2	0.556	2.11	
3	12	0.477	16.02	32.84
	10	0.501	11.80	
	8	0.550	3.17	
	6	0.552	2.82	
	2	0.556	2.11	

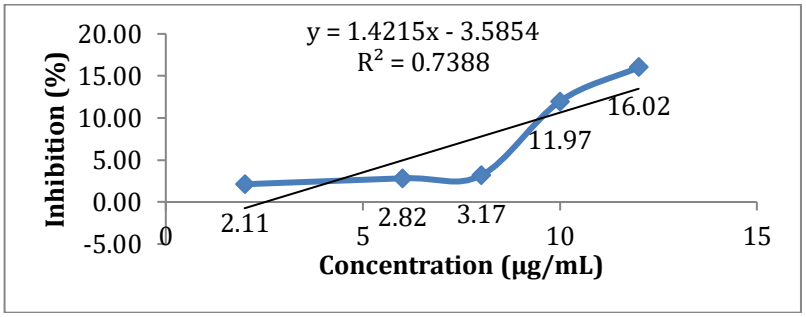

Figure 2. Correlation Curve of Concentration with % Inhibition of *Areca catechu* L. Stalk Extract (Replication 1)

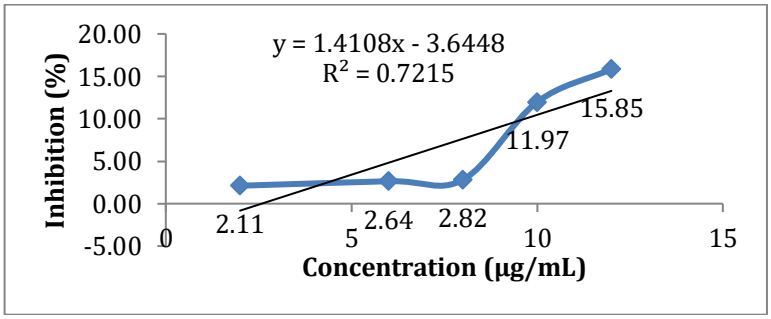

Figure 3. Correlation Curve of Concentration with % Inhibition of *Areca catechu* L. Stalk Extract (Replication 2)

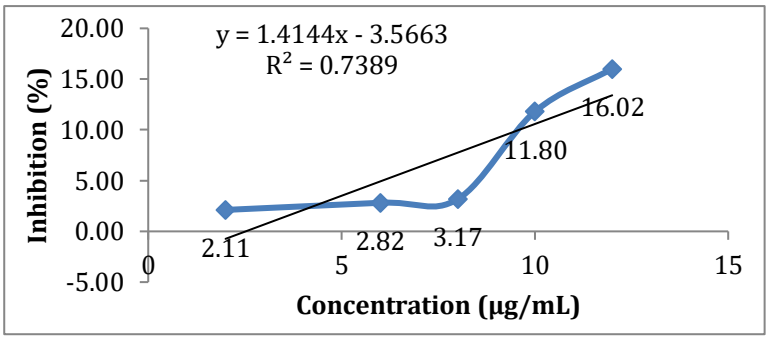

Figure 4. Correlation Curve of Concentration with % Inhibition of *Areca catechu* L. Stalk Extract (Replication 3)

Table II shows that the absorbance values for each concentration of each replicate decreased. The higher the extract concentration, the lower is the absorbance value. Research conducted by Moniung *et al*., (2022), that the lower absorbance obtained is due to the higher concentration value. Low absorbance indicates that more free radicals are inhibited. The percentage of inhibition and IC_{50} value of the antioxidant activity of the areca nut stalk extract were obtained by calculating the obtained absorbance value. The inhibition percentage is the ability of the sample to ward off free radicals, which is related to the sample concentration. The results of the analysis of the ethanol extract of areca stem stalks showed that there was antioxidant activity which was known from the IC_{50} value, namely replication 1 = 32.66µg/mL, replication 2 = 32.88µg/mL, and replication 3 = 32.84µg/mL. According to Li'aini *et al.* (2021), antioxidant compounds tested using the DPPH method have a level of antioxidant power that can be categorized as very strong if IC_{50} < µg/mL, strong if IC_{50} = 50-100µg/mL, moderate if IC_{50} 100 -150, and weak if IC_{50} is 150-200µg/mL.

Table III. Results of Antioxidant Activity Testing of Quercetin Comparative Solution

No	Concentration (µg/mL)	Absorbance	Antioxidant activity (%)	IC_{50} value (µg/mL)
1	0.1	0.476	11.52	
2	0.2	0.469	12.83	
3	0.3	0.457	15.06	1.49
4	0.4	0.431	19.89	
5	0.5	0.419	22.12	

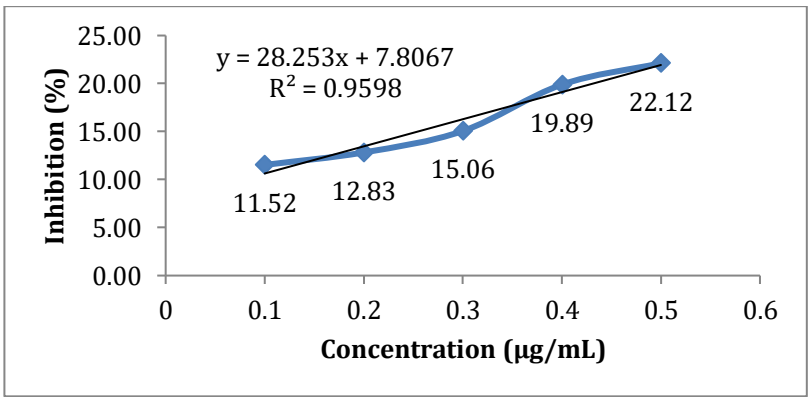

Figure 5. Curve of the relationship between concentration and % inhibition of quercetin

The quercetin comparison solution showed very strong antioxidant activity, with an IC_{50} value obtained for quercetin, namely 1.49 µg/mL. Its antioxidant activity is very strong, with an IC_{50} of <50 µg/mL. A large concentration of the comparator will make the absorbance value of the comparator lower so that the % antioxidant activity value becomes greater (Sari & Sari, 2023).

CONCLUSION

The ethanol extract of *Areca catechu* L. stalks contained flavonoids, alkaloids, tannins, and steroids. The ethanol extract of *Areca catechu* L. stalks also has very strong antioxidant activity, namely replication I of 32.66µg/mL, replication II of 32.88µg/mL, and replication III of 32.84µg/mL.

ACKNOWLEDGEMENT

The author would like to express his thanks to the Pharmacy Study Program, UNIMUDA Sorong Integrated Laboratory, and UNIMUDA Sorong Natural Ingredients Pharmacy Laboratory for facilitating the conduct of this research.

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