J-CEKI : Jurnal Cendekia Ilmiah
Vol.5, No.1, Desember 2025

The Effect Of Maceration Extract And Sonication Extract Of Seeds Areca Nut (*Areca Catechu* L.) On Antioxidant Activity On Dpph Free Radicals (*1,1-Diphenyl-2-Picrylhydrazyl*)

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Article History:
Received: 10 Desember 2025
Revised: 23 Desember 2025
Accepted: 26 Desember 2025

Keywords: *Effect of extract, antioxidant activity*

Abstract: *Free radicals are relatively unstable molecules, having one or more unpaired electrons in their outer orbitals. These molecules are reactive in the emergence of various diseases such as inflammation, atherosclerosis, cancer and premature aging. The activity of these radicals can be inhibited by the work of antioxidants. Areca nut seeds are proven to contain phenolic compounds and have activity as free radical scavengers . This research is an experimental study because an experiment was conducted on the samples used, namely areca nut seeds. The results of the antioxidant activity test showed that the IC $_{50\ value}$ of the sonication extract was smaller than the maceration extract. Therefore, when compared with the IC $_{50\ category\ table,}$ the sonication extract is included in the group of substances that have stronger antioxidant activity than the maceration extract. This is indicated by the IC $_{50\ value}$ of 97.34 ppm which is classified as strong in the IC $_{50\ category\ table.\ While\ the\ IC\ 50}$ value of the maceration extract is 145.063 ppm which is included in the medium category. However, the IC $_{50\ values}$ of both extracts are still far from the IC $_{50\ value}$ of vitamin E which is classified as a very strong antioxidant with an IC $_{50\ value}$ of 0.514 ppm.*

INTRODUCTION

The busyness and activities of daily life make many people unaware of the presence and impact of free radicals on the body's organs, especially in the development of various chronic and acute diseases. Air pollution, ultraviolet radiation, smoking, stress, unhealthy diets, high-fat foods, and other factors can unknowingly enter the body and lead to increased free radical production.

Free radicals are relatively unstable molecules with one or more unpaired electrons in their outer orbitals. These molecules are reactive and contribute to various diseases such as inflammation, atherosclerosis, cancer, and premature aging. The activity of these radicals can be inhibited by antioxidants (Munim *et al* ., 2008) .

Simbala and Tallei (2010) state that antioxidants are free radical scavengers that play a crucial role in inhibiting lipid oxidation. Antioxidants play a crucial role in neutralizing and destroying free radicals that can cause cell damage and biomolecules such as DNA, proteins, and

lipoproteins in the body, which can ultimately trigger degenerative diseases such as cancer, heart disease, arthritis, cataracts, diabetes, and liver disease.

Areca nut *(Areca catechu* L. *)* contains secondary metabolites such as alkaloids, flavonoids, triterpenoids, steroids, and tannins, which have anticancer, antitumor, antibacterial, and antifertility effects (Simbala, 2007). These effects of areca nut seeds are thought to be due to the presence of antioxidant compounds.

Betel nut seeds have been shown to contain phenolic compounds and act as free radical scavengers (Satolom *et al* ., 2015). Flavonoids are a type of phenolic compound found naturally in nature, possessing antioxidant potential and medicinal bioactivity (Rohyami, 2009). In addition to its medicinal properties, the antioxidant properties of betel nut seeds are also used in cosmetic products, including transparent soaps for skin care and beauty treatments.

Research conducted by Mokoginta *et al.* (2013) demonstrated that different extraction methods influence the antioxidant activity of areca nut seeds. Based on this, the researchers used maceration and sonication methods to extract areca nut seeds.

Both maceration and sonication are effective extraction methods for extracting secondary metabolites from medicinal plants. However, of the two extraction methods, sonication is thought to produce extracts with higher antioxidant activity than maceration. This is because the sonication method uses ultrasonic waves to extract the active ingredients from the sample. To demonstrate this, this study will compare the maceration and sonication extraction methods on the antioxidant activity of areca nut seeds.

THEORETICAL BASIS

plant, especially the seeds, is widely used traditionally to treat various ailments such as scabies, diphtheria, worms, menstrual pain, and to strengthen teeth and gums . Furthermore, betel nuts have been shown to be beneficial as free radical scavengers due to their antioxidant content , including flavonoids, alkaloids, steroids , and tannins.

Betel nuts contain antioxidants that are beneficial for the skin . One way to improve the quality of betel nut extract and achieve high antioxidant activity is by selecting an appropriate extraction method. Several studies have shown that different extraction methods significantly affect the antioxidant activity of betel nuts. Possible extraction methods include maceration and sonication. The extracts obtained from both extraction methods were then tested for antioxidant activity using the DPPH *(1,1-diphenyl-2-picrilhydrazil) method* . This method was chosen because it is easy, fast, simple, and has a high level of sensitivity, and can analyze a large number of samples in a short period of time. Antioxidant activity is expressed in IC50 , which is the concentration required to inhibit free radicals by 50%.

RESEARCH METHODS

type of research is experimental research because an experiment was carried out on the sample used, namely areca nut seeds. This research uses a comparative descriptive research design.

RESULTS AND DISCUSSION

1. Organoleptic Test of Powder

Table 1. Organoleptic Test Results of Areca Nut Powder

No	Characteristics	Observation result
1	Color	Brownish Red
2	Smell	Pinang Specialty
3	Form	Powder
4	Texture	Fine

2. Powder Moisture Content Test

Table 2. Results of Water Content Test of Areca Nut Powder

Powder Weight Before Oven (grams)	Weight of Powder After Oven (grams)	Water content
2	1,905	4.75%

3. Extract Yield Calculation

Table 3. Results of Extract Yield Calculation

Extraction Type	Maceration			Sonication		
Extract	A	B	C	A	B	C
Yield	52.20%	49.00%	48.40%	54.66%	63.04%	52.39%
Average		49.86%			56.69%	

The average yield of areca nut methanol extract obtained from maceration and sonication extraction was 49.86% and 56.69%, respectively. This average indicates that the sonication extract yield was higher than the maceration extract yield.

1. Antioxidant Activity Test

The antioxidant activity test used the DPPH method, and antioxidant activity is expressed as an IC50 value . Maceration and sonication of areca nut extracts were tested for antioxidant activity, resulting in absorbance values. The inhibitory activity (% inhibition) was then calculated and compared with the absorbance of the control to obtain the IC50 value for each sample.

The results of the antioxidant activity of each sample can be seen in table 4.4 below:

Table 4. Results of IC50 Values of Maceration and Sonication Extracts

Extraction Method	IC_{50} (Mean ± SD)	*T-test p value*
Maceration	145,063 ± 103,2048	0.557
Sonication	97.34 ± 77.6612	

Based on data processing, the data obtained was normally distributed with a significance value of 0.456. Next, the data was tested for homogeneity and the results were homogeneous data with a significance value of 0.547. Because the data obtained was normally distributed and homogeneous, the statistical test used was the T-Test. The results of the T-Test showed a significance value of 0.557, which means that there was no significant difference between the two. significant differences between maceration and sonication extraction methods on antioxidant activity in areca nut seeds.

The antioxidant activity test of macerated and sonicated areca nut extract was conducted using the DPPH (*1,1-dyphenil-2-picrylhydrazil*) method. This method was chosen because it is simple, easy, fast, and sensitive to determine the antioxidant activity of natural compounds. The antioxidant activity test begins with determining the optimum wavelength of DPPH absorption or absorbance using a blank solution. DPPH solution shows maximum absorbance at a wavelength of 512-520 nm (Molyneux, 2004). In this study, the best absorbance was obtained at a wavelength of 520 nm with an absorbance value of 0.756. This wavelength was then used to measure the absorbance of DPPH which was reacted with each sample whose antioxidant activity would be measured.

The DPPH absorbance value obtained was used to calculate the percentage of free radical inhibition (IC50) . The IC50 value is a number that indicates the extract concentration (ppm) that is able to inhibit the oxidation process by 50%. The smaller the IC50 value , the higher the antioxidant activity (Zuhra *et al* , 2008). The IC50 value can be determined using a linear regression equation.

The DPPH method is based on the reduction of a DPPH solution in methanol, a colored free radical, by a free radical quencher. The DPPH radical quenching test is a decolorization test to measure the ability of antioxidants to directly react with (quench) DPPH radicals by monitoring their absorbance at their optimum wavelength with a spectrophotometer. DPPH radicals are free radicals with a stable organic nitrogen center that are dark purple in color and become colorless when reduced to a non-radical form by antioxidants (Antolovich *et al* , 2001, Shivaprasad, 2005). Color quenching occurs due to the presence of compounds that can provide hydrogen radicals to DPPH radicals so that they are reduced to DPPH-H (*1,2 diphenyl-2-picrylhydrazyl*) (Rahim, 2012).

Descriptively, the results of the antioxidant activity test in table 4.4 show that the IC 50 value of the sonication extract is smaller than that of the maceration extract. Therefore, when compared to the IC 50 category table , the sonication extract is included in the group of substances that have stronger antioxidant activity than the maceration extract. This is indicated by the IC 50 value of 97.34 ppm, which is classified as strong in the IC 50 category table. Meanwhile, the IC 50 value of the maceration extract is 145.063 ppm, which is included in the medium category. However, the IC 50 values of both extracts are still far from the IC 50 value of vitamin E, which is classified as a very strong antioxidant with an IC 50 value of 0.514 ppm.

The difference in IC50 of the two extracts is possible due to the extraction method used. According to Oktavia (2011), the sonication extraction method utilizes ultrasonic waves which can speed up the contact time between the sample and the solvent due to the cavitation process, namely the process of forming small bubbles due to the transmission of ultrasonic waves to help the diffusion of the solvent into the plant cell walls. This causes the process of mass transfer of bioactive compounds from within the plant cells to the solvent to be faster, so that in a short time the bioactive compounds in the sample are extracted well.

The bioactive compounds found in betel nuts that act as antioxidants are flavonoids. The

methanol extract of betel nuts from sonication may contain higher levels of flavonoids than the methanol extract from maceration, thus resulting in stronger antioxidant activity. However, the flavonoid levels of the sonication and maceration extracts were not examined in this study, requiring further investigation into the flavonoid content of betel nuts.

CONCLUSION

The results of the antioxidant activity test on the macerated extract of areca nut showed antioxidant activity with an IC50 value of 145.063 ppm and is classified as a moderate antioxidant. The results of the antioxidant activity test on the sonicated extract of areca nut showed antioxidant activity with an IC50 value of 97.34 ppm and is classified as a strong antioxidant. There was no effect between maceration extraction and sonication extraction on the antioxidant activity of areca nut.

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