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Ultrasonic Extraction of Betara's Areca Nuts' Antioxidants

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ABSTRACT
Betara's areca nuts are high quality areca nuts from Betara, Tanjung Jabung Barat, Jambi, Indonesia. Areca contains many nutrients and active compounds such as flavonoids, phenolics, catechin, quercetin, and a small percentage of tannins, beneficial for natural antioxidants. The natural antioxidants can be separated through Ultrasonic Assisted Extraction or UAE technique. The advantages of the UAE technique are that it uses low temperature, reduces solvent use, and is cost-effective and quick. The ultrasonic waves in the UAE technique will accelerate the penetration of the liquid into the cell membrane wall so that the immediate release of active compounds. This study aims to determine the effect of excitation time on antioxidant activity in extracting antioxidants using the UAE method. The result showed that increasing excitation time increased the water content from 78% to 89% and increased the yield from 6.5% to 7.1%. The antioxidant activity (IC50) ranged from 4.9 to 3.5 ppm (db), suggesting that the areca nuts have a very high antioxidant activity.

Keywords: *Areca, Antioxidant, Betara, Excitation time, Extraction, Ultrasonic*

1. INTRODUCTION

Indonesia has high biodiversity, one of which is Betara, Jambi Province, Indonesia. Betara is rich in local sources, such as areca, coconut, nypa, and palm oil [1]. Areca nuts are a natural resource found in the peatland area of Betara. Farmers in Jambi Province plant Betara cultivars because of their high yield and ability to adapt to substandard land.

Areca nut includes nutrients such as B vitamins, vitamin C, antioxidants, and various active chemicals that help boost the immune system. Areca nut contains free radical scavengers and antioxidants such as tannins, phlorotannin, saponins, flavonoids, terpenoids, cardiac glycosides, and anthocyanins. Extraction can be used to isolate the areca nut's inherent antioxidants. The goal of extraction is to get an active component, a collection of similar-structured molecules, or secondary metabolites. The purpose of extraction is to get an active component, an accumulation of similar-structured molecules, or secondary metabolites.

Ultrasonic aided extraction is one of the extraction procedures used (UAE). This is an extraction process in which ultrasonic waves of 20 kHz are applied to the substance [2]. One of the advantages of the ultrasonic approach is that it can produce a more extensive antioxidant content in a shorter amount of time without causing damage to the extract compound's structure. The ultrasonic vibrations created will tear down the material's cell walls and speed up membrane diffusion, allowing organic chemicals in plants to dissolve more quickly into the solvent [3-5]. The solvent employed, starting with the kind, concentration, volume, and appropriateness of the polarity level with the solute, determines the dissolution in the ultrasonic process. Ethanol is a common solvent in the food and pharmaceutical sectors for extracting components.

The ultrasonic extraction technique is required to determine the kinetic data utilizing mathematical modeling. Extraction kinetics modeling can be used to determine how bioactive chemicals are extracted [6]. Knowing the extraction kinetics will also make it easier for researchers to determine the reaction rate, allowing them to speed up the extraction process by changing the treatment and enhancing separation efficiency [7]. The extraction time is one element that affects extraction in the ultrasonic technique.

The contact period between the ultrasonic wave and the substance and solvent to be extracted is known as extraction

time, or excitation time in ultrasonic technology. Longer excitation times can raise the temperature, speeding up the antioxidant dissolving process until equilibrium [8]. Research on antioxidant extraction of areca nut with the study of excitation time is required as a platform for further investigation. The best excitation duration will result in a high yield, low water content, and the most potent antioxidant activity is determined.

2. MATERIALS AND METHODS

2.1. Materials and Equipment

Materials used in this study included Areca nut (Figure 1), distilled water, 70% ethanol, filter paper, aluminum foil, tissue, and label paper were used in this experiment. Blue tip, yellow tip, white tip, 1.5 ml microtube, ethanol pro analysis, distilled water, and DPPH (2,2-diphenyl-1-picrylhydrazil) were utilized to investigate the antioxidant activity.

Analytical scales, cabinet dryer, blender, 40 mesh sieve, spoon, 300 ml beaker, measuring cup, funnel, stopwatch, dropper, baking sheet, oven, porcelain cup, oven, desiccator, ultrasonic cleaner brand Power-Sonic 405 bath model, and rotary evaporator are among the equipment used in the production of areca nut extract. Spatula, micropipette, centrifuge spindown, microplate reader, and microplate 96-well plate are instruments used to measure antioxidant activity.

Figure 1 Seed of areca nuts

2.2. Experimental set-up

The study utilized the single-factor approach with only one variable: the length of excitation time (T) (minutes). T1: 10 minutes, T2: 20 minutes, T3: 30 minutes, T4: 40 minutes, T5: 50 minutes, and T6: 60 minutes were the six (6) degrees of excitation time therapy. Each treatment was done three times, producing 18 experimental units in total.

Preconditioning, size reduction, and drying of the areca nuts were used in this study to make the extraction procedure easier. Per experimental unit, 3.5 kg of areca nuts were required. The seeds were used without the shell in this investigation. The areca nuts were then cut and dried for 12 hours in a cabinet dryer at 55°C. The size was reduced after drying and sieved to 40 mesh to acquire areca nuts powder.

The ultrasonic aided extraction (UAE) method extracted the areca nuts with 70% ethanol. The following are the stages of the areca nuts extraction process:

1. A scale was used to weigh up to 15 grams of areca nuts, which was then placed in a beaker with 70 percent ethanol solvent in a capacity of 150 ml (1/10 w/v) and homogenized.
2. The UAE method was used to extract areca nuts antioxidants using an ultrasonic cleaner bath model with a frequency of 40 kHz, a temperature of 25°C, and an excitation time of 10 minutes, 20 minutes, 30 minutes, 40 minutes, and 50 minutes, depending on the treatment level.
3. The sample is cooled to room temperature and cured for 15 minutes to create a residue once the extraction procedure is completed.
4. The fine filter paper was used to filter the liquid.
5. A rotatory evaporator was used to concentrate the filtrate from the filtration process at a temperature of 500°C for 45 minutes at a rotation speed of 75 rpm.
6. The concentrated filtrate's yield, water content, and antioxidant activity were then determined.

The set-up for the UAE experiment is shown in Figure 2.

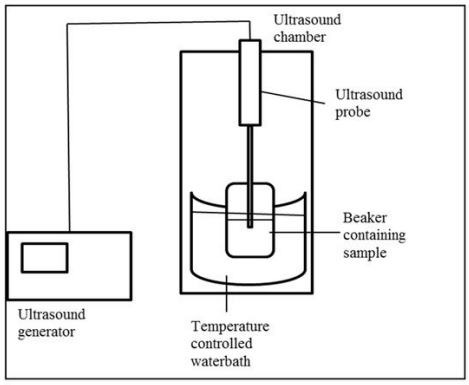

Figure 2 Ultrasound-assisted extraction set-up [9]

2.3. Antioxidant analysis

For the antioxidant (DPPH) test, we employed the following methodology in a 96-well plate. A 24 mg of DPPH was dissolved in 100 mL methanol to make DPPH stock solutions. After that, the solution was sonicated for 20 minutes. After that, the working solution was made by diluting 20 mL of the stock solution with 80 mL of methanol (5X dilution). The standard curve was made with Trolox (1 mM) and was linear between 0 and 800 M. 285 l of DPPH was added to 15 l of a sample. After that, the microplate was placed in the dark for 15-30 minutes. The data were represented as percent inhibition (percent I) or mol Trolox equivalents per gram of material (TE)/g after the plate was read at 517 nm. The absorbance of the blank

corrected raw data was used to compute percent inhibition using the following formula:

A linear regression curve was constructed using the percent inhibition data. R^2>0.95 (α<5%), seen in the dilution concentration range of the sample, was utilized to calculate the IC50 value.

2.4. Statistical analysis

Analysis of Variance (ANOVA) and DMRT were carried out using Statistical analysis tools SPSS 10.0.

3. RESULTS AND DISCUSSION

3.1. Yield of Areca Seed Extract

Table 1 shows the average yield of areca nuts' extract. Yield is the proportion of the difference between the product's dry weight and the weight of the raw materials used. The bioactive substances in the product increased with yield [10].

Table 1. The yield of areca nut extract

Excitation time (Minute)	Yield (%)
10	6.51 ± 0.17
20	7.05 ± 0.16
30	7.48 ± 0.09
40	7.65 ± 0.84
50	7.38 ± 0.04
60	7.16 ± 1.09

Table 2 reveals that the lowest yield percentage, 6.51 %, was obtained after 10 minutes of stimulation, while the highest percentage of yield, 7.65 %, was acquired after 40 minutes. The yields achieved in this investigation are consistent with the literature, which suggests that the ultrasonic extraction method generates higher yields than the maceration approach [11]. Only 13.56 % yield was obtained by Rahmah et al. [12] utilizing the maceration method for areca nut extraction for 5 x 24 hours.

According to Cunico et al. [13], the material's solubility will continue to rise until it reaches equilibrium is reached, which occurs at the optimal extraction time. At a 40-minute excitation duration, the rise in areca nut extract production reached its peak. Because of the long contact time between the solvent and the material, cell diffusion from the material matrix to the solvent is increased [14].

Cavitation also contributed to the increase in yield. Cavitation happens when pressure from a spreading shock wave causes a phase transition from water to steam. The cavitation bubbles burst and hit the cell surface as a result of this pressure, which creates periodic back and forth movements [15]. This increases the permeability of solids, making it easier for solvents to pass into the material cell and increasing the yield [16]. The drop in yield was observed at the 50-minute and 60-minute excitation time. It was considered to be caused by an increase in the temperature of the ultrasonic bath caused by the collision of ultrasonic waves, causing the heat to be challenging to manage and allowing damage to the extracted chemicals [17]. This drop might also be caused by an extraction solution that has achieved saturation, making it unable to improve yield [18].

3.2. Water content

The goal of determining the water content of an extract is to establish the minimal limit or range of water content. The wet basis moisture content of the areca nut powder employed in this extraction is 10.66 %. Extracts with high-water content will allow fungi or mold to develop more quickly, reducing shelf-life [19]. Table 2 shows the typical water content of areca nut extract. Table 2 reveals that the lowest water content was achieved at a 20-minute excitation time of 70.88 %, while the highest water content was obtained at a 60-minute excitation time of 89.60 %. At a 20-minute stimulation time, the water content dropped but then increased once the excitation time was raised by 10 minutes. Differences in the volume of the filtrate after ultrasonic extraction and the yield after the concentration process are assumed to be the cause of this fluctuating situation.

Tabel 2. The water content of areca extract

Excitation time (Minute)	Water content (%)
10	78.85± 0.1
20	70.88 ±1.4
30	73.93 ±7.9
40	84.39 ±6.4
50	87.46 ±1.6
60	89.60 ±2.4

The fluctuating water content of areca nut extract produces results that are consistent with the literature. The longer the extraction period, the higher the water content. The increase in water content is aided by an increase in yield, causing the water content to subsequently increase. Furthermore, due to variances in extraction time in each iteration, the findings of this variable water content can be altered by the length of raw material storage. The temperature and humidity of the storage space, as well as the surrounding environment, might influence changes in water content during storage [20]. Substantial impact on the water content of the areca nut extract was observed with the treatment with a 50-minute excitation period being the best according to the DMRT test.

3.3. Antioxidant Activity

The IC50 value in the areca extract was calculated using the DPPH technique as a stable free radical. The IC50 number, or 50% Inhibition Concentration, indicates how much sample concentration is required to lower free radicals by 50%. Table 3 shows the average IC50 value of areca nuts extract.

Tabel 3. IC50 value of areca extract

Excitation time (Minute)	IC50 Value (dry-bulb) (ppm)
10	4.92 ± 1.4
20	4.89 ± 0.9
30	4.49 ± 1.1
40	4.35 ± 1.7
50	3.87 ± 1.5
60	3.51 ± 0.4

Table 3 shows the lowest IC50 value, 4.92 ppm, acquired after 10 minutes of excitation, and the highest IC50 value, 3.51 ppm, obtained after 60 minutes of excitation. The stronger is the antioxidant activity contained in the substance, the lower the IC50 value [21, 22].

This shifting average IC50 value is assumed to be caused by an increase in temperature in the ultrasonic system. The higher the ultrasonic temperature, the longer the excitation duration and the higher the amplitude employed. Excessive heat can cause the extraction temperature to rise to the labile point of antioxidant-active chemicals. This may allow for a gradual increase in extract concentration due to cell-wall breakdown, evaporation, and antioxidant component breakdown [23]. However, in other case the antioxidant may decline. The decline in antioxidant activity could be due to the concentrated extract's storage duration prior to the antioxidant activity test. Antioxidant activity in extracts may decrease because secondary metabolites that act as antioxidants become unstable or damaged [24].

According to the results of the ANOVA test, the excitation duration had a significant effect on the IC50 value of the areca nuts extract, and the optimal treatment according to the DMRT test was 60 minutes of excitation time.

4. CONCLUSION

The results showed that the ultrasonic wave excitation period had a significant effect on the yield, water content, and IC50 value of areca nuts extract with a confidence level of 95 % ($\alpha<0.05$). At a 50-minute excitation period, the highest average yield and lowest water content were achieved, with 17.48 percent and 54.27 %, respectively. Treatment with a 60-minute excitation duration generated the lowest IC50 value, 311.04 ppm, which was inactive. In the ultrasonic extraction method, the development, growth, and bursting of cavitation bubbles causes the diffusion of bioactive chemicals to occur more quickly.

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