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Research paper

Comparison of Anti-inflammatory Activity of Ethanol Extracts of Young and Old Areca Seeds (*Areca catechu* L.) in vitro

Perbandingan Aktivitas Anti-Inflamasi Ekstrak Etanol Biji Pinang (*Areca catechu* L.) Muda dan Tua Secara in Vitro

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ABSTRACT

Areca catechu L. has been widely used as a traditional medicine in medicinal practices in various countries around the world. Areca nut contains secondary metabolite compounds such as alkaloids, flavonoids, triterpenoids, steroids, and tannins so it has great potential to be developed as a medicine. The use of anti-inflammatory drugs from natural ingredients is widely developed because synthetic non-steroidal anti-inflammatory drugs cause side effects to the body such as nausea, diarrhea, headaches, and stomach ulcers. This study aims to determine the content of secondary metabolites and determine the anti-inflammatory activity of ethanol extracts of young and old areca nut seeds. Old areca nut seeds used are areca nut fruits that have an orange color while young areca nut seeds are areca nut fruits that have a green color. The anti-inflammatory activity test method was carried out using the protein denaturation inhibition method at 5, 10, 15, and 20 ppm extract concentrations. The results of this study indicate that young and old areca nut seeds positively contain alkaloids, flavonoids, tannins, and saponins and negatively contain steroids. The results of anti-inflammatory activity testing show that ethanol extracts of young and old areca nut seeds have the potential as anti-inflammatory because they have a percentage value of protein denaturation inhibition greater than 20%. A comparison of anti-inflammatory activity between areca nut seeds can be seen from the IC50 value of ethanol extract. The IC_{50} results show that young areca seeds have an IC_{50} value of 7.42 ppm which is higher than the old areca seeds of 11.52 ppm.

***Keywords:**: herbal medicine, anti-inflammatory, areca nut*

INTRODUCTION

Inflammation is a protective response to damage to body tissues caused by physical trauma, chemicals, and microorganism interference (Harianto et al., 2021). Inflammation aims to destroy or reduce agents that damage tissues. Non-steroidal anti-inflammatory drugs (NSAIDs) are an anti-inflammatory therapy used to treat pain and fever. According to the National Disease and Therapeutic Index, non-steroidal anti-inflammatory drugs (NSAIDs) are the medications that doctors most frequently prescribe. Non-steroidal anti-inflammatory drugs (NSAIDs) are often used because of their good effectiveness as analgesics, anti-inflammatories,

and antipyretics (Konda & Jayanti, 2021). Inflammatory effects can also be treated using commercial steroid drugs. However, this type of widely used anti-inflammatory drug has side effects that are bad for the body if used in the long term. Steroid drugs can reduce the body's immune response to infection, reduce glucocorticoid synthesis, hypertension, osteoporosis, and moonfaces. Non-steroidal anti-inflammatory drugs (NSAIDs) can also interfere with platelet function, prevent pregnancy induction, and cause gastrointestinal (GI) ulcers, perforation, and obstruction (Fatimah et al., 2022) & (Shaikh et al., 2016).

A popular alternative therapy with significantly less negative side effects is the use of traditional medicinal plants with anti-inflammatory effects (Nifinluri et al., 2019). Areca nut (*Areca catechu L.*) is a medicinal plant that has long been used by people in Indonesia. Areca nut seeds are used as a mixture for eating betel nuts and as an elixir in traditional ceremonies. Betel nuts and areca nuts are chewed to strengthen teeth (Fitriani et al., 2023). Areca nut plants are also widely used as a source of cosmetic, health, and textile coloring ingredients (Fitriani et al., 2023).

Areca nut seeds are widely utilized because they contain many secondary metabolites. Areca nut seeds contain alkaloid and flavonoid secondary metabolite compounds that have the activity to inhibit the growth of *Streptococcus mutans* bacteria, which are the cause of dental plaque and bad breath (Wenetu & others, 2011). The 92% ethanol extract of areca nut contains flavonoids, tannins, and terpenoids and has antibacterial activity (Asrianto et al., 2021). The dichloro methanolic extract of areca nut has anthelmintic activity and contains metabolite compounds of alkaloids and flavonoids, tannins, saponins, monoterpenes, sesquiterpenes, phenols, and quinones (Dedwydd et al., 2021).

Several studies related to the anti-inflammatory activity of areca nut have been reported. The methanol extract of areca nut methanol extract has anti-inflammatory activity and has the potential to be developed as a medicine (Fitriani et al., 2023) & (Sharaf et al., 2021). Ethanol extract of yellow areca nut seeds has anti-inflammatory activity against white rat knee joints and ethyl acetate fractions also have anti-inflammatory activity (Akrama Yuda et al., 2022).

Another study concluded that the phytochemical content in the extract of young and old betel nut bark samples is relatively the same but the antioxidant activity is different where the IC_{50} value of young betel nut bark is 56.6 ppm while the IC_{50} value of the old betel nut bark sample is 67.50 (Hidayah et al., 2019). This shows a difference in the activity of young and old areca nuts. Therefore, this study will discuss the difference in anti-inflammatory activity between young and old areca nut seeds using the in vitro method.

METHODOLOGY

Tools

Analytical balance (Mettler Toledo), blender, thermometer, oven (Memmert UN55), water bath (Memmert UN55), pH meter (Mettler Toledo), filter paper, aluminum foil, dropper pipette, measuring flask, beaker (Pyrex), test tube, 250 ml separating funnel (Pyrex), test tube rack, stir bar, spatula, micropipette (Dragon Onelab), vacuum rotary evaporator (Buchi), UV-Vis double beam spectrophotometer (Shimadzu UV-1900 Series).

Materials

Young and old areca nuts (*Areca catechu L*) were obtained from Kelurahan Sei Selayur, Segaran Alley 3 Palembang City. Bovine Serum Albumin (Sigma-Aldrich), Ethanol 96% (Merck), Aquadest NaCl (Merck), Tris base (Biogear), glacial acetic acid (Merck), chloroform (Merck), HCl 2 N (Merck), H_2SO_4 (Merck), $FeCl_3$ (Merck), Mg powder (Merck), Lieberman- Burchard reagent (Merck), Dragendorff reagent (Merck).

Sample Preparation and Extraction

The areca nut (*Areca catechu L*) which has been taken is green and orange in color. Young areca nut has a green color and old areca nut has an orange color. The areca fruit is peeled for its fibers and the seeds are taken. Areca nut seeds are gathered, purified of any remaining contaminants, and then dried. The dry ingredients were then blended, crushed, and passed through sieve no. 60. The test sample was filtered before being weighed once more.

Areca nut seed ethanol extract was prepared using the maceration method. A liter of 96% ethanol solvent was used to extract the 240,96 grams of areca seed powder, which was then macerated for three to twenty-four hours. The filtrate was then collected after the extract had been filtered using filter paper. Use a rotary vacuum evaporator and then evaporate or separate the solution at a temperature of 50°C. The extract

obtained was then weighed to obtain the percent yield (Marzuki et al., 2019).

Qualitative analysis

Qualitative analysis of the chemical content of Areca nut seeds, including tests for flavonoids, alkaloids, saponins, Steroid and triterpenoids, and tannins (Setyawaty, 2020).

- **Flavonoids test:** The sample extract was put into a test tube and then added to 2 grams of magnesium powder and 3 drops of concentrated HCl. The extract was shaken and observed for changes in color. Positive flavonoids are indicated by changes in color to yellow, orange, and red.
- **Alkaloids test**: The sample extract was put into a test tube and then added with 1-2 mL of dragendorff's reagents. Positive extracts containing alkaloids are indicated by the formation of a reddish-brown precipitate.
- **Saponin test**: The sample extract of 2 ml was put into a test tube and then added to 10 mL of hot distilled water. After it chilled the filtrate in the test tube is shaken vigorously for about 30 seconds. Positive of saponin test if the formation of foam with a height of at least 1 cm and persistent for 10 minutes and not lost on the addition of 1 drop of dilute hydrochloric acid
- **Steroid and triterpenoid test**: The sample extract of 2 ml was put into a test tube and then added with Lieberman-Burchard reagent. When a green-blue color appears, this means that steroid group chemicals are present, and when a purple color develops, this means that triterpenoid group chemicals are present.
- **Tannins test**: The sample extract of 2 ml was put into a test tube and then added with 10% iron (III) chloride solution if dark blue, black blue, or greenish black showed the presence of tannin compounds.

In Vitro Assay of Anti-inflammatory Activity Test

Comparative testing of the anti-inflammatory activity of young and old areca seed extracts (Areca catechu L) was carried out by in vitro test.

- The first step for the anti-inflammatory in vitro test is the preparation of a TBS (Tris Buffer Saline) solution. Weigh 1.21 grams of tris base and 8.7 grams of NaCl, then add 900 ml of distilled water. The pH was stabilized by adding glacial acetic acid to a pH of 6.2-6.5. Then add distilled water up to 1000 ml in a measuring flask.
- The second stage is the preparation of a 0.2% BSA (Bovine Serum Albumin) solution. Weigh 0.2 grams of BSA and put it into a 100 ml volumetric flask, then add the TBS solution to a volume of 100 ml.
- The third step is to prepare a test solution by taking 2.5 mg of young and old areca seed extracts dissolved in ethanol in a 25 mL volumetric flask to obtain a concentration of 100 ppm. The test solution with a concentration of 100 ppm was diluted into a test solution at concentrations of 5, 10, 15, and 20 ppm.

The volume of 0.2% Bovine Serum Albumine solution in Tris Buffer Saline solvent pH 6.2-6.4 was increased by 40 µL of each concentration of ethanol extract solution of Areca seed (5, 10, 15, 20 ppm) until it reached 5 mL. The solution has been heated for 10 minutes at 80-85°C after 20 minutes of incubation at 30°C in a water bath. then allow to stand at room temperature for 25 minutes. After cooling, the solution was vortexed, and the UV-visible spectrophotometer was used to detect absorbance at 660 nm, the maximum wavelength for protein. The percentage of protein denaturation inhibition was measured using the equation formula :

$$\%\text{Inhibisi} = \frac{Absorbansi\ kontrol\ negatif\ -\ absorbansi\ larutan\ uji}{absorbansi\ kontrol\ negatif} x100$$

Compounds were classified as anti-inflammatory and might be used as an indicator for drug development if they inhibited protein denaturation by more than 20%. Making a linear regression equation between concentration (X) and % inhibition yields the IC_{50} value (Fatahillah et al., 2022).

RESULTS AND DISCUSSION

Extraction Results

The extraction method used in this study used the maceration method because this method is simple and prevents compound damage due to heating. In the maceration process, stirring is carried out to increase the contact area between the solvent and the sample surface. Solvents can enter plant cells so that secondary metabolites in the cells can be bound in the solvent. The number of secondary metabolites bound in the solvent is expressed in percent yield. The percent yield of young and old areca nut (*Areca catechu L.*) seed ethanol extract can be seen in Table 1.

Table1.Percent yield of young and old areca nut (Areca catechu L.) seed ethanol extract

Sample	Weight of dry simplicia	Extract weight obtained	% yield
Young areca nut	240,96 g	95,64 g	39,69 %
Old areca Nut	240,96 g	92,65 g	38,45 %

The yield value of an extract aims to determine the amount of bioactive compounds contained in dry simplicial. The % yield of young and old areca nut ethanol extracts from Table 1 shows that the % yield of young areca nut seeds is higher than that of old areca nut seeds. This shows that young areca nut seeds contain more secondary metabolites than old areca nut seeds.

Qualitative test results

The results of the qualitative tests were used to identify the chemicals that compose the secondary metabolites. The classes of secondary metabolites tested were flavonoids, alkaloids, saponins, terpenoids, and tannins. The qualitative test results for the secondary metabolites contained in the young and old areca seed extracts can be seen in Table 2.

Table 2. Qualitative test results for the secondary metabolites contained in the young and old areca seed extracts

Compound	A young areca seed extract	An old areca seed extract	indicating positive test
Flavonoids	(+)	(+)	Red, Yellow, and Orange
Alkaloids	(+)	(+)	a reddish-brown precipitate
saponins	(+)	(+)	Foam
Steroid and triterpenoid	(-)	(-)	Red Purplish/green
tannins	(+)	(+)	Dark blue or blackish green

The results of phytochemical screening of young and old areca nut extracts show that the content of secondary metabolite compounds is the same, namely containing flavonoids, alkaloids, tannins, and saponins.

Anti-Inflammatory Activity Test

Comparative testing of the anti-inflammatory activity of ethanol extracts of young and old areca nut seeds was carried out in vitro against protein denaturation. The protein used in this study is Bovine Serum Albumin (BSA) a standard protein for anti-inflammatory testing (Hidayah et al., 2021). The percent inhibition of denatured proteins can be used to calculate a compound's anti-inflammatory effectiveness. When proteins are exposed to external agents such strong acids, strong bases, strong organic salts, organic solvents, and heat, proteins lose their secondary and tertiary structures. Anti-inflammatory drugs use ingredients that can prevent the denaturation of proteins that cause inflammation.

The percentage inhibition of protein denaturation of young and old areca nut seed extracts can be seen in Table 3.

Table 3. Percent Inhibition of Young and Old Areca Seed Extracts

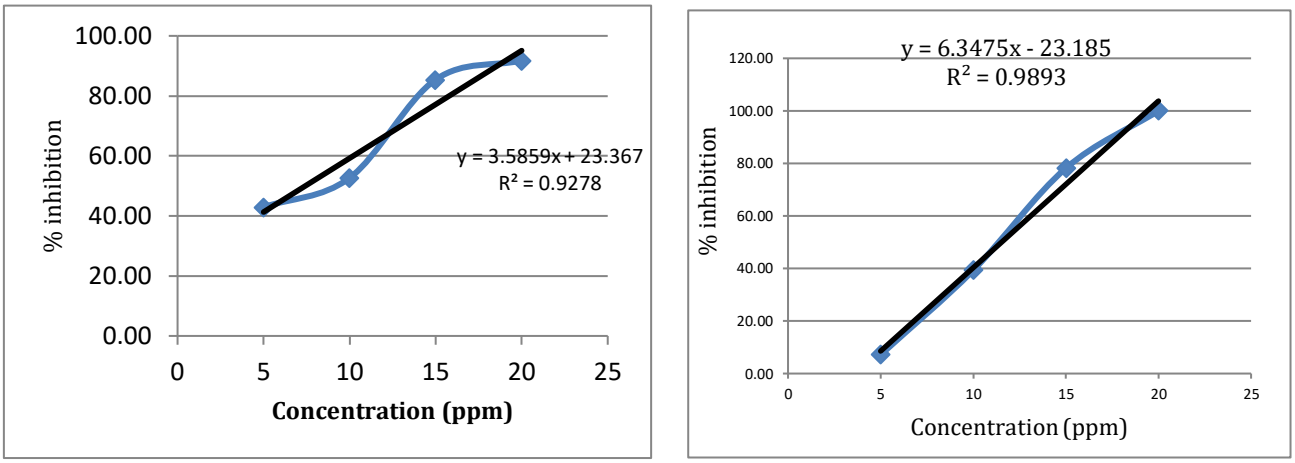
	% Inhibition	
Concentration (ppm)	**Young Areca Seed Extract (%)**	**Old Areca Seed Extract (%)**
5	42,91	7,15

10	52,66	39,32
15	85,43	78,16
20	91,76	91,82

Table 3 shows that young areca nut seeds at a concentration of 5 ppm have a percent inhibition of 42.91% while old areca nut seeds only have a percent inhibition of 7.15%. This shows that the % inhibition value of old areca nut seeds at a concentration of 5 ppm does not meet the minimum standard of an ingredient to be used as an anti-inflammatory, which has a % inhibition value exceeding 20%. Whereas at a concentration of 5 ppm, young areca nut seeds have a percent inhibition of 42.91% and exceed 20% so young areca nut seeds have better potential as an anti-inflammatory.

IC_{50} Value Calculation Results

The IC_{50} value is a parameter used to interpret the results of anti-inflammatory tests. The IC_{50} value is defined as the concentration of the test compound that can inhibit inflammation by 50%. IC_{50} was calculated by referring to the linear regression equation between concentration X and percentage inhibition Y. The linear regression of protein denaturation of ethanol extracts of young and old areca nut seeds is presented in Figure 1.



(A) old areca nut seeds (B) old areca nut seeds

Figure1. Results of linear regression of protein denaturation of ethanol extracts of young and old areca nut seeds

Extracts of young and old areca nut seeds have secondary metabolites that have the potential as anti-inflammatories namely flavonoids and alkaloids. Flavonoids are one of the secondary metabolites that can act as inflammatory agents. The mechanism of flavonoid activity as an anti-inflammatory is by inhibiting COX and lipooxygenase enzyme activity directly in inflammation. This leads to inhibition of eicosanoid biosynthesis and inactivation of inflammatory mediators (Williams et al., 2008).

One of the major categories of secondary metabolites are alkaloids. Alkaloids work to reduce inflammation by preventing the production of prostaglandins and the enzymes lipoxygenase, COX-1, and COX-2 (Fatahillah et al., 2022).

A comparison of anti-inflammatory activity between areca nut seeds can be seen from the IC50 value of ethanol extract. The IC50 results of ethanol extracts of young and old areca nut seeds are shown in Table 4.

Table 4. IC_{50} results of ethanol extracts

Test Solution	IC_{50}
Young Areca Seed Extract	7,42 µg
Old Areca Seed Extract	11,52 µg

The IC_{50} calculation data in Table 4 shows that the anti-inflammatory activity of ethanol extract of young areca nut seeds (7.42 µg/ml) is smaller than that of old areca nut seeds (11.52 µg/ml). This indicates that the anti-inflammatory activity of ethanol extract of young areca nut seeds is

better than that of old areca nut seeds. This is because young areca seeds contain higher percent yield that the percent yield of old areca seeds, meaning that young areca seeds contain more bioactive components.

CONCLUSIONS

In this study, it can be seen young and old areca nut seeds positively contain alkaloids, flavanoids, tannin, and saponins and negatively contain steroids. The result of anti-inflammatory activity testing show that ethanol extract of young and old areca nut seeds have the potential as the anti-inflammatory because they have a percentage value of protein denaturation inhibition greater than 20%. However, young areca nut seeds had a better percent inhibition at the low concentration of 5 ppm. This shows that at a concentration of 5 ppm, young areca nut seeds have the potential to be developed as an anti-inflammatory medicinal material. A comparison of anti-inflammatory activity between areca nut seeds can be seen from the IC_{50} value of ethanol extract. The IC_{50} results show that young areca seeds have an IC_{50} value of 7.42 ppm which is higher than the old areca seeds of 11.52 ppm.

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