THE EFFECT OF DIFFERENT DRYING METHODS ON THE ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACTS *Annona squamosa* L. LEAVES

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Abstract. One medicinal plant believed to possess anticancer activity is the srikaya leaf (*Annona squamosa* L.). According to empirical data, srikaya leaves can be used as an astringent, anti-inflammatory, anthelmintic, antifertility agent, abscess-ripening agent, and antitumor agent. Srikaya leaves contain antioxidants such as flavonoids, total phenols, glycosides, alkaloids, saponins, and tannins. The objective of this study was to determine the antioxidant activity of ethanol extracts from leaves of *Annona squamosa* L. using oven-drying and air-drying methods. Methods: The extraction method used was maceration with ethanol as the solvent. Evaluations included moisture content testing and phytochemical screening. The antioxidant activity of the extract was determined using UV-Vis spectrophotometry at 515 nm over 52 minutes. Results: The IC_{50} of the ethanol extract of leaves of *Annona squamosa* L. obtained via air-drying was 344.11 ppm, whereas that obtained via oven-drying was 372.66 ppm. Conclusion: The ethanol extracts of leaves of *Annona squamosa* L. obtained via air- and oven-drying methods, at 344.11 ppm and 372.66 ppm, respectively, exhibit weak antioxidant activity. Keywords: Ethanol extract of srikaya leaves (*Annona squamosa* L.), Antioxidant, UV-Vis spectrophotometry.
Keywords: *Annona squamosa* L, antioxidant, drying, IC_{50}, UV-Vis spectrophotometer

INTRODUCTION

Free radicals are molecules that have an unpaired electron in their outermost orbital, making them highly reactive. The number of free radicals can increase due to stress, radiation, cigarette smoke, and environmental pollution, rendering the body's existing defense system inadequate, so the body requires additional antioxidants from external sources to protect against free radical attacks (Chandimali et al., 2025). DPPH (2,2-diphenyl-1-picrylhydrazine) is a stable radical that is widely used to test the free radical scavenging capacity of various natural compounds, such as phenolic compounds, anthocyanins, or crude extracts. The DPPH radical is scavenged by antioxidants through the donation of a hydrogen atom from the antioxidant, thereby forming reduced DPPH (Baliyan et al., 2022).

Antioksidan didefinisikan sebagai senyawa yang dapat menunda, memperlambat, dan mencegah proses oksidasi lipid. Specifically, antioxidants are substances that can prevent free radical (peroxide) reactions during lipid oxidation. Bioactive compounds are an effective and relatively safe source of antioxidants, such as flavonoids, vitamin C, beta-carotene, and vitamin E. This has spurred an increasing number of studies exploring natural substances as sources of antioxidants (Rahaman et al., 2023).

The use of natural ingredients for traditional medicine in Indonesia has been on the rise recently; in fact, some are now being produced on a large scale. The use of traditional medicine is considered to have fewer side effects than medicines derived from chemical compounds; furthermore, it is more affordable. One such plant believed to possess antioxidant activity is the srikaya leaf (*Annona squamosa* L.). Srikaya leaves contain antioxidants such as flavonoids, total phenols, glycosides, alkaloids, saponins, and tannins (Nurmiati et al., 2024).

One of the post-harvest processes that plays a crucial role in the quality of crude drugs is the drying process (Depkes RI, 2000). The drying process affects both the chemical compound content and the pharmacological effects of a medicinal plant, particularly compounds that act as antioxidants. The total phenolic and flavonoid content in a crude drug with antioxidant activity—and its stability— can be influenced by the drying process (Purnomo et al., 2023). Various drying methods—such as oven drying, sun drying, or air drying—can affect the total flavonoid content, total phenol content, and antioxidant activity of certain herbal extracts (Rad et al., 2025).

This study aims to determine the effects of different drying methods on antioxidant activity using the DPPH immersion method.

METHODS

A. Sample Preparation, chemical materials, and instruments

Annona squamosa L. were obtained from Margoyoso, Pati, Central Java, Indonesia. Taxonomic identification was conducted in the Biology Laboratory, Faculty of Sains dan Teknologi Terapan, Universitas Ahmad Dahlan. Materials used: Ethanol, Aquadest, Quercetin, DPPH (Merk, Darmstadt, Germany). Instruments used: rotary evaporator (Biobase Re-100-Pro), UV-Vis spectrophotometer (Shimadzu UV Mini 1240), moisture balance (Ohaus MB90).

B. Preparation of crude drugs

Leaves of *Annona squamosa* L. were weighed at 2,500 grams, then wet-sorted, washed under running water, drained, and divided into two portions (one for oven drying and one for air-drying), then weighed as wet weight and thinly sliced to speed up drying. Drying was performed in an oven at 50 °C and by air-drying. The dried *Annona squamosa* L. leaves were weighed again, and the percentage of drying loss was calculated; they were then ground into powder using a blender without damaging or removing their chemical content and sieved through a No. 40 mesh sieve, after which they were weighed as powder weight.

C. Maceration and Soxhlet extraction of *Annona squamosa* L.

200 grams of *Annona squamosa* L. leaf powder, air-dried in the open air, and 200 grams of leaf powder dried in an oven at 500C were macerated; 1200 mL of 70% ethanol was added in a ratio of 1:6; allowed to stand for 1×24 hours, stirring occasionally; then filtered using Whatman paper; the filtrate was collected in a bottle or Erlenmeyer flask, and the residue was macerated again for approximately 3 days until the solution was clear. The maceration process was carried out in a tightly sealed container at room temperature. The sample solution was filtered through Whatman paper, and the resulting filtrate was evaporated using a rotary vacuum evaporator at 40°C and a speed of 100 rpm. The *Annona squamosa* L. leaf extract was tested for moisture content, weighed, and the yield percentage was calculated.

D. Phytochemical screening

The phytochemical screening conducted involved qualitative testing of the compounds contained in plants. This study was used to identify flavonoids using the Wilstatter test, the Bate-Smith test, and the 10% NaOH test, as well as to identify alkaloids and saponins. The procedure is as follows:

1. Flavonoid Test

a. Wilstatter Test

Weigh 0.1 grams of the ethanol extract of *Annona squamosa* L. leaves, dissolve it in ethanol, and place it in a test tube. Magnesium powder (0.1 grams) and 5 drops of concentrated HCl were added to the solution. If a color change to yellow occurs, the extract is positive for flavonoids (Pujiastuti & Ma'rifah, 2022).

b. Bate-Smith Test

Weigh 0.1 grams of the ethanol extract of *Annona squamosa* L. leaves, dissolve it in ethanol, and place it in a test tube. Add 5 drops of concentrated HCl to the solution. The mixed sample was heated for 15 minutes in a water bath. If the color change to red occurs, the extract is positive for flavonoids (Pujiastuti & Ma'rifah, 2022)

c. 10% NaOH Test

Weigh 0.1 grams of the ethanol extract of *Annona squamosa* L. leaves, dissolve it in ethanol, and then transfer it to a test tube. Add 2–4 drops of a 10% NaOH solution to the solution. If the color changes to orange, then the extract is positive for flavonoids (Pujiastuti & Ma'rifah, 2022).

2. Alkaloid Identification

A 0.1-gram sample of the ethanol extract of *Annona squamosa* L. leaves was weighed, dissolved in ethanol, placed in a test tube, and 1 mL of HCl was added; the mixture was then heated over a

Bunsen burner. Mayer's reagent was then added to the mixture. If a white precipitate formed, the extract was positive for alkaloids (Sabdoningrum et al., 2021).

3. Identification of Saponins

A 0.1 g of the ethanol extract of *Annona squamosa* L. leaves was weighed, dissolved in ethanol, and placed in a test tube, and 1 mL of hot water was added. The solution is then shaken vigorously for several seconds, and 2–3 drops of HCl are added. A positive result is indicated by the formation of a stable white foam that persists for 1 minute (Sabdoningrum et al., 2021).

E. Determination of antioxidant activity of ethanol extract of *Annona squamosa* L

1. Preparation of a 0.4 mM DPPH solution

A total of 0.01577 g of DPPH was dissolved in ethanol p.a. to a final volume of 100 mL in a volumetric flask, resulting in a solution with a concentration of 0.4 mM. Determination of the maximum wavelength (λ) of DPPH.

2. Determination of the DPPH operating time

Add 1 mL of a 0.4 mM DPPH blank solution in analytical-grade ethanol to the 5 mL volumetric flask up to the mark. Measure the absorbance of the solution using a UV-Vis spectrophotometer at wavelengths ranging from 400 to 800 nm.

3. Quantitative DPPH assay using UV-Vis spectrophotometry.

DPPH blank absorbance. Pipette 1 mL of a 0.4 mM DPPH solution into a 5 mL volumetric flask, add p.a. ethanol up to the mark, and measure the maximum wavelength (λ) and the operating time obtained.

A reference stock solution of quercetin was prepared at a concentration of 100 ppm; in the next step, a series of concentrations of 2, 4, 6, 8, and 10 ppm was prepared. A total of 1 mL of 0.4 mM DPPH standard solution was added to the quercetin standard solution until the mark on a 5 mL volumetric flask was reached, then left to stand in the dark for the operating time of 52 minutes. The absorbance was read at the maximum wavelength of 515 nm.

A 1000 ppm extract solution was diluted to concentrations of 100, 200, 300, 400, and 500 ppm. A total of 1 mL of 0.4 mM DPPH solution was added to each sample solution concentration up to the mark. The solutions were left to stand in the dark for 52 minutes. The absorbance of the solutions was measured at a maximum wavelength of 515 nm.

F. Data analysis

Free radical scavenging activity can be determined using the DPPH method, and can be calculated using the formula:

$$\text{DPPH inhibition percentage} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{x } 100\%$$

RESULTS AND DISCUSSION

Annona squamosa L. leaves were selected at random based on the criteria that they were still fresh, green in color, and free of caterpillar damage. The results of the crude drug preparation, the moisture content of the powder and yield content are shown in Table 1.

Table 1. Results of the preparation of crude extracts and moisture content of *Annona squamosa* L. Leaves

Method	Drying Loss	Average Moisture Content (n=3) ± SD	Average Yield Content (n=3) ± SD	Color of the Crude Drug
Air drying	68.6	9.27 ± 0.42	4.818	Brownish Green
Oven drying	86.0	3.63 ± 0.39	4.922	Green

A total of 2,500 grams of *Annona squamosa* L. leaves were weighed and then wet-sorted. Wet sorting

is an initial separation process performed after harvest, without any treatment, using water; the goal is to minimize and separate *Annona squamosa* L leaves from impurities and foreign objects such as soil, pebbles, grass, damaged leaves, and other debris that must be removed. The next step is washing with running water to remove soil and other debris that is still clinging to the leaves and could not be removed during the wet sorting process (Blanchard & Works, 2013).

Annona squamosa L. leaves were dried using air-drying and oven-drying methods at 50°C; the purpose of drying at 50°C was to obtain crude drug with active compounds that are not easily degraded. The powder was prepared using a blender to reduce the size of the crude drug for easier extraction, then sieved through a No. 40 mesh sieve. The No. 40-mesh sieve was used to obtain a fine, uniformly sized powder of the crude drug, thereby facilitating compound extraction (Sholehah et al., 2024).

The purpose of testing moisture content is to determine whether the moisture content in the crude drug meets applicable requirements, so that it does not affect subsequent processes. Moisture content testing is conducted to reduce and halt enzymatic reactions, thereby preventing the degradation or spoilage of the crude drug. The moisture content in the crude drug must not be excessive—that is, it must be less than 10%. If a crude drug has a moisture content exceeding 10%, this can affect the quality of the material and accelerate fungal growth. A moisture content of less than 10% is desired, as this is expected to ensure optimal stability of the material, reduce microbial growth, and prevent disruption of the active compounds. Enzymatic reactions do not occur when the moisture content in the crude drug is less than 10% (Arslan & Alibas, 2024).

This method was chosen because it is a simple process that involves soaking the powdered crude drug in a solvent for a specific period of time. Furthermore, the maceration method does not involve heating during the extraction process; thus, there are no temperature factors to accelerate reactions or affect the active compounds in the extract, and the potential degradation of the chemical components in the sample can be avoided. Additionally, maceration is an easy method to perform and requires only simple equipment (Zhang et al., 2023). Extract yield is used as a measure of extraction efficiency and a quality standard for extracts to estimate the amount of dried herbal material needed to produce a specific volume of concentrated extract. Yield is expressed as a percentage (%); the higher the yield, the greater the amount of extract obtained (Milenkovi et al., 2025).

The results of the comparative phytochemical screening of ethanol extracts from *Annona squamosa* L. leaves using the air-drying and oven-drying methods are shown in Table 2

Table 2. Phytochemical screening results of ethanol extracts from *Annona squamosa* L. leaves using the air-drying and oven-drying Methods

Methods	Secondary metabolites	Results
Air drying	Flavonoids	
	Wilstatter Test	+
	10% NaOH Test	+
	Bate-Smith-Metcalfe Test	+
	Alkaloids	+
	Saponins	+
Oven drying	Flavonoids	
	Wilstatter Test	+
	10% NaOH Test	+
	Bate-Smith-Metcalfe Test	+
	Alkaloids	+
	Saponins	+

The Wilstatter test using HCl and Mg powder from both drying methods produced a yellow colour in both cases, indicating the presence of flavonol-type flavonoids. The addition of HCl and Mg in flavonoid identification aims to reduce the benzopyrone ring present in the flavonoid structure, resulting in a colour change to yellow, orange, or red. The addition of HCl causes an oxidation-reduction reaction between magnesium (Mg) as a reducing agent and the flavonoid compounds (Doloking et al., 2022).

Identify the flavonoids using the Baeyer–Smith–McClelland reagent with HCl, then mix and heat for 15 minutes. Both drying methods produced a red color, indicating the presence of flavonoid compounds in both cases. The orange/red color formed in the Baeyer–Smith–McClelland test is caused by the

formation of a flavilium salt. HCl reacts with the carbonyl group on the flavone, which undergoes resonance, resulting in the formation of a new bond—specifically, the breaking of a double bond and the formation of a hydroxyl group. The reaction involves the formation of a new bond, in which HCl dissolves the flavone, allowing the flavonoid to be separated from other chemical groups to form a salt. A positive result is indicated by a color change ranging from red to orange, indicating a flavone compound; a deep red color indicates a flavonol or flavonone (Hardiningsih et al., 2025).

Alkaloids are identified using two types of reagents: the Mayer and Dragendorff reagents, where a positive result is indicated by a white precipitate for the Mayer reagent and an orange precipitate for the Dragendorff reagent. A positive result for alkaloids in the Mayer test is indicated by the formation of a white precipitate. It is believed that this precipitate is a potassium-alkaloid complex. In the preparation of the Mayer reagent, a solution of mercury (II) chloride mixed with potassium iodide forms a red precipitate of mercury (II) iodide. If an excess of potassium iodide is added, potassium tetraiodomercurate (II) is formed. Alkaloids contain nitrogen atoms that have a lone pair of electrons, allowing them to form coordinate covalent bonds with metal ions (Sabdoningrum et al., 2021). Alkaloid test using Mayer's reagent: it is expected that the nitrogen in the alkaloid will react with the K+ metal ions from potassium tetraiodomercurate(II) to form a potassium-alkaloid complex that precipitates (Hasairin et al., 2022).

Saponins are compounds that contain both hydrophilic and hydrophobic groups. When agitated, saponins form foam because the hydrophilic groups bond with water, while the hydrophobic groups bond with air. The addition of HCl serves to increase polarity, allowing the hydrophilic groups to form more stable bonds and stabilizing the resulting foam (Timilsena & Phosanam, 2023).

The measured wavelength of 0.4 mM DPPH in the 400–800 nm range was 515 nm at an absorbance of 0.482. The measured operating time ranged from 52 to 56 minutes with a consistent absorbance value of 0.657; 52 minutes was used. The absorbance of the control was 0.781. The DPPH scavenging activity of the quercetin control and sample is shown in Table 3 and Table 4.

Table 3. Results of DPPH Inhibition by Quercetin

Concentration (ppm)	Average DPPH inhibition (%) n=3 ± SD	Linear regression equation	IC_{50} (ppm)
2	27.82 ± 0.0005	y = 5.218x + 18.230	6.088
4	41.48 ± 0.0006		
6	48.14 ± 0.0006		
8	59.02 ± 0.0004		
10	71.23 ± 0.0005		

The IC_{50} value obtained from that equation is 6.088, which means that the quercetin reference solution has very strong antioxidant activity. Comparative testing of the antioxidant activity of quercetin, with quercetin serving as a positive control to determine whether the method used to test antioxidant activity is reliable or not. Results of antioxidant activity: The reference compound quercetin exhibits very strong antioxidant activity and demonstrates that the antioxidant activity assay method is reliable (Carrillo-martinez et al., 2024)

Table 4. Results of DPPH inhibition by ethanol extracts of *Annona squamosa* L. leaves using oven drying and air-drying Methods

Method	Concentration (ppm)	Average DPPH inhibition (%) n=3 ± SD	Linear regression equation	IC_{50} (ppm)
Air drying	100	18.85 ± 0.003	y = 0.1116x + 8.411	372.66
	200	30.21 ± 0.001		
	300	44.47 ± 0.001		
	400	52.32 ± 0.006		
	500	63.59 ± 0.002		
Oven drying	100	35.33 ± 0.005	y = 0.0763x + 28.957	275.243
	200	45.58 ± 0.002		
	300	52.62 ± 0.003		

400	58.89 ± 0.003
500	66.83 ± 0.003

Antioxidant activity testing in this study used the DPPH (2,2-diphenyl-1-picrylhydrazil) method. The DPPH method was chosen because it requires only a small sample, is simple, easy, fast, and sensitive for evaluating the antioxidant activity of natural compounds (Baliyan et al., 2022; Santoso & Nursafitri, 2023).

According to Putri et al., (2024), antioxidant activity is classified as very strong if the IC50 value is < 50 ppm, and as weak if the IC50 value is between 250 and 500 ppm. Based on the IC50 values obtained, the ethanol extract of *Annona squamosa* L. leaves prepared by air-drying and oven-drying exhibited weak antioxidant activity, whereas quercetin yielded an IC50 value of 6.088 ppm, indicating very strong antioxidant activity. The antioxidant activity of the air-dried and oven-dried ethanol extracts of *Annona squamosa* L. is lower than that of quercetin. This low antioxidant activity is likely due to several factors, including extraction methods that do not sufficiently extract the antioxidant-containing chemical components from the leaves of *Annona squamosa* L. The extracts may also exhibit weak activity because they are not yet pure and consist of various chemical compounds (Snoussi et al., 2021).

Statistical analysis using the paired samples t-test on the drying methods for herbal crude drugs—the oven method and the air-drying method—yielded a p-value of 0.017 < 0.05, indicating that there is a significant difference between the two methods. This significant difference indicates that the drying method has a tangible effect on the characteristics of the resulting crude drug. Drying is a crucial post-harvest step because it determines the stability of the raw material, moisture content, enzyme activity, and the content of secondary metabolites such as phenolics and flavonoids.

A study by Snoussi et al., (2021) reported that the drying method affects the amount of phenolic compounds and the antioxidant capacity of the extract. The study showed that excessively high drying temperatures cause a decrease in phenolic compound content, whereas more moderate temperatures are still able to preserve the plant's phytochemical quality. These findings indicate that the selection of drying temperature and method must be tailored to the characteristics of the target compounds to be preserved. This aligns with the research by (Rad et al., 2025), which suggests that the choice of drying method should consider the intended end use of the crude drug—whether to achieve high yield, preserve active compound content, or enhance the biological activity of the extract.

Careful consideration must be given to the choice of drying method, as it has a significant impact on the quality of the crude drug. If the objective of the research is to preserve the content of bioactive compounds—such as phenolics and flavonoids with antioxidant activity—then low-temperature drying or air-drying is generally more advantageous. Conversely, if the priority is time efficiency and rapid reduction of moisture content to prevent microbial growth, oven drying may be the preferred option, provided that the temperature is optimized to minimize the degradation of active compounds.

CONCLUSION

There were significant differences in the antioxidant activity of the ethanol extract of *Annona squamosa* L. leaves between the air-drying and oven-drying methods.

Not applicable

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