

# Phytochemical Content Analysis of Simplex and Ethanol Extract of *Pandanus amaryllifolius* Roxb. Leaves as Potential Natural Ingredients

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## Abstract

This study aimed to compare the secondary metabolite profiles of crude drug (simplicia) and refluxed ethanol extract of *Pandanus amaryllifolius* Roxb. leaves through qualitative phytochemical screening. Fresh fragrant pandan leaves were processed into dried simplicia, powdered, and extracted using the reflux method with 96% ethanol (1:10 w/v) at  $\pm 78^\circ\text{C}$  for 3 hours. The extract was concentrated using a rotary evaporator and analyzed for alkaloids, flavonoids, phenolics, saponins, terpenoids, and steroids based on standard color reactions and precipitate formation. The drying process resulted in a weight loss of 37.58%, indicating effective moisture removal, while extraction yielded 9% thick extract. Phytochemical screening revealed the presence of alkaloids, flavonoids, and terpenoids in both simplicia and extract, whereas phenolics, saponins, and steroids were not detected under the applied conditions. These findings indicate that 96% ethanol reflux extraction effectively isolates polar and semi-polar bioactive compounds without significant thermal degradation. The results provide a scientific basis for the development of pandan-based phytopharmaceuticals and natural cosmetic formulations.

**Key words:** *Pandanus amaryllifolius*, phytochemical screening, reflux extraction, secondary metabolites, ethanol extract

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## Abstrak

Penelitian ini bertujuan untuk membandingkan profil metabolit sekunder antara simplisia dan ekstrak etanol refluks daun *Pandanus amaryllifolius* Roxb. melalui skrining fitokimia kualitatif. Daun pandan wangi segar diolah menjadi simplisia kering, dihaluskan, kemudian diekstraksi menggunakan metode refluks dengan pelarut etanol 96% (1:10 b/v) pada suhu  $\pm 78^\circ\text{C}$  selama 3 jam. Ekstrak dipekatkan menggunakan rotary evaporator dan dianalisis terhadap kandungan alkaloid, flavonoid, fenolik, saponin, terpenoid, dan steroid berdasarkan reaksi warna dan pembentukan endapan standar. Proses pengeringan menghasilkan susut bobot sebesar 37,58%, sedangkan rendemen ekstrak sebesar 9%. Hasil skrining menunjukkan adanya alkaloid, flavonoid, dan terpenoid pada simplisia maupun ekstrak, sementara fenolik, saponin, dan steroid tidak terdeteksi pada kondisi pengujian ini. Metode refluks dengan etanol 96% efektif mengekstraksi senyawa polar dan semi-polar tanpa degradasi termal yang signifikan. Hasil ini mendukung pengembangan fitofarmaka dan kosmetik alami berbasis pandan wangi.

**Kata kunci:** *Pandanus amaryllifolius*, skrining fitokimia, ekstraksi refluks, metabolit sekunder, ekstrak etanol

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## INTRODUCTION

The use of medicinal plants as a source of natural ingredients in the development of traditional medicines and phytopharmaceuticals continues to increase globally. This trend is driven by increasing resistance to synthetic drugs and awareness of the safety and sustainability of natural ingredients (Buddhakala & Yongkhamcha, 2025; Nurbaiti et al., 2024). World health organizations are also encouraging the exploration of tropical Asian plants as potential sources of bioactive compounds for modern therapies (Sa'adah et al., 2023).

One plant with potential for development is the fragrant pandan leaf (*Pandanus amaryllifolius* Roxb.), widely known as an aromatic and traditional medicinal ingredient in Southeast Asia. Its distinctive aroma is produced by volatile compounds such as 2-acetyl-1-pyrroline (Wakte, K. V., et al. 2009). Pharmacologically, fragrant pandan leaf extract has been reported to possess antioxidant, antibacterial, antidiabetic, and anti-inflammatory activities (Buddhakala & Yongkhamcha, 2025). Several studies have also shown that ethanol extract of pandan leaves is active against *Staphylococcus aureus* and *Escherichia coli* and has mild antifungal and antidiabetic activity. This activity is associated with the presence of phenolic compounds, flavonoids, and alkaloids.

Phytochemical screening is an important initial step in identifying secondary metabolites of medicinal plants, such as alkaloids, flavonoids, saponins, tannins, phenolics, and steroids/triterpenoids. Ethanol was chosen as a solvent because it can efficiently extract polar and semi-polar compounds and is safe and non-toxic compared to other organic solvents.

Although various studies have reported the biological activity of pandan leaf extract, comparative studies examining the differences in secondary metabolite profiles between the crude drug and refluxed ethanol extract are still very limited. This is despite the fact that differences in material form and extraction process have the potential to influence the composition and concentration of the resulting bioactive compounds.

Based on this, this study aimed to conduct phytochemical screening of crude drug and

refluxed ethanol extract of pandan leaf to identify differences in secondary metabolite content. The results are expected to provide a scientific basis for developing pandan as a source of bioactive compounds in phytopharmaceutical formulations and natural cosmetics.

## RESEARCH METHODS

This is an experimental laboratory study using a qualitative descriptive approach. The aim was to identify secondary metabolites in crude drugs and ethanol extracts of pandan wangi (*Pandanus amaryllifolius*) leaves through phytochemical screening.

Extraction was performed using the reflux method with 96% ethanol as a solvent in the Pharmaceutical Biology Laboratory, Kader Bangsa University, Palembang, from September 25 to November 7, 2025. The extracted filtrate was evaporated using a rotary evaporator and thickened in a water bath to obtain a thick extract.

The thick extract was then analyzed qualitatively through phytochemical screening to identify alkaloids, flavonoids, saponins, tannins, and terpenoids/steroids based on color reactions and precipitate formation according to standard methods (Harborne, 1987; Farmakope Indonesia).

## Tools and Materials

The main equipment includes an analytical balance, blender (grinder), No. 60 sieve, glass jar, reflux extraction apparatus, filter paper, rotary evaporator, water bath, beaker, Erlenmeyer flask, graduated cylinder, test tube, tripod, Bunsen burner, wire gauze, funnel, and porcelain cup.

The materials used include fresh pandan leaves, 96% ethanol, distilled water, and Mayer, Dragendorff,  $\text{FeCl}_3$ , Liebermann–Burchard, and Mg–HCl reagents.

## Simplisia Process

Fragrant pandan leaves were obtained from the Pusri area, Palembang City, South Sumatra. Samples were selected from healthy, fresh leaves, then wet sorted to remove dirt and damaged parts. The leaves were washed under running water until clean, sliced into small pieces, and air-dried at room temperature, covered with a black cloth, until dry leaves were obtained.

The dried simplex was then ground using a blender and sieved through a 60-mesh sieve to obtain a powder with a uniform particle size. The powdered simplex was weighed and stored in a tightly closed container until used in the extraction stage.

### Extraction Process Using The Reflux Method

A total of 100 g of powdered medicinal plants was extracted using 1 L of 96% ethanol (1:10 w/v ratio) using the reflux method for 3 hours at  $\pm 78^{\circ}\text{C}$ . The filtrate was separated by filtration and evaporated with a rotary evaporator at 45–50°C, then condensed using a water bath until a thick extract was obtained.

The resulting thick extract was weighed and its concentration calculated using the following equation:

$$\text{Yield} = \frac{\text{extract weight}}{\text{simple drug weight}} \times 100\%$$

### Phytochemical Screening Of Simplisia and Ethanol Extract Of Fruitful Pandan Leaves

Phytochemical screening was performed on the simplicia and extract to qualitatively identify secondary metabolites, including alkaloids, flavonoids, phenolics, saponins, terpenoids, and steroids.

#### Alkaloid testing

Used Mayer, Dragendorff, and Liebermann–Burchard reagents; a positive result was indicated by the formation of a white, orange, or brown precipitate.

#### Test for Phenolic Compounds and Their Derivatives

The simplicia and extract samples were each extracted using ethanol, then separated with a 1:1 mixture of chloroform and water to form two layers. The water layer was used to test for phenolic compounds, flavonoids, and saponins, while the chloroform layer was used to test for terpenoids and steroids.

- The phenolic test used  $\text{FeCl}_3$  reagent (purple-blue color).
- The flavonoid test was carried out by adding HCl and magnesium powder to the water layer. A positive reaction is indicated by a color change from orange to red.
- The saponin test is performed by vigorous shaking until a stable foam forms, indicating saponin. The sample is declared positive for

saponin if the foam does not disappear immediately.

- The terpenoid and steroid tests use the Liebermann–Burchard reagent, which produces a brownish-red color for terpenoids and a green color for steroids.

## RESULTS AND DISCUSSION

**Table 1. Results of making fragrant pandan leaf simplicial**

No.	Parameters	Results
1.	Initial weight of fresh leaves	801 g
2.	Weight of dried medicinal plants	500 g
3.	Weight loss	37.58%

Description: The drying process was carried out naturally under light protection. The shrinkage value corresponds to the range of moderately water-soluble materials.

The pandan leaf drying process resulted in a weight loss of 37.58%, indicating high water removal efficiency without visual degradation of the material. The hot air drying method for pandan leaves can reduce the water content by 35–40%, depending on the leaf age and slice thickness. A water content reduction below 10% of the wet weight is considered optimal to prevent microbial growth and maintain the stability of secondary metabolites during storage (Adhamatika et al., 2021).

Overall, the weight loss values indicate that the drying process was optimal and suited the physiological characteristics of pandan leaves, which have a moderate to high initial water content. These results align with a study (Ghazali et al., 2024) which found that controlled drying at 45–55°C maintained the stability of flavonoids and volatile compounds such as 2-acetyl-1-pyrroline (2-AP), which determine the characteristic aroma of pandan.

Thus, proper drying plays an important role in maintaining the balance between reducing water content and preserving key bioactive compounds, including flavonoids and essential oils, which are indicators of the quality of the medicinal plant for further phytochemical research.



**Figure 1. Dried simplisia of *Pandanus amaryllifolius* Roxb leaves.**

**Table 2. Results of pandan leaf extraction using the reflux method**

No.	Parameters	Results
1.	Solvent and Amount Used	1 Liter of 96% Ethanol
2.	Weight of the Drug Used	100 g
3.	Extract Weight	9 g
4.	Yield	37.56%

Note: Extraction was performed using the reflux method at a temperature of  $\pm 78^{\circ}\text{C}$ . The filtrate was evaporated at  $45\text{--}50^{\circ}\text{C}$  using a rotary evaporator.

Extraction of *Pandanus amaryllifolius* leaves using the reflux method with 96% ethanol produced a yield of 9%, indicating moderate efficiency in extracting polar and semi-polar active compounds from the crude drug. These results align with a report by (Qomarudin, 2023), which showed that pandan extraction with ethanol yields between 8–12%, depending on the material ratio and extraction temperature. The reflux method was chosen because it maintains the solvent at its boiling point, allowing continuous contact between the solvent and the ingredients, thus enhancing the diffusion of bioactive compounds.

According to (Ghazali et al., 2024), the use of ethanol at high concentrations (95–96%) effectively dissolves semi-polar compounds such as flavonoid aglycones and terpenoids, but is less optimal for highly polar compounds such as phenolic glycosides. This explains why the 9% yield obtained was relatively lower than that obtained with an ethanol-water mixture (70–

80%), which has better polar compound extraction capabilities. Water in the mixture acts as a cell wall softener, aiding the release of secondary metabolites from plant cell walls.

Thus, the 9% extract yield reflects a balance between dissolution efficiency and thermal stability of bioactive compounds. This value is considered efficient for a pure ethanol solvent system, although it is not optimal for extracting all phytochemical groups. For further research, it is recommended to explore the use of solvents with varying polarities or the application of non-thermal methods such as ultrasound-assisted extraction (UAE), which has been shown to increase yield without degrading the quality of pandan phytochemical compounds (Azhar et al., 2022).

**Table 3. Characterization of fragrant pandan leaf simples**

No	Parameters	Results	FI Herbal Compliance
1.	Shape	Homogeneous fine powder	in accordance
2.	Color	Light green	in accordance
3.	Odor	Typical pandan leaves (2-AP)	in accordance
4.	Taste	Kelat (shoe)	in accordance

Description: Organoleptic characteristics according to the Indonesian Pharmacopoeia, Edition VI (2020).

The fine, homogeneous powder form indicates that the pollination and sieving processes have been optimal without causing physical degradation of the plant tissue. Uniform particle size increases the contact surface area between the material and the solvent during extraction, thereby increasing the efficiency of secondary metabolite extraction. This is consistent with a report (Rohaeni et al., 2025) that homogeneous granulation of aromatic leaf simplisia can increase the solubility of bioactive compounds such as flavonoids and phenolics.

The light green color indicates that the drying process took place at a moderate temperature (45–55°C) so as not to damage natural pigments such as chlorophyll a and b. According to (Ghazali et al., 2024), drying at temperatures above 60°C risks reducing the intensity of the green color due to the degradation of chlorophyll to pheophytin. The fresh color indicates that the pigment structure remains stable, indicating good quality simplicia for further phytochemical analysis.

The distinctive aroma of pandan leaves comes from the volatile compound 2-acetyl-1-pyrroline (2-AP), which is the main component that determines the "glutinous rice" odor of pandan. This compound belongs to the group of volatile nitrogen heterocycles, but is relatively stable at moderate drying temperatures. (Buddhakala & Yongkhamcha, 2025) reported that 2-AP concentrations can be maintained when drying is carried out slowly below 60°C. Thus, the presence of this distinctive aroma indicates that post-harvest processing is under control without loss of key volatile compounds.

The astringent taste indicates the high content of tannins and polyphenolic flavonoids in the herbal medicine. These compounds are astringent because they can precipitate proteins in the oral mucosa, causing an astringent sensation. (Wahyuni et al., 2024) stated that the astringent taste can be used as an early indicator of the presence of phenolic metabolites, which play a key role in the antioxidant and antimicrobial activity of pandan leaves.

Overall, these characterization results indicate that the resulting herbal medicine meets the organoleptic standards of the Indonesian Herbal Pharmacopoeia and maintains the integrity of key phytochemical compounds, such as chlorophyll, flavonoids, and 2-AP. These stable physical and sensory characteristics are essential prerequisites for maintaining the reliability of phytochemical screening test results and supporting the development of fragrant pandan natural ingredients as a source of natural bioactives. These findings emphasize the importance of controlling organoleptic parameters

as a basis for developing fragrant pandan natural ingredients in standardized phytochemical research.



**Figure 2. Powdered Simple Leaf Powder *Pandanus amaryllifolius* Roxb.**

**Table 4. Characterization of ethanol extract of pandan wangi leaves**

No	Parameters	Results	Information
1.	Shape	Thick, high viscosity	High solute content
2.	Color	Dark green	Dominant chlorophyll a and b
3.	Odor	Typical pandan leaves (2-AP)	Stable during extraction
4.	Taste	Chelate (astringent)	Indications of tannins and flavonoids

The ethanol extract of *Pandanus amaryllifolius* leaves has a thick, high-viscosity consistency, indicating a high solute content, particularly flavonoids, tannins, and chlorophyll. The viscosity of the extract is directly proportional to the concentration of the bioactive compounds produced by the solvent. (Qomarudin et al., 2025) reported that extraction using 96% ethanol resulted in a higher extract viscosity than water, due to ethanol's ability to attract semi-polar metabolites such as flavonoid aglycones and terpenoids. Furthermore, the reflux process maintains the stability of the heat-soluble

compounds, preventing degradation of the active structure.

The dark green color of the extract indicates the dominance of chlorophyll a and b, the two main pigments in pandan leaves. The intensity of the green color increases with the extract concentration and the solvent's efficiency in dissolving these pigments. (Harun Nurashid et al., 2025) stated that ethanol solvents with moderate heat energy allow for optimal chlorophyll release without causing oxidation to pheophytin. Thus, the dark green color can be used as an indicator of pigment stability and the quality of pandan extract during extraction and storage.

The strong, distinctive aroma of pandan leaves indicates the stability of the volatile compound 2-acetyl-1-pyrroline (2-AP), the main aromatic component of pandan. The highest 2-AP concentration was obtained at 80–96% ethanol concentration and moderate extraction temperature (60–70°C). This indicates that the reflux method is able to retain volatile compounds through a vapor condensation system, thereby reducing aroma loss. These results align with (Qomarudin, 2023), which confirms that 2-AP stability increases when extraction is carried out in a closed system with ethanol as a solvent.

The astringent taste of the extract indicates a high content of tannins and polyphenolic flavonoids, which are astringent because they can precipitate proteins in the oral mucosa. (Adhamatika et al., 2021) explained that the intensity of the astringent flavor increases in pandan leaf extract, which is rich in phenolic

compounds, especially after moderate drying (50–55°C), which maintains the stability of the aromatic hydroxyl groups. These compounds play an important role as natural antioxidants and are major contributors to the pharmacological activity of fragrant pandan.

Overall, the physical and sensory characteristics of the ethanol extract of fragrant pandan leaves indicate a high concentration of active phytochemical compounds that are stable to moderate heat. The viscous texture, deep green color, distinctive 2-AP aroma, and astringent flavor are indicators of good extract quality and consistent with the standards of the Indonesian Herbal Pharmacopoeia (2020). These characteristics strengthen fragrant pandan's potential as a natural source of antioxidants and a base ingredient for cosmetic and botanical pharmaceutical formulations.



**Figure 3. Ethanol Extract of *Pandanus amaryllifolius* Roxb Leaves**

**Table 5. Results of phytochemical screening of simple and ethanol extracts of fragrant pandan leaves**

No	Class of compounds	Reagent	Color According to Literature	Screening Results			
				Simple ingredients		Extract	
				Color	Note	Color	Note
1.	Alkaloid	Mayer	White precipitate forms	There are white lumps	+	White precipitate forms	+
		Liebermann-burchard	Formed reddish brown	There is brown sediment	+	Reddish brown sediment	+
		Dragendorff	Orange sediment formed	Orange sediment formed	+	Orange sediment formed	+

2.	<b>Fenolik</b>	<b>FeCl<sub>3</sub></b>	<b>Dark blue / blackish green</b>	<b>No blue/blackish green color is formed</b>	-	<b>No dark blue formed</b>	-
3.	<b>Flavonoid</b>	<b>HCL+ logmMg</b>	<b>Red orange</b>	<b>A red-orange color is formed</b>	+	<b>A red-orange color is formed</b>	+
4.	<b>Saponin</b>	<b>Hot water</b>	<b>Stable foam is formed</b>	<b>No foam formed</b>	-	<b>No foam formed</b>	-
5.	<b>Terpenoid</b>	<b>Lieberman-burchard</b>	<b>bluish green</b>	<b>Green color is formed</b>	+	<b>Green color is formed</b>	+
6.	<b>Steroid</b>	<b>Lieberman-burchard</b>	<b>Purplish red</b>	<b>No purplish red color is formed</b>	-	<b>No purplish red color is formed</b>	-

Description: (+) = detected (-) = not detected

Phytochemical screening results showed that both the crude drug and the ethanol extract of *Pandanus amaryllifolius* leaves contained alkaloids, flavonoids, and terpenoids, but no phenolics, saponins, or steroids. This pattern indicates that 96% ethanol is more selective for polar and semi-polar compounds such as alkaloids and flavonoids, while non-polar compounds such as steroids and saponins are less optimally extracted. These results align with findings (Sa'adah et al., 2023), which found that ethanol extraction yielded a broader spectrum of secondary metabolites than n-hexane.

A positive reaction for alkaloids in the Mayer, Dragendorff, and Liebermann–Burchard reagents is characterized by the formation of a white to reddish-brown precipitate, indicating the presence of heat-stable aromatic nitrogen bases. (Thanebal et al., 2021) reported that pandan leaf alkaloids play a role in hepatoprotective activity by increasing liver antioxidant enzymes. The reflux process, carried out at temperatures close to the boiling point of ethanol, helps release protein-bound alkaloids, thereby increasing the solubility of the compound without damaging its structure (Wahyuni et al., 2024).

A positive flavonoid result, indicated by a red-orange color change in the magnesium-HCl test, indicates the presence of flavonoid aglycones such as quercetin and luteolin. These compounds are known to act as antioxidants and antibacterial agents against *Staphylococcus aureus* and *Escherichia coli* (Wahyuni et al., 2024). The stability of the positive results in both the crude drug and the extract indicates that the reflux process does not cause significant degradation of

the aromatic hydroxyl groups, which are important in the biological activity of flavonoids. (Buddhakala & Yongkhamcha, 2025).

A positive reaction for terpenoids in the Liebermann–Burchard reagent, with a bluish-green color, indicates the presence of volatile compounds such as linalool, β-ionone, and terpineol, the main components of pandan essential oil. (Mohamed Zakaria et al., 2020) stated that terpenoids are responsible for the distinctive aroma of pandan as well as its antioxidant and antimicrobial activities. The condensation system in the reflux method helps maintain the stability of these volatile compounds during the heating process (Burhana et al., 2023).

Conversely, negative results in the phenolic, saponin, and steroid tests are likely due to differences in solvent polarity and thermal degradation during the reflux process. Free phenolic compounds are easily oxidized at high temperatures, while saponins and steroids are more soluble in semi-polar solvents such as 70% ethanol or n-hexane (Bhuyan & Sonowal, 2021). Therefore, negative results do not indicate the complete absence of these compounds, but rather limitations in the extraction conditions used.

Overall, the secondary metabolite profile indicates that pandan leaves are rich in alkaloids, flavonoids, and terpenoids, which are heat-stable and contribute to the plant's key biological activities. The differences in phytochemical profiles between the crude drug and the extract underscore the importance of optimizing solvents and extraction methods to maximize bioactive compounds. These results support the potential of pandan leaves as a base material for the

development of natural pharmaceutical and cosmetic preparations.

## CONCLUSION

The process of producing crude drugs and ethanol extracts of *Pandanus amaryllifolius* Roxb. leaves using the reflux method yielded high-quality extracts containing primary secondary metabolites, including alkaloids, flavonoids, and terpenoids. These compounds have the potential to act as bioactive components supporting antioxidant, antimicrobial, and anti-inflammatory activities.

Scientifically, the results of this study confirm that the combination of the reflux method and 96% ethanol solvent is effective in extracting polar and semi-polar compounds without causing significant thermal degradation, thus serving as a baseline model for standardizing tropical natural product extraction processes.

These findings contribute to the development of pandan wangi-based phytopharmaceuticals by providing a basis for comprehensive phytochemical characterization. Further research is recommended to identify and isolate specific active compounds and evaluate their biological activities, to strengthen the therapeutic value and industrial applications of *Pandanus amaryllifolius* as a source of natural ingredients.

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