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DETERMINATION OF TOTAL CONTENTS OF FLAVONOIDS AND PHENOLIC FRACTIONS *PANDANUS JULIANETTII* MARTELLI

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ABSTRACT

Pandanus julianettii Martelli is a type of plant endemic to mountainous areas in Papua that has long been used by people as a food ingredient. P. julianettii contains nutrients such as vitamin E, vitamin C, crude fiber, protein, carotene and fat; however, the research about the fruit is limited. The aim of this research is to carry out phytochemical screening tests and determine the total levels of flavonoids and phenolics of this pandanous in fruit. The method began with the extraction simplicia using ethanol, followed by fractionation using three variations of solvent: n-hexane, ethyl acetate and ethanol. The series of methods continued with phytochemical screening, then continued with determining the total flavonoid and phenolic percentage from the ethanol extract, ethyl acetate fraction and ethanol fraction. Determining the flavonoid content using the Chang method with quercetin as a comparison with standard. The results showed that the percentage of flavonoid in the extract was 0.58%, the ethyl acetate fraction was 0.44% and the ethanol fraction was 3.93%, while for the total content of phenolic of ethanol extract at 1.56%, the fraction of ethyl acetate 1.82% and ethanol fraction 1.20% calculated for gallic acid. This study found substantial levels of flavonoids and phenolics in extracts and solvent fractions derived from the fruit of the endemic Papuan plant P. julianettii. The enriched ethanol extract and fractions represent promising sources for further analysis of the nutritional and medicinal bioactives in this understudied of this fruit.

Keywords: *Pandanus julianettii* Martelli, Total Flavonoids, Phenolics, UV-Vis Spectrophotometry, Lanny Jaya

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INTRODUCTION

Pandanus julianettii Martelli is a type of plant endemic to the mountainous regions of Papua, Indonesia, especially areas such as Tolikara, Lanny Jaya, Puncak Jaya and Wamena. Local people call *P. julianettii* with coconut nut in Indonesian, tuke in Dani-Baliem or karuka in Papua New Geunia. This plant typically grows at altitudes between 1,800 m–2,600 m, with occasional sightings as low as 1,450 m and as high as 2,800 m above sea level. *P. Julianettii* is a pandan tree which has multipurpose uses—the fruit is consumed as food, the roots are utilised for weaving noken bags, the leaves for cigarettes and umbrellas, and the stems for firewood (Zebua *et al.* 2020).

The drupes (especially the endosperm) of *P. julianettii* have been traditionally eaten by highland communities across New Guinea for their psychoactive effects, locally known as "*karuka* madness" (Keim & Sujarwo 2021). According to oral history accounts never before documented in literature, elders of the Dani tribe used to administer endosperm extracts from roasted drupes as anesthetics. By rendering patients' unconscious, traditional surgeries could be performed to treat injuries and wounds resulting from frequent tribal wars in the Baliem Valley prior to the introduction of Western medicine (Lekitoo *et al.* 2017).

P. julianettii fruit is dominated by fatty acids and vitamin [5–6], with composition of 13%−15% crude protein dry weight, 52.39% oleic acid, 44.90% palmitic acid, 0.19% stearic acid and some unidentified acids. As reported by Lekito *et al*. (2017), this plant harbors nutrients like vitamin C, crude fiber, protein, fat and water (Lekito *et al.* 2017). Its levels of protein, fat and vitamin C typically exceed values for common Indonesian fruits such as passion fruit, avocado, soursop, snake fruit, papaya and rambutan. Existing data documents total phenols at 48.55 ppm, carotene at 2.75 µg/g, vitamin E at 5.03 mg/100 g, and antioxidant activity IC_{50} at 45.83 mg/mL from the fruit oil (Lekitoo *et al.* 2017; Kogoya and Guritno 2014; Zebua and Purnamasari 2018).

Thus far, research on *P. julianettii* remains scarce. Zebua and Purnamasari (2018) tested the phenol content of the oil, but there are no studies quantifying phenols or flavonoids of the fruit itself. As *P. julianettii* is widely consumed by locals for both its oil and as whole fruit, further investigation is warranted, especially given the promising antioxidant data. Therefore, this study aims to determine total phenolic and flavonoid levels in *P. julianettii* fruit, helping to expand the limited scientific knowledge around this nutritionally and culturally important plant.

METHODS

The scheme of methodology is described in Figure 1.

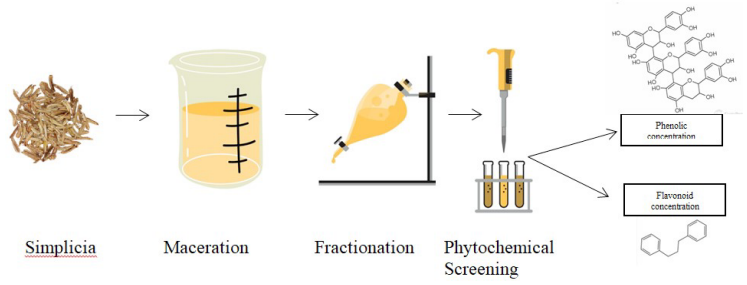

Figure 1: Flow chart for the phytochemical analysis of *P. julianettii.*

Sample Collection and Preparation

P. julianetti fruit samples were obtained from the Lani district. The samples were cut into pieces and then dried in an oven at 50°C for 3 days. The dried samples were ground into a simplicia powder.

Preparation of Extracts

A total of 1000 g of the *P. julianettii* simplicia powder was macerated using 4 L of ethanol as the solvent. The simplicia was left to macerate for 3 cycles of 24 hours each, then filtered and the macerate was collected after each cycle. All the macerate was combined and evaporated at 50°C using a rotary evaporator to obtain a concentrated extract (Raho Ghalem and Mohamed 2021).

Fractionation of Extract

The crude ethanol extract, weighing 10 g, was fractionated using n-hexane, followed by ethyl acetate and finally ethanol-water. The solvents were evaporated to obtain the corresponding hexane, ethyl acetate, and ethanol fractions (Rebryk *et al.* 2022).

Phytochemical Screening

Preliminary phytochemical tests were carried out on the ethanol extract and fractions to screen for the presence of alkaloids, flavonoids, saponins, polyphenols and tannins using standard procedures as follows:

1. Alkaloid test

   A total of 0.5 g of each sample was dissolved in 1 mL of 2N HCl and diluted with water to 10 mL. The solution was divided into 3 aliquots and tested with Mayer's, Wagner's and Dragendorff's reagents (Alqethami and Aldhebiani 2021). Formation of white/yellowish, brown and orange-red precipitates indicate positive results (Harborne 1989).

2. Flavonoid test

   Each extract and fraction, weighing 0.5 g, was dissolved in a few drops of ethanol. Then, 5 to 6 drops of concentrated hydrochloric acid (HCl) were added to each ethanol solution (Das *et al.* 2022). The development of a red coloration indicates the presence of flavonoids in the sample. Meanwhile, an orange colour denotes the presence of flavones (Harborne 1989).

3. Saponin test

   A total of 0.5 g of each extract and fraction was placed in test tubes. Hot water (10 mL) was added to each test tube, and the solutions were shaken vigorously for 10 minutes. Foam formation was observed. If foam persisted even after the addition of 2N HCl, it indicated the presence of saponins in the extract (Toklo *et al.* 2023).

4. Polyphenol test

   A total of 0.5 g of extract and fractions were reacted with 10% iron (III) chloride solution. If a dark blue, blackish blue or greenish black colour occurs, it indicates the presence of polyphenolic compounds (Harborne 1989).

5. Tannin test

   A total of 0.5 g of each sample fruit extract and fraction were treated with 5 drops of ferric chloride solution. The development of a green to blackish blue coloration indicates the presence of tannins in the sample (Toklo *et al.* 2023).

Determination of Flavonoid Levels

Preparation of sample solutions

A total of 25 mg of extract and each fraction were dissolved in 10 mL of ethanol. Then, 0.5 mL of each was pipetted into a test tube and 1.5 mL of 96% ethanol, 0.1 mL of $AlCl_3$, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water were added. The sample solution was incubated for 30 minutes at room temperature and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 440 nm. The sample solution was prepared in three replications (Pavun *et al.* 2018).

Quercetin standard solution

The standard solution was prepared by dissolving 25 mg of quercetin standard in 25 mL of ethanol. This standard solution was diluted to 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. From each quercetin standard solution concentration, 0.5 mL was pipetted and placed in a test tube, then 1.5 mL ethanol, 0.1 mL $AlCl_3$, 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water were added. After that, it was incubated for 30 minutes at room temperature and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 440 nm (Khan *et al.* 2018).

Determination of Phenolic Content

Determination of the gallic acid standard curve

Gallic acid, weighing 50 mg, was dissolved in 5 mL ethanol and diluted to 100 mL with distilled water. From this, 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL and 0.5 mL were pipetted into volumetric flasks and diluted with distilled water to 10 mL. This resulted in concentrations of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L gallic acid. A total of 3 mL of each concentration was taken, and 0.6 mL Folin Ciocalteu Reagent was added, shaken and allowed to stand for 3 minutes. Then, 0.6 mL of Na_2CO_3 solution was added, shaken until homogeneous and the absorbance was measured at 656 nm to create a calibration curve (Wabaidur *et al.* 2020).

Preparation of sample solutions

Half a gram of ethanol extract and each fraction were placed in a 50 mL volumetric flask and dissolved in distilled water. Then, 1 mL of each solution was diluted to 10 mL with distilled water. A 3 mL portion of each dilution was taken, followed by the addition of 0.6 mL of Folin-Ciocalteu reagent. The mixture was shaken and left to stand for 3 minutes. Next, 0.6 mL of 7.5% Na_2CO_3 was added. The sample solutions were then incubated for 30 minutes at room temperature. Absorbance was measured using a UV-Vis spectrophotometer at 656 nm wavelength (Yamin *et al.* 2021).

RESULTS

P. julianettii is found in the forests of Lanny Jaya, typically growing in groups in humid conditions with moist soil (Inoue *et al.* 2013). We dried the sample until it reached a brownish colour (Figure 2).

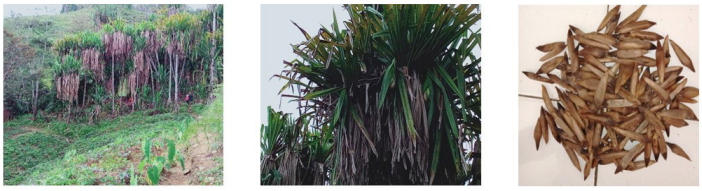

Figure 2: Habitat, trees and fruit of *P. julianettii.*

Preparation Simplicia

Table 1 presents the data for the crude extract obtained from *P. julianettii* fruits through maceration, yielding 37.38 g, corresponding to a modest percent yield of 3.738%. This relatively low yield suggests that the constituents of *P. julianettii* fruits may exhibit limited solubility in polar solvents, indicating a propensity for greater solubility in semi-polar or non-polar solvents, as inferred from the extracted fractions.

Table 1: Recovery of extracts and fractions of *P. julianettii* fruit.

***P. julianettii* fruit samples**	**Mass (g)**	**Yield (%)**
Simplicity	1,000	–
Ethanol extract	37.38	3.74
Ethanol fraction	1.01	2.67
Ethyl acetate fraction	2.11	5.35

Table 2: Phytochemical screening of *P. julianettii* extracts: Presence of alkaloids, flavonoids, saponins, phenolics and tannins.

Inspection	Ethanol extract	Hexane fraction	Ethyl acetate fraction	Ethanol fraction
Alkaloids				
-Meyer	+	–	+	+
-Dragendrof	+	–	+	+
-Wanger	+	–	+	+
Flavonoids	+	–	+	+
Saponins	-	–	–	–
Phenolic	+	–	+	+
Tannin	+	–	+	+

Determination of Phenolic Content

The data from Table 3 unveil a strong linear relationship between absorbance and concentration, as evidenced by the regression equation of y = 0.0176x + 0.0757 and a highly significant correlation coefficient (r = 0.9973). This coefficient, being proximate to unity, signifies an exceptionally high correlation between absorbance and the level of compounds, elucidating the direct proportionality of the variables.

Table 3: Measured absorbance of gallic acid standards for calibration curve.

Concentration (ppm)	Absorbance (λ) 656 nm
10	0.26
20	0.45
30	0.60
40	0.79
50	0.94

Table 4: Total phenolic contents by gallic acid equivalence in *P. julianettii* fruit extracts and fractions.

Sample *P. julianettii* fruit	Initial phenolic content (mgGAE/L)	Total phenolics (mgGAE/g)	% Phenolic content
Ethanol extract	1.56	15.57	1.56
Ethyl acetate fraction	1.83	18.18	1.82
Ethanol fraction	1.19	11.97	1.19

Note: n = 3

Determination of Flavonoid Levels

Flavonoids, as one of the groups of phenolic compounds, play an important role in various biological activities such as antioxidant, anti-inflammatory and anti-cancer. Therefore, this study will measure the total flavonoid content in *P. julianettii* to evaluate its potential benefits. The determination of total flavonoid content in this study will be conducted using the UV-Vis spectrophotometry method. This method was chosen due to its sensitivity and speed in measuring flavonoid compounds.

Table 5: Quercetin solution absorbances for construction of calibration curve.

Concentration (µg/mL)	Absorbance (λ) 440 nm
20	0.29
40	0.44
60	0.66
80	0.78
100	0.93

Table 6: Results of measurement of total flavonoid levels of *P. julianettii* fruit extracts and fractions.

Sample	Flavonoid content initial (mgQE/l)	Total flavonoids (mgQE/mg)	% Flavonoid content
Ethanol extract	5.90	0.005	0.58
Ethyl acetate fraction	4.46	0.004	0.44
Ethanol fraction	39.81	0.039	3.93

Note: n = 3

DISCUSSION

The examination of chemical compounds using n-hexane as a solvent, as detailed in Table 1, yielded no detectable compounds. This outcome is attributed to the non-polar nature of n-hexane, which selectively dissolves non-polar compounds. Consequently, this observation reinforces the hypothesis that the compounds of interest are predominantly soluble in polar solvents such as ethanol, and to some extent, in semi-polar solvents like ethyl acetate. Ethyl acetate ($CH_3CH_2OC(O)CH_3$), an ester derivative of ethanol and acetic acid, is particularly noted for its affinity towards polar chemical compounds. This includes flavonoids, which belong to the polar phenol group and demonstrates solubility in both polar and semi-polar solvents. Other secondary metabolites, such as tannins, saponins and alkaloids, exhibit varying degrees of polarity that is slightly lower than that of flavonoids, yet they are effectively solvated by ethanol and ethyl acetate.

The quantification of total phenolics in our study employed gallic acid as a standard reference, leveraging its stability and classification as a simple hydroxybenzoic acid derivative (Zhang *et al.* 2006). This methodology provided a reliable means of phenolic determination, as presented in Table 4. Furthermore, the quantification of quercetin was performed across five concentrations (20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm),

revealing a positive correlation between quercetin concentration and absorbance. This relationship aligns with Lambert-Beer's law, affirming that the absorbance remained within the optimal range of 0.2–0.8 for the concentrations tested.

Phenolics and flavonoids, pivotal secondary metabolites pervasive in the plant kingdom, have been identified as potent antioxidants. The higher the concentration of these phenolic compounds, the more pronounced their antioxidant effect. This underscores the critical role of flavonoids and other phenolics in the development of natural medicines, especially for their antioxidant properties (Ghasemi *et al.* 2023; Zhang *et al.* 2022; Qian *et al.* 2023). Our investigations into the flavonoid and phenolic contents of *P. julianettii* Martelli not only contribute to the understanding of its chemical composition but also underscore the potential of these compounds in natural medicine development, particularly as antioxidant.

CONCLUSION

The key findings and implications of the present study can be summarised in the following points. Phytochemical analyses demonstrated the presence of several bioactive secondary metabolites across the prepared *P. julianettii* fruit extracts and solvent fractions. Specifically, alkaloids, flavonoids, phenolics and tannins were detected in the crude ethanol extract, ethyl acetate fraction and ethanol fraction. In contrast, these phytochemical classes were absent in the n-hexane fraction. Quantification of flavonoids revealed levels of 0.583% in the ethanol extract, 0.440% in the ethyl acetate fraction and 3.934% in the ethanol fraction. For total phenolics, concentrations reached 1.556% in the extract, 1.817% in the ethyl acetate fraction and 1.196% in the ethanol fraction. Taken together, these results confirm that the ethanol extract and semi-polar fractions derived from *P. julianettii* fruit are rich sources of antioxidant flavonoids and phenolic compounds. In conclusion, this study presents the first reported phytochemical profiling and flavonoid-phenolic quantitation data specifically for *P. julianettii* fruit, complementing existing compositional analyses of the oil. The enriched concentrations found in the ethanol extract and fractions highlight the potential of these materials for further investigation into the nutritional and functional food applications of this plant and possible bioprospecting of this underutilised Pandanaceae species endemic to Papua.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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