

Quantification of Total Phenolics and Alkaloids in Durian Seed Extract and Their Relevance to Functional Food Development

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ABSTRACT

*Plants offer considerable potential as natural sources of raw materials for the development of functional foods. Durian seeds (*Durio zibethinus* L.), which are often underutilized and treated as waste, present a promising resource due to their content of bioactive compounds with potential health benefits. This study aimed to determine the total phenolic and alkaloid contents in durian seed extract. Quantitative analysis was conducted using UV-Vis spectrophotometry. The results showed that the extract contained 64.1 ± 4.1 mg GAE/g of total phenolics and 445.19 ± 0.106 ppm of total alkaloids. These findings highlight the potential of durian seeds as a natural ingredient for developing functional food products. This research provides a scientific foundation for future studies, including in vitro and in vivo biological activity evaluations, to support the incorporation of durian seed extract into functional food formulations that contribute to improved health outcomes.*

Keywords: *Durian seed; Phenolic; Alkaloid; Functional Food; Bioactive Compounds*

INTRODUCTION

The rising global demand for functional foods has underscored the importance of identifying and exploiting natural resources rich in bioactive chemicals. Functional foods are characterized as goods that offer supplementary health benefits beyond their fundamental nutritional value, including the capacity to diminish the risk of chronic diseases and enhance general health and well-being (Frumuzachi, Flanagan, Rohn, & Mocan, 2025). Indonesia's abundant biodiversity presents considerable potential for development as a primary source of functional foods—natural food products that are increasingly acknowledged for their health benefits, especially in disease prevention and management (Oluwafemi et al., 2022; Puri et al., 2022). Plants suitable for functional food production generally possess important nutrients and are abundant in bioactive chemicals, including phenolics, flavonoids, alkaloids, and saponins (El-Ramady et al., 2022; Riaz et al., 2023). Nonetheless, actualizing this promise necessitates thorough assessments centered on their chemical composition, biological characteristics, and bioactive component concentration.

Durio zibethinus L., commonly referred to as durian, is a promising candidate for the development of functional foods. Durian is a tropical fruit indigenous to Southeast Asia, recognized for its unique flavor and potent scent. Durian, commonly known as the 'king of fruits,' possesses a soft and sweet pulp appreciated by many; however, its strong and pervasive odor is deemed unpleasant by others (Ketsa, 2018). Durian contains significant amounts of carbohydrates, fats, proteins, and vitamin C, establishing it as a valuable energy source. Besides its macronutrient composition, durian includes several bioactive compounds that may provide health advantages, such as dietary fiber and secondary metabolites like phenolics and alkaloids (Khaksar, Kasemcholathan, & Sirikantaramas, 2024). Two bioactive compounds have been documented to demonstrate a range of biological activities, such as antioxidant, anti-inflammatory, anti-cholesterol, antidiabetic, and antimicrobial properties (Chen et al., 2016; Ho & Bhat, 2015; Irwandi, Sartika, & Putra, 2022; Nugerahani, Suteja, Widharna, & Marsono, 2017).

In the past several decades, the body of research concerning functional foods that are sourced from indigenous plant-based resources has experienced substantial growth. This phenomenon can be attributed to the heightened public consciousness regarding the importance of healthy and preventive dietary practices (Arwanto et al., 2022). In the context of Indonesia, the employment of indigenous flora as foundational components for the advancement of functional food is experiencing a notable increase in interest; nevertheless, this initiative continues to encounter a range of scientific and technological obstacles. The challenges encompass a scarcity of quantitative data, the inadequacy of extraction technologies, and the absence of thoroughly developed downstream products that are supported by robust scientific validation. The limitations above significantly impede the optimal utilization of the nation's biodiversity, notwithstanding its substantial potential to function as a global epicenter for innovation in functional foods (Purwaningsih, Hardiyati, Zulhamdani, Laksani, & Rianto, 2021).

An innovative strategy in the realm of functional food development involves the valorization of agro-industrial byproducts that are abundant in bioactive compounds. This approach is in accordance with the principles of sustainability and the circular economy, reflecting a commitment to resource efficiency and environmental stewardship. In this context, the seeds of the durian, frequently regarded as mere byproducts and consequently disposed of, emerge as a previously overlooked resource that warrants comprehensive investigation. In addition to contributing to waste reduction and promoting environmental sustainability, the utilization of durian seeds has the potential to enhance the economic value associated with this particular fruit substantially. Prior research has demonstrated that extracts derived from durian seeds are rich in phenolic compounds, flavonoids, alkaloids, and triterpenoids (Amir & Saleh, 2014; Chen et al., 2016), all of which are linked to a diverse array of advantageous biological activities. Nevertheless, contemporary scholarly investigations have predominantly concentrated on qualitative identification, thereby exhibiting a deficiency in robust quantitative evidence that would substantiate their practical implementation. Consequently, more rigorous investigations must be conducted, focusing on the quantitative evaluation of bioactive compound concentrations. Such studies are crucial for generating measurable data that will support the advancement of functional foods derived from durian seeds (Kurniawati, 2023; Sultana et al., 2025).

Based on these considerations, this study aims to quantify the total phenolic and alkaloid contents in durian seeds as a preliminary step to assess their bioactive potential. The findings aim to establish a scientific foundation for the use of durian seeds as alternative raw materials in the development of functional foods.

METHODS

Sample Preparation

Durian seeds were sourced from the Simpang Raya District in Simalungun Regency, North Sumatra, Indonesia. The collected seeds underwent initial cleaning to remove residual fruit pulp, followed by washing under running water, thin slicing, and sun-drying for several days to reduce moisture content significantly. Following the drying process, the seeds were ground with a grinder and subsequently sieved to achieve a fine powder. The resultant powder was maintained in a securely sealed container at ambient temperature before extraction.

Extraction Process

The extraction process utilized the maceration technique, a traditional method effective for isolating bioactive compounds from plant materials. In this procedure, 500 grams of finely ground durian seed powder were placed in a sealed container and immersed in 70% ethanol at a solvent-to-sample ratio of 10:1 (v/w). The mixture was allowed to stand at ambient temperature for 72 hours to facilitate the diffusion and solubilization of phytochemical constituents into the solvent. Following the maceration period, the mixture underwent filtration to isolate the liquid extract from the solid residues. The filtrate was concentrated through evaporation with a rotary evaporator at 50°C, resulting in a viscous extract that was stored for further phytochemical analysis (Amir & Saleh, 2014).

Total Phenolic Content Analysis

Fifty milligrams of durian seed extract was accurately weighed and placed into a 10 mL volumetric flask to assess the total phenolic content. The sample was subsequently treated with 0.5 mL of Folin–Ciocalteu reagent, followed by the addition of 5 mL of distilled water. Following the thorough homogenization of the mixture, 1.5 mL of a 20% sodium carbonate (Na₂CO₃) solution was introduced to promote the colorimetric reaction. The mixture was allowed to stand under controlled conditions to facilitate complete color development. Absorbance measurements were conducted using a UV-Visible spectrophotometer at a wavelength of 760 nm. The analysis was conducted in three independent replicates, and the average absorbance values obtained were used to calculate the total phenolic concentration in the extract (Indriyah, Permatasari, & Pratama, 2023).

Alkaloid Content Analysis

A standard solution of quinine was prepared by accurately weighing 10 mg of quinine and dissolving it in 5 mL of 2N hydrochloric acid. The mixture was adequately combined and filtered to yield a clear solution. The filtrate underwent washing with 10 mL of chloroform, after which the organic phase was discarded. The aqueous phase was neutralized through the gradual addition of 0.1N sodium hydroxide. Subsequently, 5 mL of Bromocresol Green (BCG) reagent and 5 mL of phosphate buffer were added. The mixture was subjected to liquid-liquid extraction using 5 mL of chloroform and was magnetically stirred at 500 rpm for 15 minutes. The extraction procedure was conducted twice to optimize yield. The chloroform layers from each extraction were combined and evaporated using a nitrogen stream. The residue was subsequently re-dissolved in chloroform to achieve a final volume of 10 mL. A calibration curve was established by preparing standard solutions at concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.125 ppm, with absorbance values recorded at 470 nm using a UV-Vis spectrophotometer (Kurniawan, Nurbaeti, Ih, Nugraha, & Fajriaty, 2024).

RESULT AND DISCUSSIONS

Durian seeds are frequently regarded as a byproduct following the consumption of the pulp, even though they represent an underappreciated element of the fruit (Mustofa, Asmoro, & Handayani, 2024). Recent research demonstrates that durian seeds possess considerable nutritional significance, comprising roughly 80.61% carbohydrates, 6.26% protein, 0.57% fat, 0.85% fiber, and 41.42% starch (Permatasari, Witoyo, Masruri, Yuwono, & Widjanarko, 2021). Beyond their nutritional composition, durian seeds encompass a range of bioactive

compounds that could confer physiological advantages. The characteristics of durian seeds indicate their potential as a significant raw material for the formulation of functional food ingredients, especially in light of the increasing interest in natural products that confer health benefits (Oluwafemi et al., 2022).

This analysis seeks to ascertain the concentration of total phenolics and alkaloids present in durian seed extracts, thereby substantiating their potential as components of functional foods. The quantification of bioactive compounds generally commences with an extraction process designed to isolate the target molecules from the plant matrix. The efficacy of this procedure is predominantly contingent upon the selection of solvent, which should correspond with the polarity and solubility attributes of the target compounds to guarantee optimal yield and analytical precision (Patel, Panchal, & Ingle, 2019). This investigation entailed the extraction of durian seeds utilizing 70% ethanol as a solvent through a maceration technique aimed at quantifying the total phenolic and alkaloid contents. Amir & Saleh (2014) presented analogous findings through the utilization of the same solvent for the effective extraction of phenolics, flavonoids, alkaloids, and triterpenoids. In contrast, Mungmai et al. (2023) utilized 95% ethanol and found no detectable alkaloids, suggesting that 70% ethanol may provide enhanced extraction efficacy for specific bioactive compounds, particularly alkaloids and phenolics.

The selection of phenolic compounds and alkaloids as the primary focus of this investigation is grounded in their extensively reported health effects, which have been substantiated through rigorous scientific research. The presence of phenolic compounds is widely recognized for their potent antioxidant and anti-inflammatory properties, which contribute significantly to the mitigation of oxidative stress and the subsequent reduction of the risk associated with chronic diseases, including cardiovascular disorders and cancer (Allo, Suryanto, & Koleangan, 2022; Boussof et al., 2017; Maheshwari & Sharma, 2023). At this time, alkaloids exhibit a range of beneficial properties, including antimicrobial, antihypertensive, and antidiabetic effects, thereby rendering them up-and-coming candidates for incorporation into functional food formulations as well as traditional medicinal practices (Gan, Feng, He, Li, & Zhang, 2017; Masitah et al., 2023; Muhammad, Rahman, Gul-E-nayab, Nishan, & Shah, 2021; Renda, Pote, & Nadut, 2023). The existence and concentration of these two compounds within durian seeds not only augment their nutritional significance but also substantiate their potential as functional food components, which are capable of delivering particular health advantages.

Total Phenolic Content Analysis

The total phenolic content in durian seed extract was determined using UV-Vis spectrophotometry, which employed gallic acid as the standard.

Figure 1 presents the calibration curves utilized to quantify the total phenolic content in the samples. Absorbance values for the standards were measured using spectrophotometry, and a linear regression model was utilized to determine the relationship between concentration and absorbance.

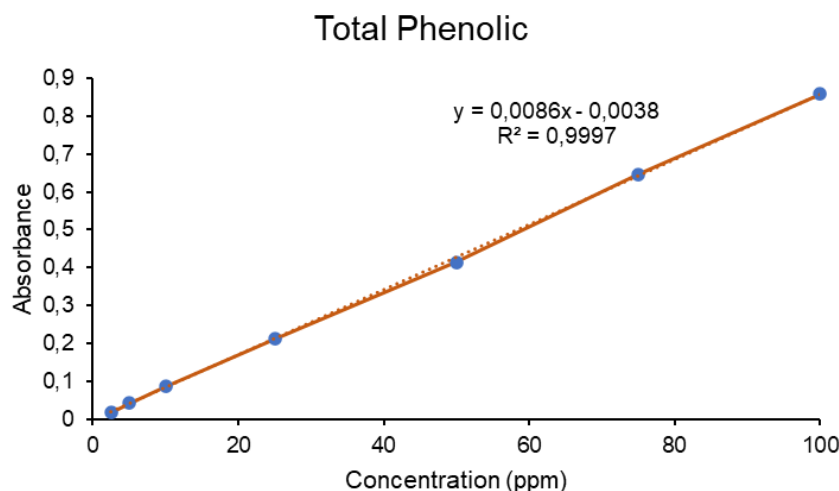


Figure 1. The Standard Curves Calibrations for Total Phenolic

The calibration curve obtained from the experiment exhibited a strong linear correlation between the concentration of the analyte and its associated absorbance. This relationship is quantitatively represented by the regression equation $y = 0.0086x - 0.0038$, which is further substantiated by a coefficient of determination (R^2) value of 0.9997, as illustrated in Figure 1. The R^2 value, approaching solidarity, indicates that the regression model utilized demonstrates an exceptionally high degree of accuracy and precision in estimating the concentration of phenolic compounds based on absorbance values (Wiyatno et al., 2024).

Table 1. Total Phenolic Content of Durian Seed Extract

Parameter	Value
Total phenolic content (mean \pm SD)	64.1 \pm 4.1 mg GAE/g
Number of replications (n)	3

A quantitative analysis of the total phenolic content in durian seed extract was performed to assess the potential bioactive compounds present within it. The data presented in Table 1 reveal that the total phenolic content in the extract of durian seeds is quantified at 64.1 \pm 4.1 mg GAE/g. The total phenolic content identified in this investigation surpassed the findings reported Maharani, Zuhro, & Mahbubah (2024), which indicated a value of 39.4 mg GAE/g. This variation could be affected by the method of extraction and the concentration of the solvent employed (Velázquez-Martínez et al., 2022). This study utilized maceration extraction with 70% ethanol, in contrast to the approach taken by Maharani et al. (2024) who implemented Soxhlet extraction using 96% ethanol. The investigation carried out by Kumalaningrum & Lestari (2024) revealed that the total phenolic concentration was greater in a 55% ethanol solution than in a 96% ethanol solution. In contrast to 55% ethanol, 70% ethanol demonstrates a superior capacity for binding phenolic compounds (Prayitno, Kusnadi, & Murtini, 2018). This phenomenon can be attributed to variations in solvent polarity, which profoundly influence the extraction efficacy of phenolic compounds. Phenolic compounds exhibit semi-polar characteristics, resulting in enhanced solubility in solvents of moderate polarity, exemplified by a 70% ethanol-water mixture. This solubility is notably greater than that observed in non-polar solvents, such as 96% ethanol, or in excessively polar solvents, such as 50% ethanol (Fauziyah, Widyasanti, & Sutresna, 2022).

Furthermore, the total phenolic content may exhibit variations that are influenced by the

specific extraction method utilized in conjunction with the concentration of the solvent employed. The methodology employed in this research involved the application of the maceration technique. The findings are consistent with the research performed by Sirumapea, Indryasari, Darwis, & Hilma (2021), which demonstrated that the process of maceration produced a higher total phenolic content when compared with the Soxhlet extraction method. The observed disparity can be ascribed to the superior efficacy of maceration in both the preservation and extraction of phenolic compounds. In contrast to Soxhlet extraction, a method that operates at heightened temperatures, specifically reaching 80°C, maceration is conducted at ambient temperature. This approach serves to mitigate the risk of thermal degradation of phenolic compounds, as noted by Mahardani & Yuanita (2021).

The specifically elevated total phenolic content identified in the extract derived from durian seeds indicates its promising potential as an organic source of antioxidants. Phenolic compounds are extensively acknowledged for their capacity to scavenge free radicals and alleviate oxidative stress, a condition that is associated with the onset of chronic diseases, including cancer, cardiovascular disorders, and diabetes (Abu-odeh et al., 2022; Bakrim et al., 2022; Vázquez-Ruiz et al., 2022). Consequently, this research establishes a robust scientific foundation for the application of durian seeds as a fundamental resource in the formulation of functional food products that assert antioxidant properties. Furthermore, the valorization of durian seeds, which are frequently regarded as agricultural byproducts, is congruent with sustainable methodologies and contributes to the economic enhancement of local food industries.

Alkaloid Content Analysis

Figure 2 presents the calibration curves employed for quantifying alkaloid content in the analyzed samples. The absorbance values corresponding to these standards were meticulously quantified using spectrophotometric techniques. Subsequently, a linear regression model was utilized to elucidate the relationship between concentration and absorbance, thereby providing a comprehensive understanding of their correlation.

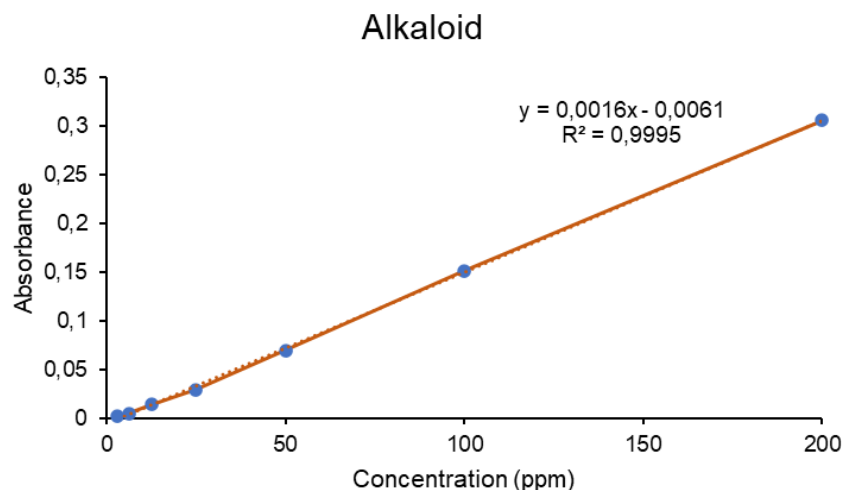


Figure 2. The Standard Curves Calibrations for Alkaloid

The determination of alkaloid content was performed through the measurement of the standard curve at a peak wavelength of 470 nm, utilizing a concentration series of 200, 100, 50, 25, 12.5, 6.25, and 3.125 ppm. The concentration of alkaloids in the extract derived from

durian seeds was determined utilizing the linear regression equation established from the corresponding calibration curve. The calibration curve obtained exhibited a robust linear relationship between concentration and absorbance, characterized by the regression equation $y = 0.0016x - 0.0061$ and a coefficient of determination (R^2) of 0.9995 (Figure 2).

Table 2. Alkaloid Content of Durian Seed Extract

Parameter	Value
Alkaloid content (mean \pm SD)	445.19 \pm 0.106 ppm
Number of replications (n)	2

The quantitative analysis of alkaloid content in the extract of durian seeds was conducted utilizing two replicates, as illustrated in Table 2. The findings indicated that the alkaloid concentration in the initial replicate was measured at 445.12 ppm, whereas the subsequent replicate recorded a concentration of 445.27 ppm. The average of these two measurements was determined to be 445.19 ppm, accompanied by a standard deviation of ± 0.106 ppm.

The analysis of alkaloid content in the extract of durian seeds revealed that the sample possessed a significant concentration of alkaloids. Nonetheless, this observation stands in opposition to the research undertaken by Azka et al. (2023), which indicated that methanol extracts of durian seeds were devoid of alkaloid compounds. The observed discrepancy can be ascribed to variations in the solvent employed throughout the extraction procedure (Hidayah, Hisan, Solikin, Irawati, & Mustikaningtyas, 2016). In the current investigation, ethanol served as the extraction solvent, characterized by a lower polarity in comparison to methanol (Ramadhan et al., 2023). Alkaloids are typically characterized as semi-polar compounds, which enhances their solubility in solvents exhibiting moderate polarity, such as ethanol. Conversely, methanol, due to its greater polarity, demonstrates enhanced efficacy in the extraction of highly polar compounds, including phenols and flavonoids (Dewi & Sari, 2024; Renda et al., 2023). The discrepancy in polarity between methanol and alkaloid compounds may yield diminished extraction efficiency, which could consequently result in the absence of alkaloid detection in methanol extracts, as articulated by Azka et al. (2023). These observations highlight the critical necessity of choosing suitable solvents in accordance with the chemical properties of the compounds of interest. In this context, ethanol has demonstrated superior efficacy compared to methanol in the extraction of alkaloids from durian seeds. This outcome offers significant understanding for enhancing the extraction processes of bioactive compounds, especially in the formulation of plant-derived natural products that encompass secondary metabolites like alkaloids.

The identification of phenolic compounds and alkaloids within the extract derived from durian seeds highlights the effectiveness of the extraction methodology utilized in this study. This research establishes an essential scientific basis for the potential application of these findings in the enhancement and development of functional food products (Masitah et al., 2023). The compounds classified as phenolic and alkaloid are extensively acknowledged for their multifaceted biological activities, which play a crucial role in the enhancement of health and the prevention of various diseases. These activities encompass antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anti-hypercholesterolemic properties (Gan et al., 2017; Liu et al., 2023; Muhammad et al., 2021; Simorangkir, Silaban, & Roza, 2022; Takó et al., 2020).

Considering their health-promoting properties, extracts from durian seeds, which are rich in phenolics and alkaloids, may be employed in the formulation of diverse functional food

products, including nutritionally enhanced beverages, plant-based snacks, or fortified cereal items (Patel & Rauf, 2017). Furthermore, the utilization of durian seeds, commonly considered waste, adheres to the tenets of sustainable food systems by minimizing agricultural by-products and optimizing the use of underutilized local biological resources (Ling, Lim, Khin, Antipina, & Ng, 2025). The findings of this study affirm that durian seed extract possesses promise as an active component in the formulation of functional meals, due to its elevated phenolic and alkaloid concentrations. The content of this compound is derived via the maceration method with a 70% ethanol solvent, which possesses appropriate polarity for the extraction of semi-polar phenolic and alkaloid compounds, while preserving the stability of thermolabile substances due to the process being conducted at ambient temperature. In contrast with several prior studies that employed high-concentration solvents or heated extraction techniques, which reported diminished results or failed to discover alkaloids (Chen et al., 2016). The study's focus remains confined to quantitatively examining total bioactive chemicals, lacking precise structure identification or biological activity assessment. Furthermore, no comparisons were conducted across solvents, extraction procedures, or variations in extraction parameters such as duration, temperature, and solvent ratio; hence, the results obtained cannot be utilized to ascertain the most optimal extraction circumstances. This work has not assessed the stability and sensory attributes of extracts in food systems, which are crucial for their practical applicability.

CONCLUSION

This study demonstrates that durian (*Durio zibethinus* L.) seed extract possesses total phenolic compounds of 64.1 ± 4.1 mg GAE/g and alkaloids of 445.19 ± 0.106 ppm, indicating significant promise as a source of bioactive ingredients for functional food production. The maceration technique utilizing a 70% ethanol solvent effectively extracts both components, aligning with the semi-polar nature of phenolics and alkaloids, while ambient temperature settings facilitate their stability. These results demonstrate a significant disparity when compared with prior investigations employing highly concentrated solvents or hot extraction techniques, yielding lower or undetectable active chemical concentrations. Future research should examine various solvents and extraction methods to optimize the extraction conditions for bioactive chemicals, thereby reinforcing and expanding these findings. Furthermore, conducting particular component characterization, biological activity assessments via *in vitro* and *in vivo* testing, and the formulation of food products derived from durian seed extract to ensure their applicability, safety, and sustainability within the functional food sector is essential.

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