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RESEARCH ARTICLE

Antioxidant Activity of Quercetin and Quercetin-3-*O*-Glucoside Isolated from Durian Seed (*Durio zibethinus* Murr.)

Dante Alighiri[1,*], Lisdiana[2], Masturi[3], Kumala Aris Tanti[1], Apriliana Drastisianti[4], Samuel Budi Wardhana Kusuma[1], Neli Syahida Ni'ma[5] and Tri Minarsih[5]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang 50229, Central Java, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang 50229, Central Java, Indonesia*
[3]*Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang 50229, Central Java, Indonesia*
[4]*Department of Chemistry Education, Faculty of Science and Technology, Universitas Islam Negeri Walisongo Semarang, Semarang 50185, Central Java, Indonesia*
[5]*Department of Pharmacy, Faculty of Medicine, Universitas Negeri Semarang, Semarang 50229, Central Java, Indonesia*

(*Corresponding author's e-mail: dante_alighiri@mail.unnes.ac.id)

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Abstract

Durian seeds (*Durio zibethinus* Murr.), an underutilised agro-industrial by-product, are a promising source of bioactive flavonoids with potential antioxidant uses. However, comprehensive studies that combine extraction optimisation, flavonoid isolation, and structure–activity relationship analysis are scarce. This research examined extraction optimisation using maceration, reflux, and Soxhlet methods with ethanol, methanol, and *n*-hexane as solvents. The extracts were characterised by proximate analysis, phytochemical screening, and determination of total phenolic and flavonoid contents by UV-Vis spectrophotometry. Flavonoid isolation was carried out by column chromatography, and antioxidant activity was assessed using the DPPH assay to determine IC_{50} values. Reflux extraction with ethanol yielded the highest phenolic (26.9451 mg GAE/g) and flavonoid (3.6363 mg QE/g) contents. FTIR and HPLC analyses, supported by retention-time comparisons with authentic standards, suggested the presence of quercetin-related and quercetin-3-*O*-glucoside-related constituents. Quercetin-related concentration estimates ranged from 37.769 to 101.979 ppm, while quercetin-3-*O*-glucoside-related peaks showed relative chromatographic peak area values of 97.01%–98.81%. Among the three isolated fractions, Isolate 4 had both the highest quercetin-related concentration and the strongest antioxidant activity (IC_{50} = 92.6346 μg/mL). In contrast, Isolate 3 showed lower antioxidant activity despite having a higher relative peak area for quercetin-3-*O*-glucoside-related constituents. This pattern indicates that relative chromatographic peak dominance alone did not explain antioxidant performance across the three fractions. The findings support the potential of durian seed biomass as a sustainable source of natural antioxidants for use in functional foods and nutraceuticals.

Keywords: Flavonoids, Durian seeds, Antioxidant activity, Quercetin, Quercetin-3-*O*-glucoside

Introduction

Durian (*Durio zibethinus* Murr.) production is increasing throughout Southeast Asia, leading to significant agro-industrial waste, mainly peels and seeds [1,2]. Although durian seeds account for a large share of the fruit's biomass, their use remains limited despite rising interest in sustainable waste utilisation and in

extracting bioactive compounds from biomass [3,4]. The limited use of durian seeds not only worsens environmental issues related to waste disposal but also misses the chance to create valuable natural products for use in functional foods and nutraceuticals [4-6].

Previous studies have mainly examined the carbohydrate-rich composition of durian seeds and their use in food products, edible coatings, biomass utilisation, and bioenergy. However, research on secondary metabolites and their biofunctional properties, particularly flavonoids and antioxidants, remains relatively scarce. This is significant because secondary metabolites are key to the biological and functional qualities of plant-based materials [7-14].

Flavonoids, a group of polyphenolic compounds among secondary metabolites, have garnered significant scientific interest due to their wide range of biological activities and potent antioxidant properties [15-18]. They are extensively used in food, pharmaceutical, and nutraceutical sectors [10,19-23]. Notably, quercetin and its glycosylated forms are increasingly studied for their exceptional ability to scavenge free radicals and donate hydrogen atoms and electrons during oxidative processes [24-27]. The antioxidant effectiveness of flavonoids is largely determined by their molecular structure, especially the quantity and placement of hydroxyl groups and glycosylation patterns, which influence electron delocalisation and stabilisation of free radicals [26-29].

Despite increasing interest in flavonoid bioactivity, there is still a lack of comprehensive studies that combine extraction optimisation, flavonoid isolation, and quantitative analysis of antioxidant structure–activity relationships specifically from durian seeds [15,17]. Most earlier research has mainly examined the antioxidant activity of crude extracts without thoroughly investigating how flavonoid composition and molecular form affect antioxidant performance. To fill this gap, the current study incorporates extraction optimisation, chromatographic fractionation, relative chromatographic evaluation, and quantitative IC_{50} analysis of selected flavonoid-enriched fractions. This integrated approach aims to clarify how molecular structure impacts flavonoid bioactivity in this underutilised biomass resource.

This study examines how extraction methods and solvent polarity affect flavonoid recovery from durian seeds. It also explores the link between flavonoid composition and antioxidant activity, with a focus on comparing quercetin and quercetin-3-*O*-glucoside. This comparison aims to deepen understanding of how flavonoid structures relate to their activity. Ultimately, the results are intended to promote the sustainable use of durian seed biomass as a source of natural antioxidants for functional foods and nutraceuticals.

Materials and methods

Materials

Durian seeds analysed in this study were obtained from Gunungpati District, Semarang, Indonesia. Silica gel G60F254 plates and silica gel G40–63 were purchased from Merck (Germany). Analytical-grade ethanol, methanol, *n*-hexane, Folin–Ciocalteu reagent, aluminium chloride ($AlCl_3$), DPPH, and other analytical-grade reagents were obtained from Merck (Germany) and used without further purification.

The instruments used in this study included a UV-Vis spectrophotometer (Shimadzu UV-1800) for total phenolic and flavonoid analysis, a Fourier Transform Infrared (FTIR) spectrometer (Bruker Alpha) for functional group analysis, and a High-Performance Liquid Chromatography (HPLC) system (Shimadzu LC-20AD) for flavonoid characterisation. A rotary vacuum evaporator (Büchi R-300), a Soxhlet extractor, and a reflux apparatus were also employed during the extraction and concentration processes.

Sample preparation

Durian seeds were washed thoroughly with distilled water, sliced into smaller pieces, and dried indoors at room temperature for 5 days to minimise moisture-induced degradation before extraction. The dried seeds were ground into a fine powder and sieved through a 60-mesh sieve to obtain a homogeneous particle size suitable for extraction [15,30,31].

Proximate analysis

Proximate analysis was performed to assess the nutritional components of durian seed powder, including moisture, ash, protein, fat, and carbohydrate contents [15,30-32]. Moisture was measured by oven drying at 105 °C, and ash content was determined by furnace incineration. Protein was analysed using the Kjeldahl method, and fat was extracted using Soxhlet

extraction with *n*-hexane as the solvent. Carbohydrates were estimated by calculating the difference.

Phytochemical screening

Phytochemical screening was conducted using standard qualitative methods to identify major classes of bioactive compounds in durian seed extracts, including alkaloids, phenolics, flavonoids, terpenoids, steroids, saponins, and tannins [15,33-35]. Alkaloids were evaluated using Dragendorff's and Mayer's reagents, while phenolics and tannins were identified using ferric chloride ($FeCl_3$). Flavonoids were analysed using the $AlCl_3$ and Shinoda tests. Terpenoids and steroids were assessed using the Salkowski and Liebermann–Burchard methods, whereas saponins were identified using the foam test.

Extraction optimisation

Durian seed powder was extracted using maceration, reflux, and Soxhlet techniques with ethanol, methanol, and *n*-hexane as solvents to obtain flavonoid-rich crude extracts [15,30,31]. In the maceration method, the sample was soaked in 1 L of solvent for 24 h at room temperature, then filtered and solvent removed using a rotary evaporator. For reflux extraction, 250 g of durian seed powder was extracted with 1 L of solvent at 60 °C for 6 h under continuous heating. Soxhlet extraction was performed using 250 g of sample with 1 L of solvent at 60 °C for 6 h under continuous extraction conditions. The resulting extracts were subsequently concentrated for further analysis.

These extraction techniques were used to evaluate the combined effects of the extraction mechanism and solvent polarity on flavonoid recovery. Maceration, reflux, and Soxhlet extraction represent diffusion-driven, thermally assisted, and continuous extraction systems, respectively, enabling a comparative assessment of mass-transfer efficiency and compound solubilisation. Ethanol, methanol, and *n*-hexane were selected based on differences in solvent polarity to evaluate extraction selectivity toward flavonoid compounds.

Total phenolic content assay

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method as previously reported [30,31]. Briefly, 1.6 mL of sample solution (100 μg/mL) was mixed with 0.2 mL of Folin–Ciocalteu reagent and 0.2 mL of 10% sodium carbonate solution. The mixture was homogenised and incubated at room temperature for 30 min before absorbance measurement at the predetermined maximum wavelength.

Total flavonoid content assay

Total flavonoid content (TFC) was determined using the aluminium chloride ($AlCl_3$) colourimetric method, as previously reported [30,31]. Briefly, 2 mL of sample solution was mixed with 0.1 mL of 10% $AlCl_3$ and 0.1 mL of 1 mM potassium acetate solution. The mixture was homogenised and incubated in the dark at room temperature for 30 min prior to analysis using a UV-Vis spectrophotometer at the predetermined maximum wavelength.

Isolation of flavonoid compounds

The extract exhibiting the highest total phenolic and flavonoid contents was selected for flavonoid isolation using Thin Layer Chromatography (TLC) and column chromatography [33]. TLC analysis was initially performed to determine the optimal eluent system for flavonoid separation. Silica gel G60F254 plates were used as the stationary phase, and a petroleum ether:ethyl acetate mixture (25:6, v/v) was used as the mobile phase. The developed plates were observed under UV light at 254 and 365 nm. Flavonoid compounds were tentatively identified based on Rf value comparison with reference flavonoid standards.

Following TLC optimisation, column chromatography was subsequently performed using silica gel G40–63 as the stationary phase and the selected eluent system obtained from TLC analysis. One gram of durian seed extract dissolved in methanol was loaded onto the column, followed by continuous elution. The resulting fractions were collected and concentrated for further characterisation and antioxidant analysis [33].

Antioxidant activity testing

The antioxidant activity of durian seed extracts, isolated fractions, quercetin, and vitamin C standards was assessed through the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay [34-36]. This assay measures the samples' capacity to reduce DPPH radicals, indicated by changes in absorbance and expressed as radical-scavenging activity. The IC_{50} value,

representing the concentration required to inhibit 50% of DPPH radicals, was calculated by linear regression of sample concentration versus inhibition percentage. All tests were conducted in triplicate.

Fourier Transform Infrared Analysis (FTIR)

Fourier Transform Infrared (FTIR) analysis was performed to evaluate the functional group characteristics of durian seed powder, extracts, and selected flavonoid-enriched fractions [36,37]. Approximately 1 mg of sample was mixed with potassium bromide (KBr) and compressed into pellets prior to analysis using an FTIR spectrometer within the spectral range of 4,000 - 400 cm^{-1}. The resulting spectra were used to evaluate changes in functional group profiles before and after extraction, as well as among the isolated flavonoid fractions.

High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) analysis was conducted to examine the chromatographic profiles and flavonoid-related peak distribution in durian seed extracts and their isolated fractions using quercetin and quercetin-3-*O*-glucoside as reference standards [33]. The mobile phase was chosen based on the optimal eluent composition determined from Thin Layer Chromatography (TLC) analysis. Separation was performed on a reverse-phase column with an injection volume of 20 μL, a flow rate of 1 mL/min, and a column temperature of 25 °C. Detection was conducted at 254 nm.

The retention times for quercetin and quercetin-3-*O*-glucoside standards were 2.818 and 3.540 min, respectively. Chromatographic peaks in the durian seed extracts and isolated fractions were compared with the retention times of the reference standards, and peak areas were integrated for relative peak-area evaluation. Repeated runs produced consistent chromatograms and retention times, supporting analytical reproducibility under the applied chromatographic conditions. Because the method was used for semi-quantitative profiling rather than as a fully validated targeted assay, the reported values are interpreted as quercetin-related concentration estimates and relative chromatographic peak areas.

Statistical analysis

All experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to evaluate significant differences among extraction methods, total phenolic content, total flavonoid content, and antioxidant activity. Exploratory Pearson's correlation analysis was used to evaluate the relationship between quercetin-related concentrations and the IC_{50} values of the selected isolates. Statistical analyses were performed using RStudio. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Characteristics of durian seed

The Montong durian seeds used in this study were obtained from plants cultivated in Gunungpati, Semarang, Indonesia. Morphological observation showed that the seeds exhibited typical characteristics of *Durio zibethinus* Rumph. ex Murray, including an elongated-oval shape, a smooth light-brown seed coat, and relatively uniform seed morphology (**Figure 1**). These characteristics are consistent with previously reported descriptions of *Durio zibethinus* and support the botanical identification of the sample [38,39].

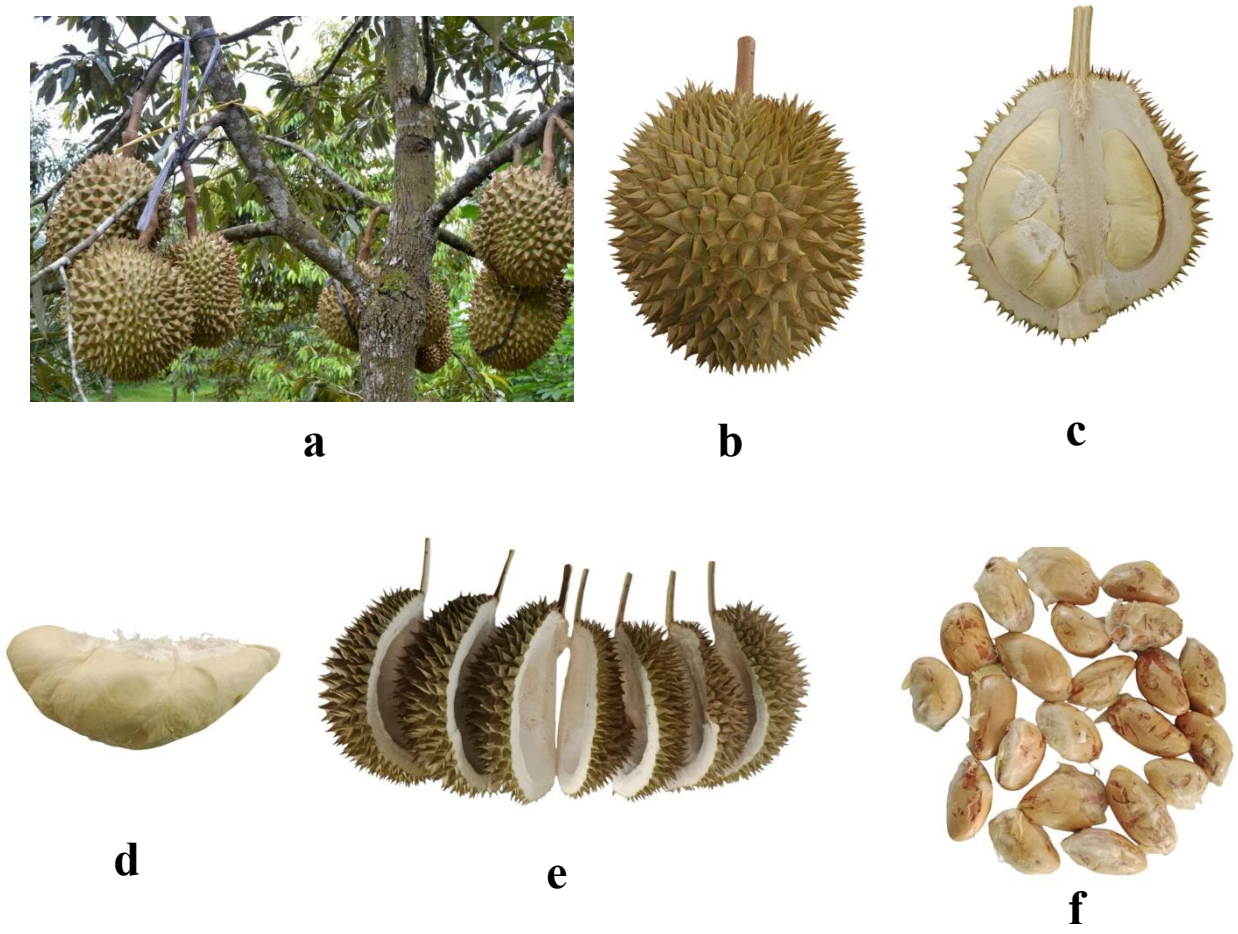


Figure 1 Morphological characteristics of Montong durian (*Durio zibethinus* Rumph. ex Murray) cultivated in Gunungpati, Semarang, Indonesia: (a) durian plant; (b) durian fruit; (c) durian pulp; (d) whole fruit; (e) rind; and (f) seed morphology.

Figure 1 presents the morphological features of the durian plant, fruit, rind, pulp, and seed. The fruit showed typical external characteristics, including an obovoid shape, grey-yellow rind, and aromatic flesh, which are commonly associated with cultivated durian varieties [40,41]. The seed morphology, as shown in **Figure 1(f)**, was characterised by an elongated oval form with a yellowish-brown outer surface. The seeds were approximately 2 - 4 cm long and 1 - 2 cm wide, although slight variations may occur depending on fruit maturity and cultivation conditions.

The observed seed characteristics indicate that the sample was suitable for further processing and extraction. Minor morphological variation compared with previous reports may be attributed to environmental and genetic factors, including cultivation conditions and domestication effects, as previously described for durian germplasm [38]. These observations provide an initial basis for confirming sample identity before proximate, phytochemical, and antioxidant analyses.

Proximate analysis of durian seed

Proximate analysis was performed to evaluate the nutritional composition of raw and dehydrated durian seed powder, including moisture, ash, protein, fat, and carbohydrate contents [15,30-32]. The results are presented in **Table 1**.

Table 1 Proximate composition of raw and dehydrated durian seed powder.

Component	Content (%)	
	Raw	**Dehydrated**
Moisture	29.00 ± 1.25[a]	4.58 ± 1.25[b]
Ash	4.78 ± 0.32[a]	5.43 ± 0.32[a]
Protein	11.01 ± 0.75[a]	11.50 ± 0.75[a]
Fat	3.15 ± 0.18[a]	3.58 ± 0.18[a]
Carbohydrate	52.06 ± 2.10[b]	74.91 ± 2.10[a]

Note: Data are presented as mean ± standard deviation (n = 3). Within each row, values followed by different superscript letters are significantly different between raw and dehydrated samples ($p < 0.05$), whereas values followed by the same superscript letter are not significantly different.

Dehydration significantly reduced the moisture content of durian seed powder from 29.00% to 4.58%, indicating improved sample stability for subsequent extraction and storage. This reduction is important because lower moisture content can minimise microbial growth and reduce moisture-induced degradation of bioactive constituents during sample handling and preparation. In contrast, ash, protein, and fat contents showed no significant differences between raw and dehydrated samples, suggesting that dehydration primarily affected water removal rather than substantially altering these nutritional components.

Carbohydrate was the predominant component in both raw and dehydrated durian seed powder, increasing significantly from 52.06% to 74.91% after dehydration. This apparent increase was mainly attributed to moisture removal, which proportionally concentrated the dry matter components. These findings are consistent with previous reports showing that durian seeds are rich in carbohydrate-based constituents, particularly polysaccharides and starch-related components [10,16-21]. Overall, the proximate profile indicates that dehydrated durian seed powder provides a stable biomass matrix for subsequent extraction and recovery of bioactive compounds.

Extraction optimisation of durian seed

Durian seed powder was extracted using maceration, reflux, and Soxhlet extraction with *n*-hexane, ethanol, and methanol as solvents to evaluate the combined effects of extraction method and solvent polarity on crude extract recovery. The extraction yields obtained from each method and solvent combination are presented in **Table 2**.

Table 2 Extraction yield of durian seed powder using different extraction methods and solvents.

Extraction method	*n*-Hexane (%)	Ethanol (%)	Methanol (%)
Maceration	1.12 ± 0.03[e]	7.63 ± 0.15[c]	7.22 ± 0.12[c]
Reflux	3.60 ± 0.05[d]	12.90 ± 0.25[a]	12.20 ± 0.20[a]
Soxhlet	1.14 ± 0.04[e]	9.40 ± 0.18[b]	8.27 ± 0.15[b]

Note: Data are presented as mean ± standard deviation (n = 3). Values followed by different superscript letters indicate significant differences among extraction treatments ($p < 0.05$), whereas values followed by the same superscript letter are not significantly different.

The extraction yield depended on both the method used and the solvent type. Reflux extraction with ethanol and methanol yielded the highest amounts, showing that heat-assisted extraction with polar solvents improves the recovery of compounds from durian seed powder. Soxhlet extraction with these solvents gave moderate yields, whereas maceration yielded lower yields than either the reflux or Soxhlet methods. This pattern indicates that moderate heating and better solvent penetration during reflux enhance mass transfer, making it easier to release intracellular components from the plant material.

Solvent polarity significantly influenced extraction efficiency. Across all methods, ethanol and methanol generally yielded more extract than *n*-hexane, indicating that durian seed compounds favour polar solvents. This aligns with the presence of polar phytochemicals such as phenolics and flavonoids, which are more easily extracted by polar organic solvents. For *n*-hexane treatments, reflux extraction yielded higher yields than maceration or Soxhlet extraction, suggesting that heat-assisted extraction enhances solvent penetration and matrix disruption, even with non-polar solvents.

Reflux extraction with ethanol was chosen as the most effective method overall, as it yielded the highest results and used a solvent with suitable polarity, lower toxicity, and relevance to food and nutraceutical applications. Consequently, the ethanol reflux extract was utilised for further phytochemical screening, flavonoid isolation, and antioxidant testing.

Phytochemical screening of durian seed extracts

Phytochemical screening was conducted to qualitatively evaluate the major classes of secondary metabolites in durian seed extracts obtained with solvents of varying polarities. The screening results are presented in **Table 3**.

Table 3 Phytochemical screening of durian seed extracts obtained using different solvents.

Phytochemical class	Assay method	*n*-Hexane	Ethanol	Methanol
Alkaloids	Dragendorff's reagent	−	−	−
	Mayer's test	−	−	−
Phenolics	$FeCl_3$ test	−	+	+
Flavonoids	HCl–Mg reaction	−	+	+
	Shinoda test	−	+	+
Terpenoids	Salkowski and Liebermann–Burchard tests	+	+	+
Steroids	Salkowski and Liebermann–Burchard tests	−	−	−
Saponins	Foam test	−	−	−
Tannins	$FeCl_3$ test	−	−	−

Note: (+) indicates detected; (−) indicates not detected under the applied test conditions.

Phytochemical screening showed distinct differences in the distribution of secondary metabolites across the extracts. Phenolics and flavonoids were present in ethanol and methanol extracts but absent in the *n*-hexane extract. This suggests that polar solvents are more effective at extracting phenolic compounds from durian seeds, consistent with the polar properties of many flavonoids and phenolic compounds. The presence of flavonoids in ethanol and methanol extracts also justifies selecting these polar extracts for further flavonoid isolation and antioxidant testing.

Conversely, alkaloids, steroids, saponins, and tannins were not detected in any of the extracts by the qualitative tests. The lack of alkaloids implies that nitrogen-containing secondary metabolites were not significant in the durian seed extracts examined. Additionally, the absence of saponins and tannins suggests that these compounds were either absent, below detection limits, or not effectively extracted under the conditions used.

Terpenoids were found in all extracts, suggesting this compound class may have greater solvent compatibility than phenolics and flavonoids. Their presence in both polar and non-polar extracts indicates that durian seeds contain phytochemicals with different polarities. Overall, the phytochemical profile shows that ethanol and methanol are more effective at extracting phenolic and flavonoid compounds, whereas *n*-hexane is less effective. These results highlight the influence of solvent polarity on phytochemical extraction and support the use of ethanol for subsequent flavonoid isolation and antioxidant testing.

Total phenolic and flavonoid contents of durian seed extracts

Total phenolic content (TPC) and total flavonoid content (TFC) were determined using UV-Vis spectrophotometry with gallic acid and quercetin as reference standards, respectively, following previously reported procedures [30,31]. The calibration curves for gallic acid and quercetin showed good linearity, with regression equations $y = 0.0164x + 0.0021$ ($R^2 = 0.9952$) and $y = 0.0418x - 0.01$ ($R^2 = 0.9962$), respectively. These high correlation coefficients indicate that both calibration curves were suitable for quantifying phenolic and flavonoid contents in durian seed extracts.

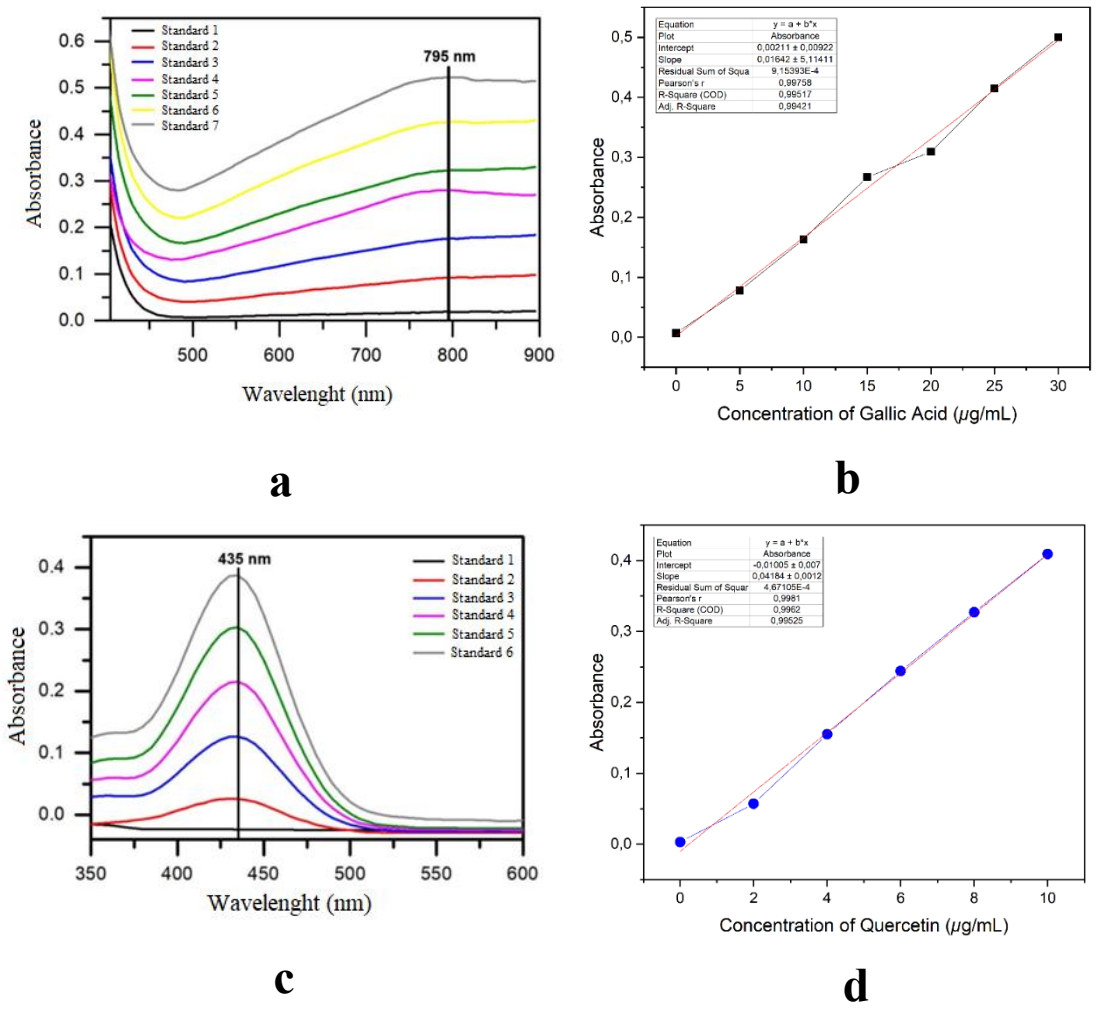


Figure 2 Optimum wavelengths and calibration curves of standard solutions for gallic acid (a), (b) and quercetin (c), (d).

The TPC values varied markedly with extraction method and solvent type (**Table 4**). Reflux extraction with ethanol produced the highest TPC value (26.9451 mg GAE/g sample), followed by reflux extraction with methanol (14.3841 mg GAE/g sample) and Soxhlet extraction with ethanol (13.7134 mg GAE/g sample). In contrast, *n*-hexane extracts showed considerably lower TPC values, indicating that phenolic compounds in durian seeds were more efficiently recovered using polar solvents. Previous studies have also reported that durian biomass contains phenolic compounds with antioxidant potential, supporting the relevance of phenolic profiling for evaluating durian-derived materials [42,43]. These findings are consistent with previous reports on the antioxidant potential and bioactive constituents of durian-derived materials [42-45].

Table 4 Total phenolic content (TPC) of durian seed extracts obtained using different extraction methods and solvents.

Extraction method	*n*-Hexane (mg GAE/g sample)	Ethanol (mg GAE/g sample)	Methanol (mg GAE/g sample)
Maceration	1.5793 ± 0.2644^{e}	8.4695 ± 0.1917^{c}	5.4817 ± 0.3959^{d}
Reflux	5.2378 ± 0.1123^{d}	26.9451 ± 0.2840^{a}	14.3841 ± 0.4628^{b}
Soxhlet	0.4817 ± 0.0468^{f}	13.7134 ± 0.3842^{b}	8.2866 ± 0.2154^{c}

Note: Data are presented as mean ± standard deviation ($n = 3$). Values followed by different superscript letters indicate significant differences among extraction treatments ($p < 0.05$), whereas values followed by the same superscript letter are not significantly different.

A similar pattern was observed for TFC (**Table 5**). Reflux extraction using ethanol yielded the highest TFC value (3.6363 mg QE/g sample), followed by Soxhlet extraction using ethanol (2.3684 mg QE/g sample) and reflux extraction using methanol (2.1053 mg QE/g sample). These findings indicate that ethanol under reflux conditions was the most effective extraction system for recovering flavonoid compounds from durian seed powder.

Table 5 Total flavonoid content (TFC) of durian seed extracts obtained using different extraction methods and solvents.

Extraction method	*n*-Hexane (mg QE/g sample)	Ethanol (mg QE/g sample)	Methanol (mg QE/g sample)
Maceration	0.3110 ± 0.0264^{h}	1.0287 ± 0.0513^{f}	1.2201 ± 0.0745^{e}
Reflux	0.7416 ± 0.0321^{g}	3.6363 ± 0.0847^{a}	2.1053 ± 0.0629^{e}
Soxhlet	0.8373 ± 0.0186^{g}	2.3684 ± 0.0452^{b}	1.4593 ± 0.0278^{d}

Note: Data are presented as mean ± standard deviation ($n = 3$). Values followed by different superscript letters indicate significant differences among extraction treatments ($p < 0.05$), whereas values followed by the same superscript letter are not significantly different.

The combined effects of crude extract yield, solvent polarity, and extraction mechanism likely governed the differences in TPC and TFC among extraction treatments. Higher extraction yield may increase the total amount of extractable constituents and, consequently, lead to higher measurable phenolic and flavonoid contents. Nevertheless, the extraction yield alone cannot fully explain the observed TPC and TFC patterns, because these values also depend on the solvent's ability to solubilise phenolic and flavonoid constituents selectively. Therefore, the superior TPC and TFC obtained from ethanol reflux extraction suggest that this condition provided both efficient matrix disruption and favourable solubilisation of phenolic-type compounds.

Solvent polarity played a central role in the recovery of phenolics and flavonoids. Ethanol and methanol generally produced higher TPC and TFC values than *n*-hexane, reflecting the polar nature of many phenolic and flavonoid compounds. The presence of hydroxyl groups in these compounds increases their compatibility with polar organic solvents, thereby enhancing their extraction from the plant matrix. Among the extraction methods, reflux extraction provided better recovery than maceration and Soxhlet extraction, probably due to moderate heating, improved solvent penetration, enhanced mass transfer, and more effective release of intracellular constituents.

The superior performance of ethanol is particularly relevant because ethanol combines effective extraction capability with lower toxicity and greater suitability for food- and nutraceutical-related applications. Although methanol also effectively extracts phenolic and flavonoid compounds, ethanol is

more appropriate for recovering the bioactive compounds intended for functional food applications. Phenolic and flavonoid compounds are important contributors to antioxidant activity because their hydroxyl groups can participate in hydrogen-donating and electron transfer mechanisms during radical-scavenging reactions [46]. Thus, the high TPC and TFC values observed in the ethanol reflux extract support its selection for subsequent flavonoid isolation and antioxidant activity evaluation.

Thin Layer Chromatography (TLC) of durian seed ethanol extract

The ethanol extract of durian seed was analysed using Thin Layer Chromatography (TLC) to evaluate its separation profile and support preliminary flavonoid screening. TLC separation was performed using petroleum ether:ethyl acetate (25:6, v/v) as the mobile phase, with quercetin as the reference standard. The developed chromatograms were observed under UV light at 254 and 365 nm, and the TLC profiles are shown in **Figure 3**.

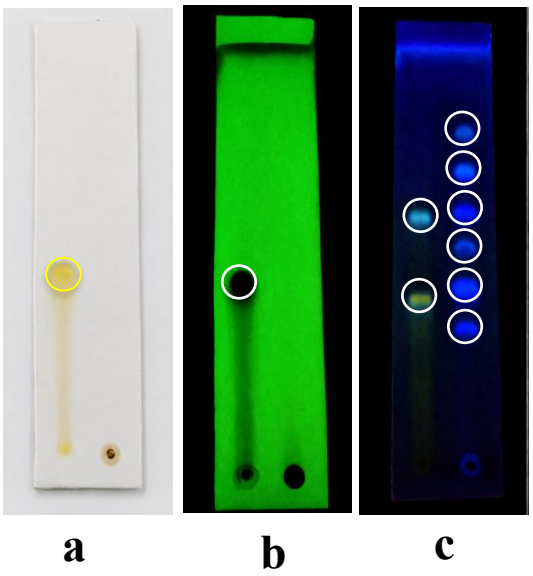


Figure 3 TLC profile of durian seed ethanol extract using petroleum ether:ethyl acetate (25:6, v/v) as the mobile phase: (a) developed TLC plate; (b) observation under UV light at 254 nm; and (c) observation under UV light at 365 nm.

At 254 nm, no clearly visible spot was observed for the durian seed ethanol extract, whereas the quercetin reference standard showed a distinct spot with an Rf value of 0.41. Under UV light at 365 nm, six distinct spots were observed in the ethanol extract, with Rf values of 0.31, 0.41, 0.48, 0.55, 0.66, and 0.78. Among these spots, the band with an Rf value of 0.41 showed chromatographic behaviour similar to the quercetin reference standard, suggesting the possible presence of quercetin-related flavonoid constituents in the ethanol extract.

The petroleum ether:ethyl acetate (25:6, v/v) solvent system provided clear separation of several constituents in the ethanol extract, with minimal tailing. This result indicates that the selected eluent had sufficient polarity to separate flavonoid-related compounds from the durian seed matrix. However, TLC results were interpreted only as preliminary evidence, and further HPLC chromatographic characterisation was required to support the tentative identification and relative evaluation of flavonoid constituents.

Column chromatography of durian seed ethanol extract

Column chromatography was performed to fractionate flavonoid-related constituents from the durian seed ethanol extract using the previously optimised solvent system from TLC. Petroleum ether:ethyl acetate (25:6, v/v) was used as the mobile phase, and silica gel as the stationary phase. This solvent system provided sufficient polarity to separate several

constituents in the ethanol extract, as indicated by distinct TLC spots in the preliminary separation.

A total of thirteen fractions were collected from the column chromatography process, as shown in **Figure 4**, and each fraction was further monitored using TLC. Among these fractions, 3 - 5 showed single, well-defined TLC spots, suggesting relatively enriched fractions compared with the crude ethanol extract (**Figure 5**). The Rf values of fractions 3 - 5 were 0.41, 0.39, and 0.40, respectively. These values were close to the Rf value of the quercetin reference standard, suggesting the possible presence of quercetin-related flavonoid constituents in these fractions.

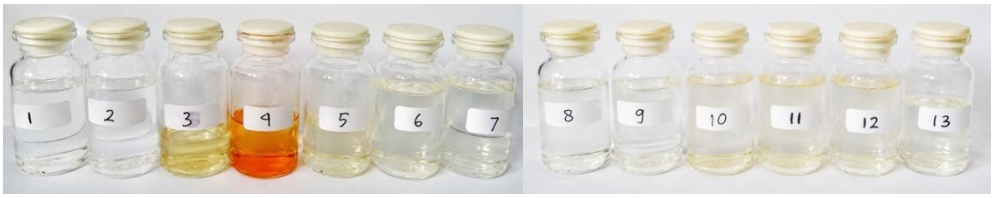


Figure 4 Fractions obtained from column chromatography of durian seed ethanol extract using silica gel as the stationary phase and petroleum ether:ethyl acetate (25:6, v/v) as the mobile phase.

The intense orange colour observed in Fraction 4 may reflect the enrichment of flavonoid-related chromophores during column chromatography. This visual characteristic is consistent with the concentration of conjugated or polyphenolic constituents in the selected fraction. Considering the carbohydrate-rich nature of durian seeds, the possible contribution of non-enzymatic browning-derived constituents, such as melanoidin-like compounds, may be considered. However, this interpretation remains speculative because specific melanoidin markers were not analysed. The higher quercetin-related concentration and stronger antioxidant activity observed in Isolate 4 suggest that this orange fraction was enriched with antioxidant-active chromophoric constituents rather than representing visual impurity.

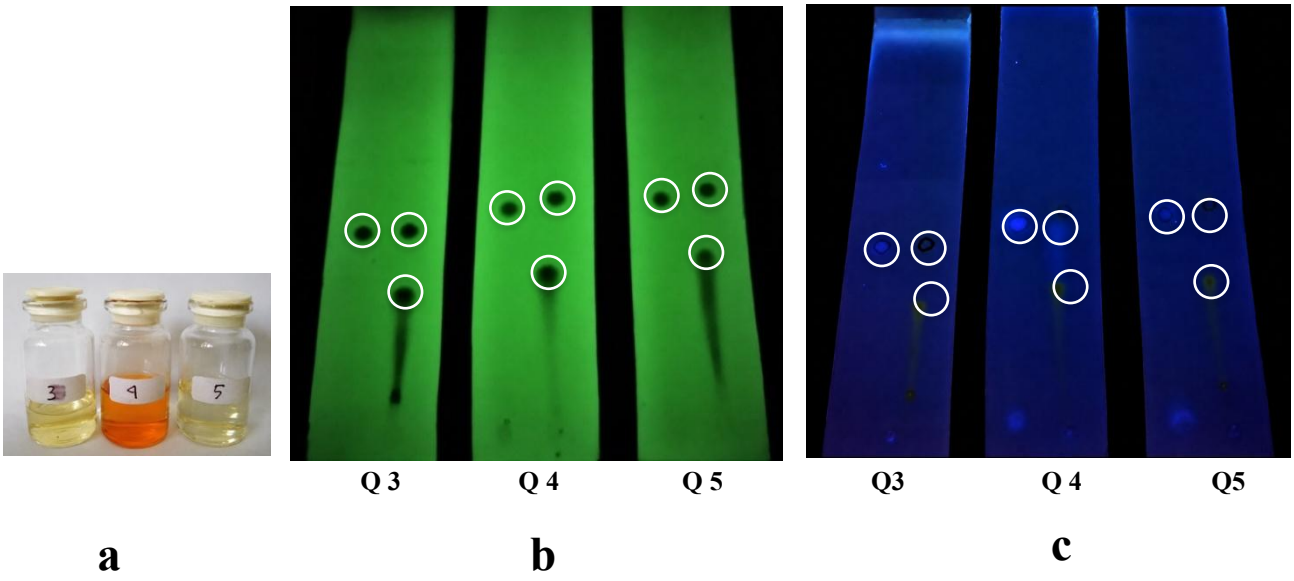


Figure 5 TLC profiles of selected column chromatography fractions: (a) developed TLC plate of fractions 3 - 5; (b) observation under UV light at 254 nm; and (c) observation under UV light at 365 nm.

The appearance of single spots in selected fractions indicates improved chromatographic separation and enrichment of flavonoid-related constituents. However, TLC-based fraction monitoring provides only preliminary evidence of compound similarity and does not provide definitive structural

confirmation. Therefore, the selected fractions were subjected to further FTIR and HPLC analyses to evaluate their functional group characteristics, chromatographic behaviour, and relative flavonoid composition.

Antioxidant activity assay of durian seed ethanol extract and isolated flavonoid fractions

The antioxidant activity of durian seed ethanol extract, isolated flavonoid fractions, quercetin standard, and vitamin C standard was evaluated using the DPPH radical scavenging assay [34-36]. This assay is based on the ability of antioxidant compounds to reduce DPPH radicals, which is reflected by a decrease in absorbance. Antioxidant activity was expressed as IC_{50}, defined as the concentration required to inhibit 50% of DPPH radicals. Lower IC_{50} values indicate greater radical-scavenging activity.

Antioxidant activity was evaluated at concentrations ranging from 20 to 120 μg/mL, with absorbance measured at 514 nm. Linear regression analysis was used to determine the IC_{50} values of each sample, as shown in **Figure 6**.

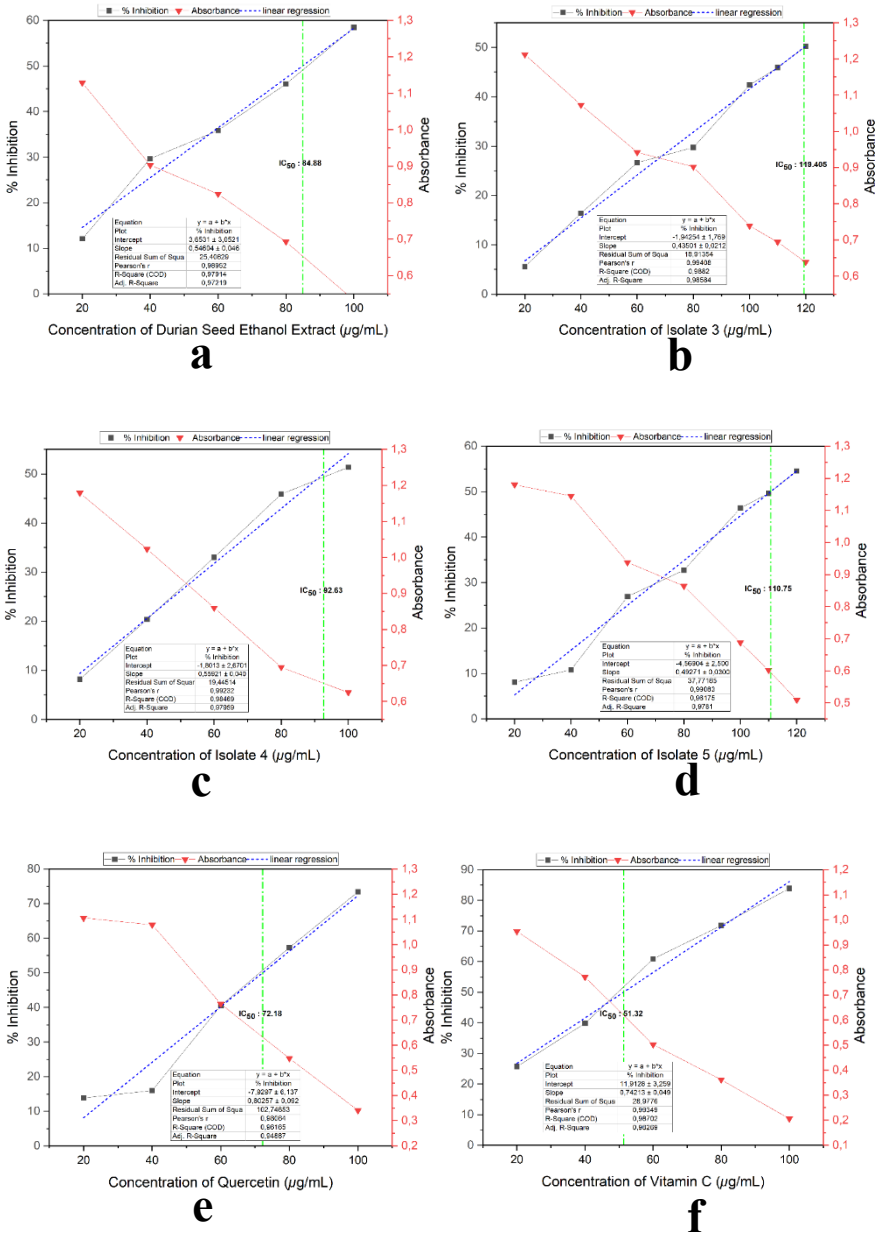


Figure 6 Linear regression analysis for IC_{50} determination of antioxidant activity: (a) ethanol extract of durian seed; (b) - (d) isolates 3 - 5; (e) quercetin standard; and (f) vitamin C standard.

The regression analysis showed good linearity between sample concentration and percentage inhibition, indicating that DPPH radical scavenging activity increased with increasing sample concentration. The IC_{50} values, regression equations, coefficients of determination, and antioxidant classifications of the tested samples are presented in **Table 6**.

Table 6 Antioxidant activity of durian seed ethanol extract, isolated fractions, and standards.

Sample	Regression equation	R^2	IC_{50} (μg/mL)	Antioxidant strength
Vitamin C	y = 0.7421x + 11.913	0.9870	51.3233	Very strong
Quercetin	y = 0.8020x − 7.9297	0.9617	72.1770	Strong
Ethanol extract	y = 0.5460x + 3.6531	0.9791	84.8844	Strong
Isolate 4	y = 0.5592x − 1.8013	0.9847	92.6346	Strong
Isolate 5	y = 0.4927x − 4.5664	0.9625	110.7497	Moderate
Isolate 3	y = 0.4350x − 1.9404	0.9756	119.4030	Moderate

Vitamin C exhibited the strongest antioxidant activity, as indicated by the lowest IC_{50} value of 51.3233 μg/mL. Quercetin also showed strong antioxidant activity, with an IC_{50} value of 72.1770 μg/mL. The ethanol extract of durian seed demonstrated strong radical scavenging activity, with an IC_{50} of 84.8844 μg/mL, suggesting that it contains antioxidant-active constituents. Among the isolated fractions, Isolate 4 showed the strongest antioxidant activity, with an IC_{50} value of 92.6346 μg/mL, whereas Isolates 5 and 3 exhibited moderate antioxidant activity, with IC_{50} values of 110.7497 and 119.4030 μg/mL, respectively.

The antioxidant activity observed in the ethanol extract is consistent with its high phenolic and flavonoid content, as previously shown by TPC and TFC analyses. Phenolic and flavonoid compounds are known to contribute to antioxidant activity through hydrogen donation and electron transfer mechanisms during radical scavenging reactions [46]. Therefore, the strong activity of the ethanol extract may be associated with the presence of phenolic-type constituents and flavonoid-related compounds extracted under ethanol reflux conditions.

The stronger activity of Isolate 4 compared with Isolates 3 and 5 suggests that antioxidant performance was not determined solely by fraction enrichment, but also by the composition and molecular characteristics of the flavonoid constituents. The lower IC_{50} value of quercetin supports this interpretation compared with the isolated fractions. In flavonoid systems, radical scavenging activity is strongly influenced by structural features, particularly the availability of hydroxyl groups and electron-donating capacity [24-29,46]. Therefore, differences in IC_{50} values among the isolated fractions may reflect variations in flavonoid composition, hydroxyl group availability, and electron-transfer efficiency.

The results also indicate that chromatographic enrichment alone does not necessarily guarantee stronger antioxidant activity. Although selected fractions showed relatively enriched flavonoid profiles, their antioxidant activities varied, suggesting that the molecular form and concentration of flavonoid constituents may influence radical-scavenging efficiency. In particular, quercetin-like aglycone structures may contribute more effectively to antioxidant activity than some glycosylated flavonoid forms because free hydroxyl groups are important for hydrogen donation, electron transfer, and radical stabilisation [24-29,46]. To examine this structure–activity relationship, Pearson correlation analysis was conducted between quercetin-related concentrations and the IC_{50} values of the selected isolates. A strong inverse trend was observed ($r = -0.991$, $p = 0.086$, $n = 3$), indicating that higher quercetin-related concentration tended to correspond to lower IC_{50} values and stronger antioxidant activity. However, because the p value exceeded 0.05 and only three fractions were included, the association was not statistically significant and should be regarded as preliminary rather than confirmatory evidence. Therefore, the observed differences in antioxidant activity may be associated

with variations in flavonoid composition, quercetin-related concentration, hydroxyl-group availability, and electron-transfer efficiency.

Overall, the ethanol extract and selected isolated fractions demonstrated promising antioxidant activity, supporting the potential of durian seed as a source of natural antioxidant compounds. The strong activity observed in Isolate 4 highlights the importance of flavonoid composition and molecular characteristics in determining antioxidant performance. These findings support the further evaluation of durian seed flavonoids as natural antioxidant candidates for functional food and nutraceutical applications.

FTIR analysis

FTIR analysis was performed to evaluate the functional group characteristics of durian seed powder, ethanol extract, selected flavonoid-enriched fractions, and quercetin standard, as FTIR spectroscopy is widely used for functional group profiling of plant-derived materials [37,47]. The FTIR spectra are presented in **Figure 7**, while the major absorption bands and their corresponding functional group assignments are summarised in **Table 7**. The analysis was used to compare functional group profiles among the samples and to support the preliminary interpretation of flavonoid-related constituents.

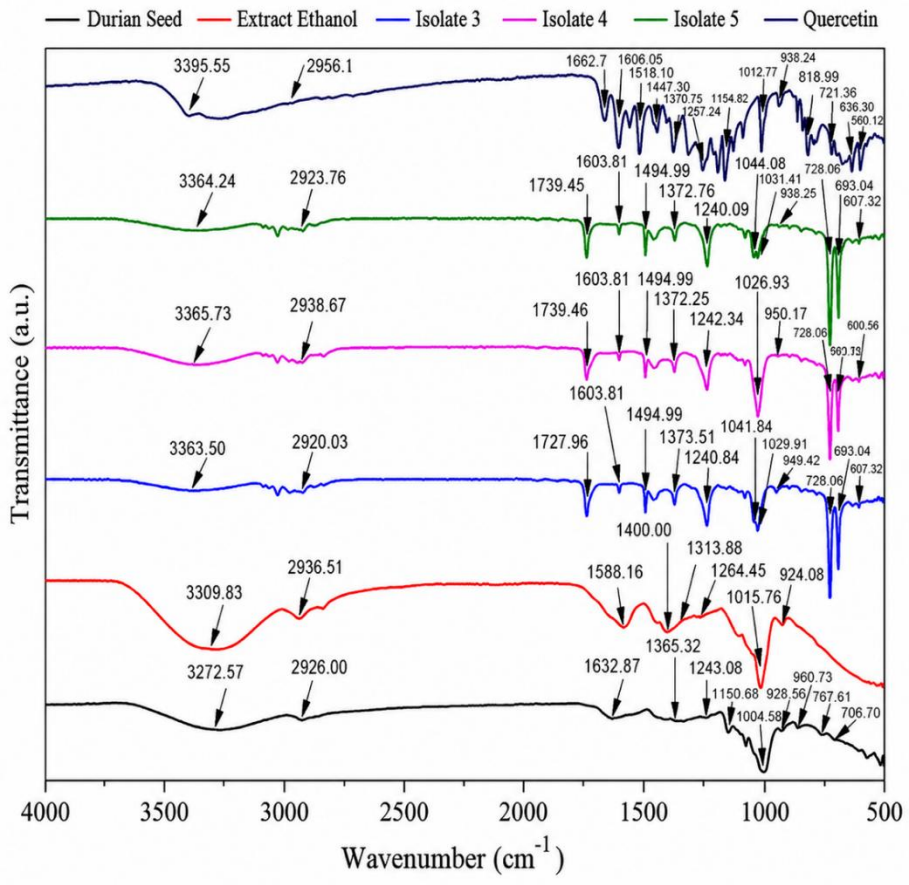


Figure 7 FTIR spectra of durian seed powder, ethanol extract obtained by reflux extraction, selected flavonoid-enriched fractions from column chromatography, and quercetin standard.

The broad absorption bands observed in the region of 3,625 - 3,200 cm^{-1} were assigned to O–H stretching vibrations, which are commonly associated with hydroxyl groups in phenolic compounds. The O–H absorption appeared at 3,272 cm^{-1} in durian seed powder, 3,309 cm^{-1} in the ethanol extract, 3,363 - 3,365 cm^{-1} in the isolated fractions, and 3,395 cm^{-1} in the quercetin standard. The presence of these bands suggests that hydroxyl-containing compounds, including phenolic and flavonoid-related constituents, were present in the durian seed samples. These hydroxyl groups are relevant to antioxidant activity because they

may participate in hydrogen-donating and radical-scavenging reactions.

The absorption bands around 2,940 - 2,920 cm^{-1} correspond to aliphatic C–H stretching vibrations, indicating the presence of organic matrix components in the seed powder, extract, and fractions. In addition, bands observed around 1,600 cm^{-1} were attributed to aromatic C=C stretching vibrations. These bands were detected at 1,632 cm^{-1} in durian seed powder, 1,588 cm^{-1} in the ethanol extract, 1,603 cm^{-1} in the isolated fractions, and 1,606 cm^{-1} in the quercetin standard. The occurrence of aromatic C=C absorption supports the presence of aromatic or polyphenolic structures, which are characteristic features of flavonoid-related compounds.

Table 7 FTIR spectral characteristics of durian seed powder, ethanol extract, selected flavonoid-enriched fractions, and quercetin standard.

Wavenumber range (cm^{-1})	Functional group assignment	DS	EE	I3	I4	I5	QS
3625 - 3200	O–H stretching, phenolic hydroxyl	3272	3309	3363	3365	3364	3395
1850 - 1700	C=O stretching, carbonyl-related group	–	–	1727	1739	1739	1662
~1600	Aromatic C=C stretching	1632	1588	1603	1603	1603	1606
1300 - 1000	C–O–C / C–O stretching	1004, 1150	1015	1029, 1041	1026	1031, 1044	1012
2940 - 2920	Aliphatic C–H stretching	2926	2936	2920	2938	2923	2956
1400 - 1300	Aromatic C–H bending	1365, 1313	1400, 1373	1374, 1494	1372, 1494	1378, 1494	1378, 1447
700 - 500	Aromatic C–H out-of-plane bending	705, 761	693, 728	693, 728	607, 728	636, 721	636, 721

Note: DS = durian seed powder; EE = ethanol extract obtained by reflux extraction; I3 = isolate/fraction 3; I4 = isolate/fraction 4; I5 = isolate/fraction 5; QS = quercetin standard.

Carbonyl-related absorption bands were observed in the selected fractions at 1,727 cm^{-1} for Isolate 3 and 1,739 cm^{-1} for Isolates 4 and 5, whereas the quercetin standard showed a carbonyl absorption at 1,662 cm^{-1}. The difference in carbonyl absorption does not negate the presence of quercetin-related constituents, but indicates that the isolated fractions should be interpreted as flavonoid-enriched fractions rather than pure quercetin. The carbonyl band was not clearly observed in the crude ethanol extract, possibly due to overlapping absorption from complex matrix components. After chromatographic separation, the increased enrichment of specific constituents may have allowed the carbonyl-related bands to become more distinguishable. In addition, potential interference from residual ethyl acetate was minimised by solvent evaporation before FTIR analysis. Therefore, the observed carbonyl absorption in the isolated fractions is interpreted as being associated with enriched flavonoid-related or carbonyl-containing constituents rather than direct evidence of a single pure compound. This interpretation is consistent with the HPLC assignment of the isolated fractions as quercetin-related constituents and supports the use of FTIR as complementary evidence of functional groups.

Absorption bands in the 1,300 - 1,000 cm^{-1} region were assigned to C–O–C and C–O stretching vibrations. These bands were observed in all samples and were particularly evident in the isolated fractions and the quercetin standard. This region is commonly associated with ether, phenolic C–O, and possible glycosidic linkages, which may occur in flavonoid derivatives. The presence of these bands supports the interpretation that the isolated fractions contained flavonoid-related constituents with hydroxyl and C–O-containing functional groups.

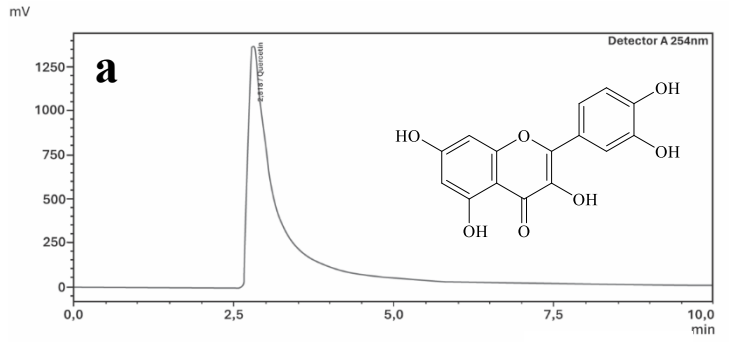
The fingerprint region below 1,000 cm^{-1} showed several absorption bands related to aromatic C–H out-of-plane bending. Similarities between the spectra of selected fractions and the quercetin standard suggest

that the isolated fractions shared certain functional group features with quercetin-related compounds. However, FTIR analysis alone cannot provide definitive structural identification. Therefore, the FTIR results should be regarded as functional group evidence that supports, but does not independently confirm, the tentative assignment of flavonoid-related constituents. Similar FTIR absorption patterns have been reported for antioxidant flavonoid compounds, including quercetin and related polyphenols [37].

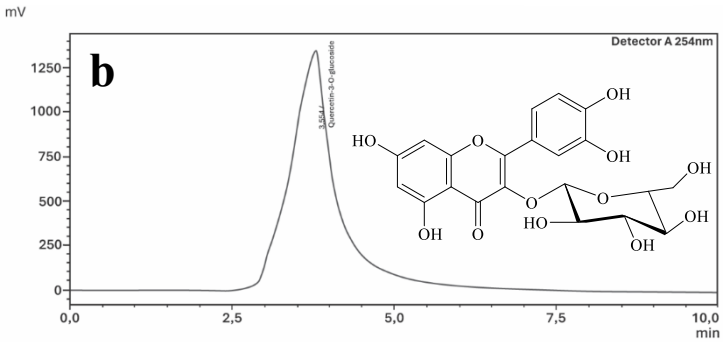
Overall, the FTIR spectra indicated the presence of hydroxyl, aromatic C=C, C–O/C–O–C, and carbonyl-related functional groups in the selected fractions. These functional groups are consistent with flavonoid-related structures and support the chromatographic evidence obtained from TLC and HPLC analyses. Thus, in the present study, FTIR was used as complementary evidence of functional groups, in conjunction with chromatographic profiling, to support the interpretation of quercetin-related and quercetin-3-*O*-glucoside-related constituents in the isolated fractions.

HPLC analysis of durian seed isolates

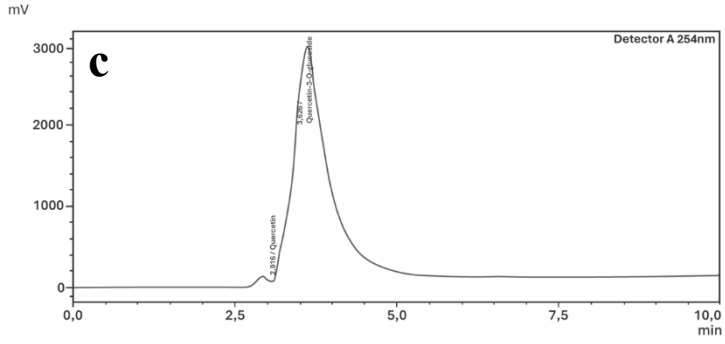
High-Performance Liquid Chromatography (HPLC) analysis was conducted to evaluate the chromatographic profiles, relative peak distribution, and quercetin-related concentration in selected durian seed isolates. Quercetin and quercetin-3-*O*-glucoside were used as reference standards to support tentative flavonoid assignment based on retention time comparison. The HPLC chromatograms of the standards and selected isolates are shown in **Figure 8**.



Quercetin



Quercetin-3-*O*-glucoside



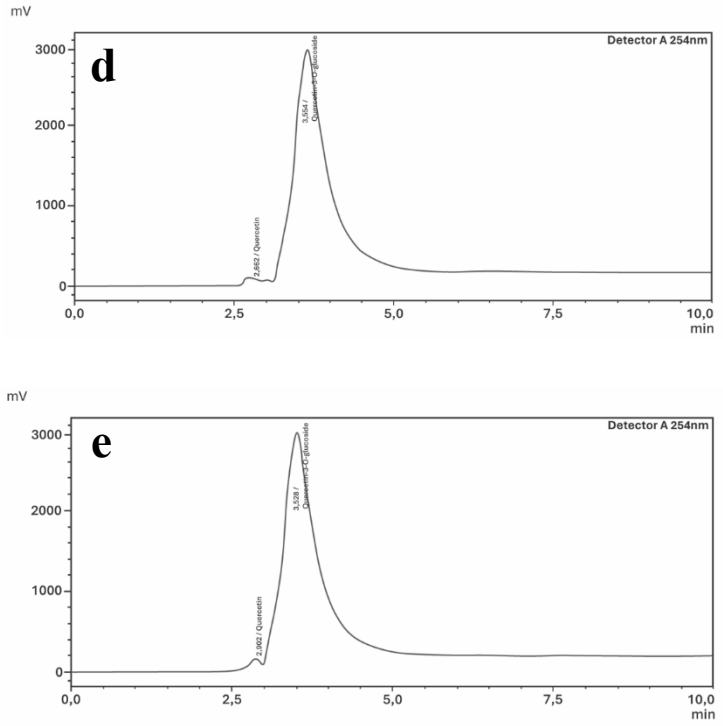


Figure 8 HPLC chromatograms at 254 nm: (a) quercetin standard, (b) quercetin-3-*O*-glucoside standard, (c) Isolate 3, (d) Isolate 4, and (e) Isolate 5.

Table 8 HPLC chromatographic characteristics of quercetin and quercetin-3-*O*-glucoside in durian seed isolates.

Sample	Retention time (min)	Relative chromatographic peak area (%)	Tentative compound assignment
Quercetin standard	2.818	96.0000	Quercetin
Isolate 3	2.916	1.1749	Quercetin-related compound
Isolate 4	2.902	2.9799	Quercetin-related compound
Isolate 5	2.662	1.5104	Quercetin-related compound
Quercetin-3-*O*-glucoside standard	3.540	98.0000	Quercetin-3-*O*-glucoside
Isolate 3	3.626	98.8105	Quercetin-3-*O*-glucoside-related compound
Isolate 4	3.528	97.0104	Quercetin-3-*O*-glucoside-related compound
Isolate 5	3.554	98.4896	Quercetin-3-*O*-glucoside-related compound

The quercetin standard showed a retention time of 2.818 min, while the quercetin-3-*O*-glucoside standard appeared at 3.540 min. In the isolated fractions, chromatographic peaks were observed at retention times close to those of the reference standards. In Isolate 3, peaks were observed at 2.916 and 3.626 min, corresponding chromatographically to quercetin and quercetin-3-*O*-glucoside, respectively. Isolate 4 showed peaks at 2.902 and 3.528 min, whereas Isolate 5 showed peaks at 2.662 and 3.554 min. These retention time similarities suggest the presence of quercetin- and quercetin-3-*O*-glucoside-related constituents in the selected isolates. The detailed chromatographic

characteristics and tentative compound assignments are summarised in **Table 8**.

The detected peaks were assigned as quercetin-related and quercetin-3-*O*-glucoside-related constituents based on their chromatographic similarity to authentic standards and their functional-group characteristics observed by FTIR. This assignment was supported by converging chromatographic and spectroscopic evidence, including TLC behaviour, FTIR profiles, HPLC retention-time comparison, and authentic quercetin and quercetin-3-*O*-glucoside standards. Accordingly, describing these peaks as related constituents provides a scientifically appropriate interpretation of the flavonoid-rich fractions within the analytical scope of this study. These findings strengthen the chromatographic and functional-group evidence for quercetin-related flavonoid constituents in the isolated fractions.

The chromatographic profiles showed that the quercetin-3-*O*-glucoside-related peak was the dominant peak in all selected isolates, with relative peak areas ranging from 97.0104% to 98.8105%. The relative peak area was calculated as the integrated area of the selected quercetin-3-*O*-glucoside-related peak relative to the total integrated peak area detected in each chromatogram. These values indicate the high chromatographic contribution of the selected flavonoid-related peak within the isolated fractions under the applied HPLC conditions. In combination with the minor accompanying peaks, this profile suggests that the selected isolates were flavonoid-enriched fractions dominated by quercetin-3-*O*-glucoside-related constituents.

To further evaluate the contribution of quercetin-related constituents, quercetin-related concentrations in durian seed isolates were calculated for the selected isolates. The results are presented in **Table 9**.

Table 9 Quercetin-related concentration in durian seed isolates.

Isolate	Peak	Retention time (min)	Concentration (ppm)	Tentative compound assignment
Isolate 3	1	2.916	37.769 ± 2.000^{c}	Quercetin-related compound
	2	3.626	–	Quercetin-3-*O*-glucoside-related compound
	3	9.768	–	Unassigned peak
Isolate 4	1	1.145	–	Unassigned peak
	2	2.902	101.979 ± 4.000^{a}	Quercetin-related compound
	3	3.528	–	Quercetin-3-*O*-glucoside-related compound
	4	9.683	–	Unassigned peak
Isolate 5	1	2.662	50.432 ± 3.000^{b}	Quercetin-related compound
	2	3.554	–	Quercetin-3-*O*-glucoside-related compound

Note: Values are presented as mean ± standard deviation (n = 3). Different superscript letters in the concentration column indicate significant differences in quercetin-related concentration among isolates ($p < 0.05$).

The quercetin-related concentration differed significantly among the selected isolates. Isolate 4 exhibited the highest quercetin-related concentration (101.979 ppm), followed by Isolate 5 (50.432 ppm) and Isolate 3 (37.769 ppm). This ordering coincided with the DPPH results, for which Isolate 4 showed the strongest antioxidant activity among the isolated fractions. Within these three fractions, the pattern suggests a possible association between quercetin-related concentration and antioxidant activity, but it does not by itself establish causality.

The relationship between HPLC profiles and antioxidant activity indicates that relative chromatographic dominance does not necessarily correspond to stronger radical-scavenging activity. Although Isolates 3 and 5 showed high relative peak areas of quercetin-3-*O*-glucoside-related constituents, their antioxidant activities were lower than that of

Isolate 4. Isolate 4 contained the highest quercetin-related concentration and exhibited the strongest antioxidant activity among the isolated fractions, a pattern consistent with—but not sufficient to establish—the contribution of aglycone-related flavonoid constituents to DPPH radical scavenging. Quercetin, as an aglycone flavonoid, contains free hydroxyl groups that can participate in hydrogen donation and electron transfer. Quercetin-3-*O*-glucoside also has reported biological activity [48], while comparative antioxidant studies show that glycosylation can alter measured antioxidant capacity and that the magnitude of this effect depends on the assay used [46,49]. Accordingly, the present DPPH results should be interpreted as a preliminary structure–activity pattern rather than definitive proof of an aglycone–glycoside effect.

Therefore, the stronger antioxidant activity of Isolate 4 may be attributed to its higher quercetin-related concentration and favourable flavonoid composition, rather than to the relative abundance of quercetin-3-*O*-glucoside-related peaks alone. The HPLC profiles, supported by TLC behaviour and FTIR functional-group characteristics, provide converging chromatographic and spectroscopic evidence for the presence of quercetin-related and quercetin-3-*O*-glucoside-related constituents in the isolated fractions. This interpretation is consistent with the observed antioxidant pattern, in which Isolate 4 exhibited both the highest quercetin-related concentration and the strongest radical-scavenging activity among the isolated fractions.

Overall, the HPLC analysis supports the presence of flavonoid-related constituents in durian seed isolates and, together with the DPPH results, indicates a preliminary association between quercetin-related concentration and antioxidant activity. Confirmation will require a larger set of fractions, a validated quantitative HPLC method, and complementary antioxidant assays.

Conclusions

This study demonstrates that durian seed (*Durio zibethinus* Murr.) is a promising underutilised biomass source of phenolic and flavonoid-rich fractions with antioxidant potential. Among the extraction conditions evaluated, reflux extraction with ethanol produced the highest crude extract yield, total phenolic content, and total flavonoid content, indicating that thermal assistance combined with a suitable solvent polarity enhanced the recovery of bioactive constituents from durian seed powder. Chromatographic fractionation of the ethanol extract yielded flavonoid-enriched fractions, with TLC, FTIR, and HPLC analyses supporting the presence of quercetin-related and quercetin-3-*O*-glucoside-related constituents. HPLC analysis showed that Isolate 4 had the highest quercetin-related concentration, which was consistent with its stronger antioxidant activity compared with the other isolated fractions. These findings suggest that antioxidant activity may be influenced not only by relative chromatographic dominance but also by flavonoid composition, molecular form, and quercetin-related concentration. Overall, the results provide insight into the relationship between extraction conditions, flavonoid composition, chromatographic enrichment, and antioxidant performance in durian seed isolates. The findings support the sustainable valorisation of durian seed biomass as a potential source of flavonoid-rich natural antioxidant fractions for functional food and nutraceutical applications. Because the compound assignments were tentative, HPLC quantification was semi-quantitative, and the exploratory correlation involved only three fractions, further LC–MS or NMR confirmation and validated quantitative analysis are required.

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Declaration of generative AI in scientific writing

The authors declare that generative artificial intelligence (AI) tools were used solely to improve the readability, clarity, and linguistic quality of this manuscript. The use of such tools was performed under full human oversight and editorial control. The authors take full responsibility for the scientific content, data interpretation, originality, and integrity of the work presented. No generative AI tools were used in the design of the study, data collection, data analysis, or

interpretation of results. Generative AI tools were not listed as authors or co-authors of this manuscript.

CRediT author statement

Dante Alighiri: Conceptualisation; Methodology; Investigation; Formal analysis; Writing – Original Draft; Project administration; Supervision; Funding acquisition. **Lisdiana**: Investigation; Data Curation; Validation. **Masturi**: Formal analysis; Visualisation; Software. **Kumala Aris Tanti**: Investigation; Resources; Data Curation. **Apriliana Drastisianti**: Writing – Review & Editing; Visualisation. **Samuel Budi Wardhana Kusuma**: Software; Validation; Formal analysis. **Neli Syahida Ni'ma**: Investigation; Data Curation; Visualisation. **Tri Minarsih**: Investigation; Data Curation; Validation.

References

[1] BPS-Statistic Indonesia, Available at: https://www.bps.go.id, accessed 13 October 2024.

[2] BT Amid, H Mirhosseini and S Kostadinović. Chemical composition and molecular structure of polysaccharide-protein biopolymer from *Durio zibethinus* seed: Extraction and purification process. *Chemistry Central Journal* 2012; **6**, 117.

[3] S Baraheng and T Karrila. Chemical and functional properties of durian (*Durio zibethinus* Murr.) seed flour and starch. *Food Bioscience* 2019; **30**, 100412.

[4] RAJ Gamay, PMN Botecario, PDC Sanchez and MC Alvarado. Durian (*Durio zibethinus*) waste: A promising resource for food and diverse applications—a comprehensive review. *Food Production, Processing and Nutrition* 2024; **6**, 27.

[5] X Fang, Y Li, YL Kua, ZL Chew, S Gan, KW Tan, TZE Lee, WK Cheng and HLN Lau. Insights on the potential of natural deep eutectic solvents (NADES) to fine-tune durian seed gum for use as edible food coating. *Food Hydrocolloids* 2022; **132**, 107861.

[6] H Mirhosseini, NFA Rashid, BT Amid, KW Cheong, M Kazemi and M Zulkurnain. Effect of partial replacement of corn flour with durian seed flour and pumpkin flour on cooking yield, texture properties, and sensory attributes of gluten-free pasta. *LWT - Food Science and Technology* 2015; **63(1)**, 184-190.

[7] ZL Chew, QQ Koh, EE Chu, YL Kua, S Gan, KW Tan and TZE Lee. Tunable durian seed gum-derived eutectogel as a novel coating material: Rheological, thermal, textural and barrier properties for enhanced food preservation. *International Journal of Biological Macromolecules* 2024; **267(1)**, 131201.

[8] S Bai-Ngew, N Therdthai and W Zhou. Microwave vacuum-dried durian flour and its application in biscuits. *Heliyon* 2021; **7(11)**, e08292.

[9] NTH Nguyen, TTT Nguyen, DTC Nguyen and TV Tran. A comprehensive review on the production of durian fruit waste-derived bioadsorbents for water treatment. *Chemosphere* 2024; **363**, 142801.

[10] H Mirhosseini, BT Amid and KW Cheong. Effect of different drying methods on chemical and molecular structure of heteropolysaccharide–protein gum from durian seed. *Food Hydrocolloids* 2013; **31(2)**, 210-219.

[11] S Rahmawati, Pathuddin, S Nuryanti, VDA Sangkota, Afadil, Anggraini and N Syawaliah. Characteristics and antioxidants of edible film from durian seeds (*Durio zibethinus*) with additions of rosella flower extract (*Hibiscus sabdariffa* L.). *Materials Today: Proceedings* 2022; **65(7)**, 3109-3115.

[12] TB Ly, AMH Trinh, HPT Tran, KN Dang, TDT Nguyen, VT Tran and PK Le. Evaluation of an operating durian shell charcoal briquette manufacturing line and development of a biorefinery process for higher value products. *Energy* 2024; **307**, 132727.

[13] Masturi, D Alighiri, P Dwijananti, RD Widodo, SP Budiyanto and A Drastisianti. Bioethanol synthesis from durian seeds using *Saccharomyces cerevisiae* in aerobic fermenter and bioethanol enrichment by batch vacuum distillation. *Jurnal Bahan Alam Terbarukan* 2020; **9(1)**, 36-46.

[14] JY Chua, KM Pen, JV Poi, KM Ooi and KF Yee. Upcycling of biomass waste from durian industry for green and sustainable applications: An analysis review in the Malaysia context. *Energy Nexus* 2023; **10**, 100203.

[15] Masturi, D Alighiri, SS Edie, A Drastisianti, U Khasanah, KA Tanti, Susilawati, RZ Maghfiroh, KGC Kirana and F Choirunnisa. Identification of flavonoid compounds and total flavonoid content from biowaste of local durian shell (*Durio zibethinus*). *Journal of Physics: Conference Series* 2020; **1567**, 042084.

[16] AM Amin, AS Ahmad, YY Yin, N Yahya and N Ibrahim. Extraction, purification, and characterization of durian (*Durio zibethinus*) seed gum. *Food Hydrocolloids* 2007; **21(2)**, 273-279.

[17] Y Liu, S Feng, L Song, G He, M Chen and D Huang. Secondary metabolites in durian seeds: Oligomeric proanthocyanidins. *Molecules* 2013, **18(11)**, 14172-14185.

[18] NAA Aziz and AMM Jalil. Bioactive compounds, nutritional value, and potential health benefits of indigenous durian (*Durio zibethinus* Murr.): A review. *Foods* 2019; **8(3)**, 96.

[19] S Charoenkiatkul, P Thiyajai and K Judprasong. Nutrients and bioactive compounds in popular and indigenous durian (*Durio zibethinus* Murr.). *Food Chemistry* 2016; **193**, 181-186.

[20] G Khaksar, S Kasemcholathan and S Sirikantaramas. Durian (*Durio zibethinus* L.): Nutritional composition, pharmacological implications, value-added products, and omics-based investigations. *Horticulturae* 2024, **10(4)**, 342.

[21] BT Amid and H Mirhosseini. Optimisation of aqueous extraction of gum from durian (*Durio zibethinus*) seed: A potential, low cost source of hydrocolloid. *Food Chemistry* 2012; **132(3)**, 1258-1268.

[22] P Singcha, G Khaksar, M Sirijan and S Sirikantaramas. Durian (*Durio zibethinus* L.) fruit: A superior dietary source of natural glutathione and γ-glutamylcysteine. *Journal of Food Composition and Analysis* 2024; **127**, 105975.

[23] N Charoenphun and WK Klangbud. Antioxidant and anti-inflammatory activities of durian (*Durio zibethinus* Murr.) pulp, seed, and peel flour. *PeerJ* 2022; **10**, e12933.

[24] L Mungmai, C Kanokwattananon, S Thakang, A Nakkrathok, P Srisuksomwong and P Tanamatayarat. Physicochemical properties, antioxidant and anti-tyrosinase activities of *Durio zibethinus* Murray and value-added for cosmetic product formulation. *Cosmetics* 2023; **10(3)**, 87.

[25] YV Rajagukguk and M Arnold. *Tempoyak*: Fermented durian paste of Malay ethnic and its functional properties. *International Journal of Gastronomy and Food Science* 2021; **23**, 100297.

[26] R Haruenkit, S Poovarodom, S Vearasilp, J Namiesnik, M Sliwka-Kaszynska, YS Park, BG Heo, JY Cho, HG Jang and S Gorinstein. Comparison of bioactive compounds, antioxidant and antiproliferative activities of Mon Thong durian during ripening. *Food Chemistry* 2010; **118(3)**, 540-547.

[27] L Fu, BT Xu, XR Xu, RY Gan, Y Zhang, EQ Xia and HB Li. Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry* 2011; **129(2)**, 345-350.

[28] LH Ho and R Bhat. Exploring the potential nutraceutical values of durian (*Durio zibethinus* L.)—An exotic tropical fruit. *Food Chemistry* 2015; **168**, 80-89.

[29] MGL Hertog, D Kromhout, C Aravanis, H Blackburn, R Buzina, F Fidanza, S Giampaoli, A Jansen, A Menotti, S Nedeljkovic, M Pekkarinen, BS Simic, H Toshima, EJM Feskens, PCH Hollman and MB Katan. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine* 1995; **155(4)**, 381-386.

[30] Masturi, D Alighiri, K Nuzulina, M Rodhiyah and A Drastisianti. Optimization of condition extraction in quantification of total flavonoid content in the seeds of Arummanis (*Mangifera indica* L.) mango from Indonesia. *Journal of Physics: Conference Series* 2019; **1321**, 022041.

[31] Masturi, D Alighiri, SS Edie, U Hanisyifa and A Drastisianti. Determination of total phenol and flavonoid contents and antioxidant activity from extract fraction of sappan wood (*Caesalpinia sappan* L.) by liquid–liquid extraction and vacuum liquid chromatography. *Asian Journal of Chemistry* 2021; **33(8)**, 1729-1735.

[32] S Priatmoko, D Alighiri, A Drastisianti, D Harjunowibowo, AN Rohman and T Sulistyaningsih. Coagulant preparation from glutaraldehyde-crosslinked durian skin cellulose for treatment of wastewater generated by sugar

palm starch industries. *Cellulose Chemistry and Technology* 2024; **58(1-2)**, 201-214.

[33] Harjono, INP Wahyuni, A Darmawan and D Alighiri. Isolasi dan karakterisasi senyawa metabolit sekunder dari serbuk kayu mahoni (*Swietenia macrophylla* King). *Journal of Farmasi Sains Indonesia* 2020; **3(1)**, 45-48.

[34] LI Humairoh and D Alighiri. Formulation and evaluation of lozenges from durian peel extract (*Durio zibethinus* Murr.) as an antioxidant. *Journal of Science and Technology Research in Pharmacy* 2023; **3(1)**, 26-34.

[35] C Pramesti and D Alighiri. Formulation of effervescent tablet from extract of durian (*Durio zibethinus* Murr.) peels and its antioxidant activity. *Journal of Science and Technology Research in Pharmacy* 2023; **3(1)**, 35-44.

[36] WT Eden, D Alighiri, N Wijayati and S Mursiti. Synthesis of chalcone derivative from clove leaf waste as a natural antioxidant. *Pharmaceutical Chemistry Journal* 2021; **55(3)**, 269-274.

[37] O Abbas, G Compère, Y Larondelle, D Pompeu, H Rogez and V Baeten. Phenolic compound explorer: A mid-infrared spectroscopy database. *Vibrational Spectroscopy* 2017; **92**, 111-118.

[38] DH Mursyidin, MI Makruf, Badruzsaufari and A Noor. Molecular diversity of exotic durian (*Durio* spp.) germplasm: A case study of Kalimantan, Indonesia. *Journal of Genetic Engineering and Biotechnology* 2022; **20(1)**, 39.

[39] X Zhou, H Wu, J Pan, H Chen, B Jin, Z Yan, L Xie and KM Rogers. Geographical traceability of south-east Asian durian: A chemometric study using stable isotopes and elemental compositions. *Journal of Food Composition and Analysis* 2021; **101**, 103940.

[40] A Susila, YA Bety, Y Hindarwati, FD Arianti, D Haskarini, A Malik, B Santoso and IG Cempaka. Characterization of durian (*Durio zibethinus*) Monthok from Blora, Central Java, Indonesia. *E3S Web Conferences* 2021; **306**, 01003.

[41] K Weerawanich, H Halbwirth and S Sirikantaramas. A novel MYB transcription factor from durian (*Durio zibethinus*), DzMYB1, regulates flavonoid biosynthesis in fruit pulp. *Scientia Horticulturae* 2024; **333**, 113246.

[42] EHK Ikram, KH Eng, AMM Jalil, A Ismail, S Idris, A Azlan, HSM Nazri, NAM Diton and RAM Mokhtar. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis* 2009; **22(5)**, 388-393.

[43] GL Chen, SG Chen, YY Zhao, CX Luo and YQ Gao. Total phenolic contents of 33 fruits and their antioxidant capacities before and after *in vitro* digestion. *Industrial Crops and Products* 2014; **57**, 150-157.

[44] P Arancibia-Avila, F Toledo, YS Park, ST Jung, SG Kang, BG Heo, SH Lee, M Sajewicz, T Kowalska and S Gorinstein. Antioxidant properties of durian fruit as influenced by ripening. *LWT – Food Science and Technology* 2008; **41(10)**, 2118-2125.

[45] MM Ali, N Hashim, S Abd Aziz and O Lasekan. Exploring the chemical composition, emerging applications, potential uses, and health benefits of durian: A review. *Food Control* 2020; **113**, 107189.

[46] K Csepregi, S Neugart, M Schreiner and E Hideg. Comparative evaluation of total antioxidant capacities of plant polyphenols. *Molecules* 2016; **21(2)**, 208.

[47] M Alauhdin, WT Eden and D Alighiri. Application of infrared spectroscopy for the analysis of plants and herbal medicines. *Inovasi Sains dan Kesehatan* 2021; **4**, 4.

[48] P Sarma, B Kashyap, S Gurumayum, S Sarma, P Baruah, D Swargiary, A Saikia, RC Deka and JC Borah. Antihyperglycemic potential of Quercetin-3-glucoside isolated from *Leucaena leucocephala* seedpods via the SIRT1/AMPK/GLUT4 signaling cascade. *ACS Omega* 2024; **9(30)**, 32429-32443.

[49] X Li, Q Jiang, T Wang, J Liu and D Chen. Comparison of the antioxidant effects of quercitrin and isoquercitrin: Understanding the role of the 6″-OH group. *Molecules* 2016; **21(9)**, 1246.