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Identification of flavonoid compounds and total flavonoid content from biowaste of local durian shell (*Durio zibethinus*)

Identification of flavonoid compounds and total flavonoid content from biowaste of local durian shell (*Durio zibethinus*)

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Abstract. Flavonoid compound from durian shell biowaste was identified by phytochemical assay and FTIR spectrophotometric methods. Total flavonoid content determined by the aluminum chloride ($AlCl_3$) method using a UV-Vis spectrophotometer. Durian shells, which are a waste that causes the environmental problem, can be used as a source of potentially valuable flavonoid compounds. Flavonoid has antioxidants ability that is beneficial and useful. Durian shell used in this research consists of three types, namely Malika, Malon, and Monti, which are from local Indonesian durian. Based on the result, proximate test analysis showed that three local durian shell samples generally had a water content of 7%, a fat content of 0.9%, the protein content of 4.9%, an ash content of 8.5%, and a 78% carbohydrate content. The results of the analysis of the three durian shell samples did not show significantly different results. Then for the phytochemical assay, three local durian shell samples contained phenols, steroids, and terpenoids, the results of the phytochemical assay showed that there were more phenolic groups than the flavonoid group. The following analysis result is the functional group of three samples using Fourier Transform Infrared (FT-IR) spectrophotometer shows that the three types of durian shell samples have a band that is slightly different from the standard, but the number of waves in this band is similar to the standard quercetin. Then for total flavonoid levels in local durian shell using the aluminum chloride ($AlCl_3$) method, the result is Monti durian shell having higher flavonoid levels, each 0.405 ± 0.00[illegible]g QE/g, compared with each other shell type namely Malika and Malon of 0.321 ± 0.003 mg QE / g and 0.324 ± 0.002 mg QE/g, respectively. Thus in this study shows that Indonesian local durian shell contains significant total flavonoid content without the need for extraction. Samples were only dissolved with ethanol solvent, then a series of tests were carried out, then a series of tests were carried out, ranging from phytochemical assessment, FTIR spectrophotometer, and $AlCl_3$ methods to determine the total flavonoid content through quantitative.

1. Introduction

Durian is one type of fruit that is popular and widely produced in Indonesia. According to the BPS (Indonesian Central Statistics Agency) in 2018 were 17,175,235 durian trees with durian fruit production reaching 1,142,102 tons/year, and durian fruit production in Central Java, Indonesia as many as 143,227 tons/year [1]. The part of durian that can be consumed is fruit flesh, which is the percentage is only around 20-35%, the other part is durian seeds as much as 5-15% and the dominant part of durian fruit is a shell with 60-75% percentage that has not been used optimally [2]. Durian shell is the most significant part of durian, which is thrown away and causing environmental waste and has no economic value [3,4]. Durian shell has beneficial secondary metabolites, one of which is a flavonoid compound [5,6].

Flavonoids are the biggest group of phenolic compounds that were found in nature and had powerful antioxidants [7,8]. An antioxidant can exert health-promoting and disease-preventing by scavenging free radicals and protect the human body and animals from their adverse effects [9-10]. Antioxidants are beneficial, widely used as ingredients and supplements of functional foods to prevent chronic diseases, such as cancer, atherosclerosis, and heart disease, for maintaining redox balance and avoiding oxidative damage to protect against lipid oxidation and off-flavor development [11-14].

Therefore, durian shells, which are only a waste that causes the environmental problem, can be used as a source of potentially valuable bioactive of flavonoid compounds. Several studies have been carried out on the benefits of durian shell. However, research on compounds in durian shells mainly from local durian fruit of Gunungpati, Central Java, Indonesia, namely varieties (*Durio zibethinus*) cultivar of Malika, Malon, and Monti, and which are local Indonesian native fruits of valuable bioactive of flavonoid compounds are still minimal. Also, research on total flavonoid content in their durian shell is rarely studied. In this study, conducted research on durian shells from Indonesian local durian types, namely Malika, Malon, and Monti, about proximate analysis to determine physicochemical of durian shell and phytochemical assay and FTIR spectrophotometer to confirm the presence of flavonoid in durian shell while total flavonoid content in durian shell can be known by aluminum chloride ($AlCl_3$) method.

2. Materials and Methods

2.1. Materials

The shell of the Indonesian durian (*Durio zibethinus*), namely Malon, Monti, and Malika, was collected from Gunungpati, Semarang, Central Java, Indonesia. The standard of quercetin was purchased from Sigma Aldrich. Aluminum chloride($AlCl_3$) and ethanol were analytical reagents from Merk.

2.2. Sample preparation

The shell of the Indonesian durian (*Durio zibethinus*), namely Malon, Monti, and Malika, was cleaned using water, then dried at room temperature (25-27℃). The process of drying a durian shell was carried out for approximately one week. The dried durian shell was pulverized using a blender. Then, the powder of the durian shell was analyzed for proximate components, phytochemicals evaluation, FTIR spectrophotometer, and total flavonoid content.

2.3. Proximate evaluation

2.3.1. Protein content analysis. 2 grams of durian shell powder added with a catalyst of K_2SO_4, 60 mg $CuSO_4$, and 10 ml 97% concentrated H_2SO_4. Then, it was stirred and destined until it was clear green color, cooled at room temperature, and 30 mL of distilled water was added. Destruction results were added with a 50% NaOH solution as much as 25 mL, and the distillation process was carried out until the yellow and distillate were collected in an Erlenmeyer containing 10 mL 0.1 N HCl solution. The distillate obtained was titrated with 0.1 N NaOH solution and added with 2 drops of phenolphthalein indicator. The protein content is calculated by the following equation [15,16]:

$$\% \text{ protein levels} = \frac{\text{mL NaOH (titration}-\text{blank)}}{\text{sample weight}} \times \text{NaOH concentration} \times 14.008 \times 100\% \quad (1)$$

2.3.2. Fat content analysis. 2 grams of durian shell powder was the soxhlet extraction method with 150 mL n-hexane for 8 hours. Then, the filtrate was evaporated and roasted at 105 ℃ for 2 hours, cooled in a desiccator for 30 minutes, and weighed to an absolute weight. Lipid content is calculated by the following equation [16,17]:

$$\% \text{ fat levels} = \frac{\text{final sample weight}}{\text{initial sample weight}} \times 100\% \quad (2)$$

2.3.3. Ash content analysis. 5 grams of durian shell powder was put in the furnace at 500ºC for 24 hours until white ash was formed. Then the ash was put into the desiccator and weighed. Ash content is calculated through the following equation [16,18]:

$$\% \text{ Ash level} = \frac{\text{final sample weight}}{\text{initial sample weight}} \times 100\% \quad (3)$$

2.3.4. Water content analysis. 2 grams of durian shell powder is put into a porcelain cup, and the heating process is carried out in the oven for 5 hours at 105 ℃. Then the cup is cooled and put into the desiccator for 15 minutes and weighed to a constant weight. Water content is calculated through the following equation [16,18]:

$$\% \text{ Water level} = \frac{\text{final sample weight}}{\text{initial sample weight}} \times 100\% \quad (4)$$

2.3.5. Carbohydrate content analysis. Carbohydrate analysis was done using by a different method, which uses the following equation [16,19]:

$$\% \text{ Carbohydrate level} = 100\% - (\text{water level} + \text{ash level} + \text{lipid level} + \text{protein level}) \quad (5)$$

2.4. Phytochemical evaluation

2.4.1. Alkaloid test. The alkaloid test was carried out with two reagents, namely Dragendorf and Mayer. For the alkaloid test with the Dragendorf reagent, the test tube was prepared. 1 gram of durian shell powder was added into the test tube, then added 5 mL of ethanol, and filtered. The filtrate was added with 2 drops of Dragendorf reagent. If an orange-red precipitate was formed, the test is positive. The test with the Mayer reagent was done by preparing a test tube, then added 1 mL of sample, 2 drops of HCl 2M, and 2 drops of Mayer reagent. If white precipitate was formed, the test is positive [20].

2.4.2. Steroid and terpenoid test
1 gram of durian shell powder was added with 5 mL ethanol, filtered, then the filtrate was added with 2 mL chloroform, and 2 drops of acetic anhydride. Then 2 drops of concentrated H_2SO_4 were added through the test tube wall. When green or blue color is formed, the extract positively contains steroids. Whereas if purple-red color is formed, the extract positively contains terpenoids [21].

2.4.3. Flavonoid test. 1 gram of durian peel powder is added 5 mL ethanol, filtered after that filtrate is added with 1 mL of hot methanol, Mg powder one end of the spatula, and 0.5 mL of concentrated HCl. The flavonoid test is considered positive if a red or orange solution is formed [21].

2.4.4. Phenolic test. 1 gram of durian peel powder is added with 5 mL ethanol, filtered, and the filtrate is added with 1 mL Iron (III) Chloride ($FeCl_3$). If a purple-blue color is formed, the extract positively contains phenolic compounds [22].

2.5. Determination of Flavonoid Compound by Fourier-transform infrared (FTIR) Spectrophotometer

The presence of durian flavonoid samples was studied by Fourier-transform infrared (FTIR) spectroscopy. A total of 1 g of durian shell powder samples were crushed with 10 g KBr in a mortar (sample: KBr in a ratio of 1:10), mixed evenly, and put into a pellet mold. Next the mold is pressed until a transparent pellet is obtained. Then, the pellets were analyzed with the Perkin Elmer Spectrum Version 10.03.06 FT-IR spectrophotometer. The spectrum is explained in the form of a transmittance curve at 4000-200 cm^{-1} wavenumbers [23].

2.6. Determination of the maximum wavelength (λ max)

The maximum wavelength determined by using UV-Vis spectrophotometer and running with quercetin as reference solutions. 1 mL of testing solution and 2 mL of quercetin solution were pipetted accurately into 25 mL volumetric flask, respectively, with 3 mL of 0.1 mol/mL aluminum chloride ($AlCl_3$) solution separately. Afterward, the mixture was diluted with 70% ethanol then shaken up. After 30 minutes, the absorption spectra determination of the reference solution and the testing solution carried out by wavelength scanning at 400–450 nm, showing 425 nm as the maximum absorption wavelength [24].

2.7. Standard curves preparation

50 mg of standard quercetin was weighed and dissolved in ethanol to a final volume of 50 ml. This solution was piped 0.25-2 ml, respectively. Then, diluted with distilled water to the final volume of 10 ml, so the concentrations of 25-200 μg/mL quercetin were produced. From each concentration, the standard quercetin solution was piped 2 mL. Then 2 mL of 2% $AlCl_3$ and 2 mL of potassium acetate were added to 120 mM. Samples were incubated for one hour at room temperature. Absorbance was measured at a maximum wavelength of 425 nm by using UV-Vis spectrophotometer against an appropriate blank solution. The specification curve was obtained from the absorbability as ordinate and the concentration as abscissa. The calibration curve was presented a linear response within the concentration range of 25-200 μg/mL. The regression equation was ($y = 0.0051x - 0.0176$, $R^2 = 0,9879$) [24].

2.8. Determination of total flavonoid content

Total flavonoid content carried out by the Aluminum Chloride ($AlCl_3$) method using a UV-Vis spectrophotometer. 2 mL of solution extracts (10 mg/mL) or standard solution of quercetin (25–200 μg /mL) was added 2 mL of 2% $AlCl_3$ solution and 2 mL of 120 mM potassium acetate. Samples were incubated for one hour at room temperature. Absorbance was measured at a maximum wavelength of 425 nm against the blank by using a UV-Vis spectrophotometer. The results were calculated according to the calibration curve for quercetin ($y = 0.0051x - 0.0176$, $R^2 = 0.9879$). The concentration of flavonoids was read (mg/mL) on the standard curve. The total flavonoid content obtained is expressed as mg QE (Quercetin Equivalent)/ g of sample. All determinations were performed in triplicate [24].

2.9. Statistical analysis

The experimental data was performed using a one-way analysis of variance (ANOVA). Data were expressed as the mean ± standard deviation (n = 3). Homogeneous groups and the least significant difference (LSD) were determined at a significance level of $p \leq 0.05$. All statistical analyzes were performed using SPSS 24 software.

3. Result and Discussion

Malika durian is one of the origin durian types from Gunungpati, Semarang, Central Java, Indonesia, which is favored by the surrounding community. While Malon durian is a local durian that grows in Malon village, Gunungpati, Semarang, Central Java, Indonesia, which is the most famous durian in Semarang, Indonesia, because of its distinctive taste and low price. Recently, Monti durian has come to the attention of durian lovers because of Monti's size like a Mon Thong durian with local durian flavor, which is sweeter, sticky texture, and unmushy. Monti durian is the product of crossing from the original durian of Gunungpati, Semarang, Central Java, Indonesia, with Mon Thong durian, Thailand. Different types of durian produce different sizes, colors, flavors, and aromas. Therefore, it may contain different compounds, although most of the compounds are still the same but with different compositions.

Durian shells from the three types of local durian also have different colors. Durian shell is usually considered a by-product or waste, whereas on the other hand, it is according to various studies contains quite a lot of bioactive compounds. Durian shell was reported to have an abundance of therapeutic benefits such as possessing anti-diabetic properties, anti-hyperlipidemic effects, anti-proliferative activity, and antimicrobial activities [25]. In this study, we evaluate durian shells from Indonesian local durian types, namely Malika, Malon, and Monti, about proximate, flavonoid content by phytochemical assay and FTIR spectrophotometer and total flavonoid content by aluminum chloride ($AlCl_3$) method.

3.1. Proximate evaluation

Durian shell was obtained from durian shell waste in the Gunungpati, Semarang, Central Java, Indonesia. Durian shell is made in the form of dry powder. Drying aims to eliminate the water content contained in the sample, which can cause enzymatic reactions. Enzymatic reactions can cause damage to the sample because the composition of compounds has changed. While refinement aims to expand the space of interaction between samples and test reagents in order to obtain optimal results [16].

Table 1. Proximate composition of durian shell from local Indonesian durian (*Durio zibethinus*)

Component	Durian shell from local Indonesian durian in this study (%)*:		
	Malon	**Malika**	**Monti**
Moisture content	7.321 ± 0.003	7.335± 0.002	7.422± 0.005
Crude protein	4.935± 0.003	4.941 ± 0.003	4.985± 0.003
Crude fat	0.904 ± 0.002	0.907 ± 0.001	0.900 ± 0.002
Total carbohydrates	78.279 ± 0.002	78.261 ± 0.002	78.073 ± 0.002
Ash	8.561± 0.002	8.556 ± 0.002	8.620 ± 0.002

*Treatment means of the ANOVA test
Values were expressed as the mean ± standard deviation of three replications
The mean difference is significant at the $p \leq 0.050$.
*Means are not significantly different in the different type of durian shell from local Indonesian durian at the $p = 0.000$

Proximate analysis on durian shell shows that in general, it has a moisture content of 7%, 0.9% fat content, 4.9% protein content, 8.5% ash content, and 78% carbohydrate content. The results of the analysis of the three durian peel samples did not show significantly different results. This proximate value aims to standardize the durian shell used for the analysis of flavonoid content in this study.

3.2. Phytochemical screening

Phytochemical compounds are secondary metabolites in plants, which have particular functions for humans. Phytochemical analysis conducted in this study is a qualitative test of flavonoids, phenols, saponins, and tannins. Phytochemical screening carried out showed that the durian shell from three types of local Indonesian durian, namely Malika, Malon, and Monti, which contained flavonoid, phenolic, saponin, and tannin compounds.

The color change resulting from the durian shell is shown by the positive reaction of phenolic compounds with a greenish-yellow color. Whereas the positive test of flavonoid compounds are shown in red color. The phytochemical test results of durian shells from local Indonesian durian are shown in Table 2. Phytochemical test results also showed a more intense color intensity in the phenolic group compared to the flavonoid group. Intense color is probably to indicate a greater number of compounds. This color shows that the amount of phenolic compounds is greater than flavonoid compounds [26].

Table 2. Phytochemical analysis of durian shell from local Indonesian durian (*Durio zibethinus*)

Component	Durian shell from local Indonesian durian in this study (%)*:			Reaction Results
	Malon	Malika	Monti	
Alkaloids	–	–	–	No Deposits Formed
Steroid	–	–	–	Red
Terpenoid	+	+	+	Red
Phenolic	+	+	+	Blackish Blue
Flavonoids	+	+	+	Red

* +: Positive/Detected
– : Negative/Not detected

3. Determination of the flavonoid compounds using Fourier Transform Infrared (FT-IR) spectra

Determination of the contents of the main bioactive compounds using the Fourier Transform Infrared (FT-IR) spectra of flavonoid compound. The presence of flavonoid compounds in the investigated durian shell samples was studied by Fourier Transform Infrared (FT-IR) spectroscopy.

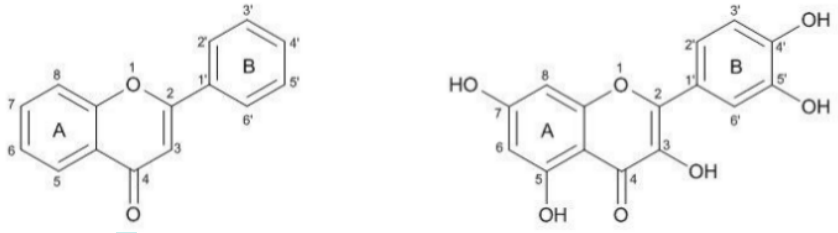

Figure 1. Compounds of (a) Flavone dan (b) Quercetin are used as standard compounds in interpreting FTIR spectra

Flavonoids are compounds that contain hydroxyl (–OH), methoxyl ($–OCH_3$), or glycoside groups, which are derived from flavones (Figure 1a). The structure of flavonoids naturally is complex, and each source will have different types of flavonoids, but most flavonoids are derived from quercetin (Figure 1b) [27]. Therefore, the presence of flavonoid compounds in the durian shell could be interpreted from IR spectra, which based on the two structures of these compounds.

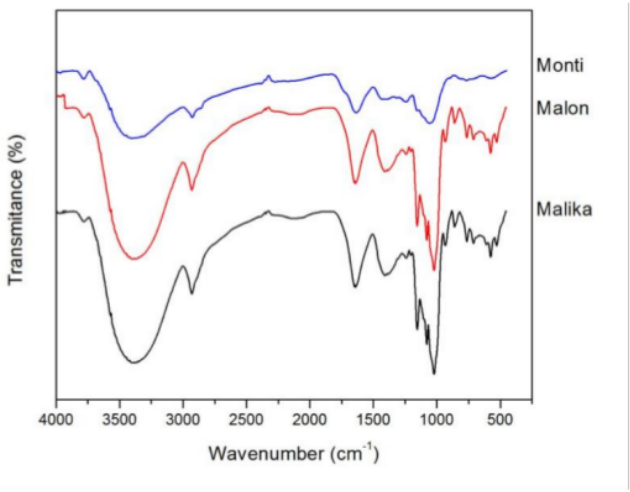

Figure 2. The spectrum of durian shell from local Indonesian durian (*Durio zibethinus*), a cultivar of (—) Malika, (—) Malon, and (—) Monti

The functional group analysis was performed by using the FTIR Spectrophotometer Frontier instrument, which can identify functional groups in material through typical IR uptake. Analysis using FTIR is done in the range of 4000-200cm^{-1}. The wavenumbers of FTIR spectra for quercetin at 827, 1039, 1115, 1143, 1286, 1478, 1511 and 1610 cm^{-1} were assigned to C–H alkenes, –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols, C–H alkanes, C=C aromatic ring and C–C alkenes. Meanwhile, gallic acid, which is a standard compound used, indicates the presence of phenolic compounds in a material showed the following wavenumbers (cm^{-1}) of 866, 1026, 1238, 1450, 1542, and 1618. It can concluded that durian shells from local Indonesian durian types, namely Malika, Malon, and Monti in the region of flavonoid, showed slightly different bands than the standards, but the wavenumbers of the bands were similar in this group (Figure 2).

3.4. Total flavonoid content in local durian shell

Quantitative analysis of total flavonoid content by using a UV-Vis spectrophotometer was carried out to find out how much total flavonoid content in local Indonesian durian (*Durio zibethinus*). Analysis of flavonoids was carried out using UV-Vis spectrophotometer because the flavonoid contained a conjugated aromatic system that showed strong absorption bands in the ultraviolet light and visible light spectrum [24].

In this study, determine the total flavonoids content in the sample used quercetin as a standard solution with a series of concentrations of 25, 50, 75, 100, and 125 ppm. Quercetin was used as a standard solution because quercetin is a flavonoid of the flavonol group that has a keto group at C-4 and a hydroxyl group on neighboring C-3 or C-5 atoms of flavones and flavonols [24]. Measurement of maximum wavelength absorption is run from wavelength 400-450 nm. The running results show the maximum wavelength of the standard quercetin at wavelength 400-450 nm.

From these measurements (Figure 3), it can be concluded that the higher the concentration used, the higher the absorbance obtained. The raw result of quercetin obtained is plotted between the levels and absorbance so that the linear regression equation was obtained y = 0051x – 0.0176, with the R^2 value, was obtained 0.9879. The quercetin calibration curve equation can be used as a comparison to determine the total concentration of flavonoids in the sample of the durian shell from local Indonesian durian (*Durio zibethinus*).

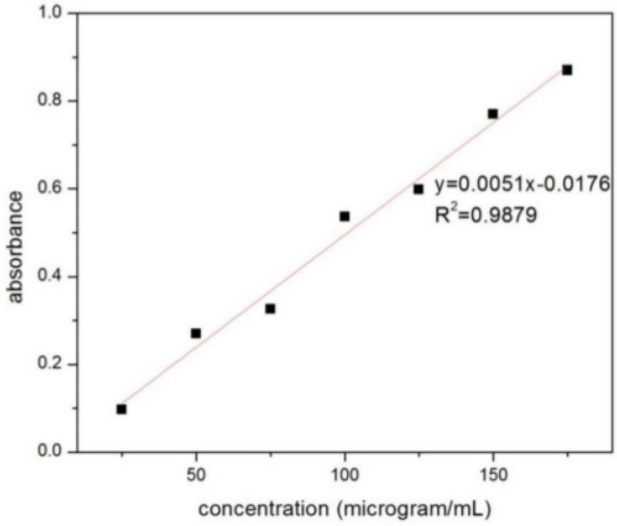

Figure 3. Quercetin calibration curve at a maximum wavelength of 425 nm

Testing of quantitative analysis with UV-Vis spectrophotometer used a blank solution as a control that functions as a check compounds that do not need to be analyzed through multiplying zero number in UV-Vis spectrophotometer instrument [24].

Table 3. The total flavonoid content of the durian shell from local Indonesian durian (*Durio zibethinus*)

Cultivar	Total Flavonoid Content (mg QE/g)
Malika	0.321 ± 0.003
Malon	0.324 ± 0.002
Monti	0.405 ± 0.002

Treatment means of the ANOVA test
Values were expressed as the mean ± standard deviation of three replications
The mean difference is significant at the $p \leq 0.050$
* Highly significant, $p = 0.000$

In measuring the total flavonoid compounds, $AlCl_3$ added to the sample solution, which can form complexes, so the wavelength shifted to visible direction while a yellower color produced in the solution [24]. The incubation treatment for 1 hour before the measurement is intended in order to run the reaction perfectly and produces maximum color intensity. The total content flavonoid of Indonesian local durian shell (*Durio zibethinus*) is shown in Table 3, according to the results of this study showing that the Monti durian shell has a higher flavonoid content compared to the other two types of durian shell, Malika and Malon.

This study only wants to show that Indonesian local durian shell contains significant total flavonoid content without the need for extraction. Samples are only dissolved with ethanol solvent, then a series of tests were carried out, ranging from phytochemical assessment, FTIR spectrophotometer, and $AlCl_3$ methods to determine the total flavonoid content through quantitative analysis. Rough results from all

methods show that the Indonesian local durian shell contains flavonoid compounds that can be extracted or even isolated in the future. Of course, the total flavonoid content in the extract will be higher than measuring the durian shell directly.

4. Conclusion

In conclusion, durian shell (*Durio zibethinus*), especially local durian like Malika, Monti, and Malon have a flavonoid content shown by proximate and phytochemical assay. FTIR analysis assigned the presence of flavonoid content with C–H alkenes, –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols, C–H alkanes, C=C aromatic ring and C–C alkenes functional group. While UV-Vis spectrophotometer analysis shows that Monti has higher flavonoid content than Malika and Malon. Rough results from all methods show that Indonesian local durian peel contains flavonoid compounds that can be extracted or even isolated in the future. That is, of course, the total flavonoid content of the extract will be higher than measuring it directly with the durian shell.

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