

Research Article

Bioactive characteristics of *Guazuma ulmifolia* lam leaf flour as a halal functional food ingredient

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<https://doi.org/xxxxx>**Abstract**

The increasing demand for plant-based functional and halal foods has encouraged the exploration of natural bioactive sources from local plants. *Guazuma ulmifolia* leaves are known to contain various bioactive compounds with potential health benefits and may be utilized as sustainable ingredients in halal functional food products. Therefore, this study aimed to evaluate the bioactive compounds, pasting properties, and morphological characteristics of *Guazuma ulmifolia* leaf powder (GULP) to assess its potential application as a plant-based halal functional food ingredient. Bioactive compound analysis was conducted by determining total phenolic content (TPC), total flavonoid content (TFC), and chlorophyll content. In addition, pasting properties were analyzed to evaluate the flour's functional characteristics during processing, while microstructural characteristics were observed via morphological analysis. The results showed that GULP contained considerable amounts of bioactive compounds, with TPC and TFC values of 105.00 ± 0.10 mg GAE/100 g and 85.65 ± 0.16 mg QE/100 g, respectively. Chlorophyll analysis revealed chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of 0.44 ± 0.01 , 0.36 ± 0.05 , and 0.80 ± 0.15 , respectively. The pasting properties indicated that GULP possessed suitable functional characteristics for food processing applications. Furthermore, microstructural analysis revealed heterogeneous morphological structures comprising dietary fiber, protein bodies, and small starch granules distributed throughout the flour matrix. In conclusion, GULP exhibited promising bioactive and functional characteristics, indicating its potential utilization as a natural ingredient in the development of plant-based halal functional foods with added nutritional and health benefits.

1. INTRODUCTION

Plant-based food processing is one approach used to maximize the use of natural resources, particularly in the development of functional foods. Functional foods are fresh or processed food products containing bioactive compounds that provide physiological health benefits, including reducing the risk of diseases and promoting overall health (Dzah et al., 2021). Bioactive compounds are key components naturally present in small quantities within food materials. Flour is considered a functional food product that can be used as a food ingredient in the preparation of various healthy food products, such as noodles, vegetarian meat analogs (e.g., shrimp, ham, and steak substitutes), bread, and cakes (Granato, et al 2020). The National Socioeconomic Survey (SUSENAS) conducted by the Badan Pusat Statistik in 2022 reported that the average per capita consumption of wheat flour among the Indonesian population was relatively high, reaching 2,750 tons per year in 2023. Floral biodiversity also reflects the diversity of chemical compounds (chemodiversity) contained within plants, including both primary and secondary metabolites. Primary metabolites are essential compounds involved in biosynthetic processes, cellular metabolism, and the breakdown of substances necessary for survival. These compounds mainly consist of carbohydrates, proteins, and lipids. In contrast, secondary metabolites are compounds synthesized by plants that do not directly contribute to the growth, development, or reproduction of the organism (Nuraeni & Darwiati, 2021). These secondary metabolites are widely recognized for their biological activities, including antioxidant, antimicrobial, anti-inflammatory, and

anticancer properties, making them important sources of functional food ingredients and nutraceuticals (Zhang et al., 2022).

In addition to their nutritional value, the bioactive compounds present in *Guazuma ulmifolia* leaves may contribute significant physiological benefits when incorporated into functional food products. Flavonoids, tannins, and saponins are known to exhibit antioxidant and anti-inflammatory activities, which are associated with the prevention of metabolic disorders and other chronic diseases. The incorporation of plant-derived bioactive compounds into flour products also offers opportunities to develop healthier food alternatives with added functional properties. Therefore, the utilization of *G. ulmifolia* leaf flour may support the growing demand for natural, plant-based functional foods with potential health-promoting effects (Shahidi & Ambigaipalan, 2015). Furthermore, the development of functional flour derived from *Guazuma ulmifolia* leaves aligns with current trends in sustainable food innovation and the utilization of local biodiversity. The exploration of underutilized plant materials as functional food ingredients not only enhances the economic value of indigenous plants but also supports the diversification of healthy food products. Hence, further studies regarding the physicochemical characteristics, nutritional composition, and bioactive potential of *G. ulmifolia* leaf flour are necessary to optimize its application in the functional food industry (Dzah et al., 2021). The development of functional flour sourced from *Guazuma ulmifolia* leaves aligns with the escalating global interest in halal-certified nutraceuticals and plant-based functional foods. By leveraging the bioactive properties inherent in botanical ingredients, there is a significant opportunity to innovate halal products that bridge the gap between religious compliance and health promotion. Furthermore, tapping into indigenous resources like *G. ulmifolia* not only fosters sustainable food innovation but also amplifies the economic potential of local biodiversity. Consequently, a rigorous evaluation of its physicochemical attributes, safety profile, and nutritional density is imperative to refine its integration as a high-value ingredient in the halal food industry (Granato et al., 2020; Muchtaridi et al., 2022)."

2. MATERIALS AND METHODS

2.1 Materials

Guazuma ulmifolia leaves from a local farmer Balai Materia Medika Batu Jawa Timur, East Java, Indonesia. The *G. ulmifolia* was taken only once during experiment to ensure the homogeneity of the samples. *G. ulmifolia* was stored in the room temperature in tight jars during the study.

2.2 Determination of Total Phenolic Content (TPC)

The total phenolic content was quantified using the colorimetric method described by Sharma et al. (2011), with slight modifications. Initially, a gallic acid stock solution (100 µg/mL) was prepared in 96% ethanol, from which a series of standard concentrations (0, 20, 40, 60, 80, and 100 ppm) was derived. To develop the calibration curve, 0.5 mL of each standard was treated with 2.5 mL of 10 Folin-Ciocalteu reagent and allowed to react for 5 minutes. Subsequently, 2 mL 7.5% Na₂CO₃ was introduced, followed by a 30 minute incubation at ambient temperature. The absorbance was then recorded at 756 nm using a spectrophotometer in triplicate.

For sample preparation, 50 g of *Guazuma ulmifolia* leaf powder (GULP) was macerated in 400 mL 90 % ethanol. The extraction was enhanced using a 200 W, 40 kHz ultrasonic bath maintained at 60 °C for 30 minutes. Following centrifugation at 5000 rpm for 5 minutes, the process was repeated twice to ensure maximum recovery. The resulting supernatants were pooled and evaporated to dryness under vacuum at 45 °C

For the TPC analysis, 0.1 g of the concentrated extract was reconstituted in 96% ethanol to obtain a concentration of 1 µg/mL. A 0.5 mL aliquot of this sample was mixed with 2.5 mL 10 % Folin-Ciocalteu reagent. After a 5 minute initial incubation, 2 mL of 5 % Na₂CO₃ was added. The final mixture was stirred and incubated for 30 minutes before measuring its optical density at 756 nm using UV-Vis spectrophotometry.

2.3 Total flavonoid content (TFC)

TFC was measured by using the modified method of Marinova et al. (2005). A stock solution of quercetin (100 ppm or 100 µg/mL) was prepared in 96% ethanol. Serial concentrations of 0, 20, 40, 60, 80, and 100 ppm were prepared by pipetting 0, 1, 2, 3, 4, and 5 mL of the stock solution into a 5 mL volumetric flask, adjusted with distilled water to the line. A total of 1 mL of standard solution was placed in a test tube and mixed with 4 mL of distilled water and 0.3 mL of 5% NaNO₂. The solution was

left at room temperature for 6 min, then 0.3 mL of 10% AlCl_3 was added. After incubation for 6 min, 2 mL of 1 M NaOH was added, followed by 2.4 mL of distilled water. Incubation was carried out for 15 min, and the intensity was measured at 340 nm using a spectrophotometer. Measurements were carried out 3 times. A standard curve was prepared to relate quercetin concentration to absorbance. GULP was extracted by ethanol using a procedure similar to TPC analysis. GULP extract (1 $\mu\text{g}/\text{mL}$) was added with 4 mL of distilled water and 0.3 mL of 5% NaNO_2 . The mixture was incubated for 6 min, and 0.3 mL of 10% AlCl_3 was added. After 6 min of incubation, 2 mL of 1 M NaOH and 2.4 mL of distilled water were added. The solution was incubated for 15 min and measured at 340 nm with a spectrophotometer.

2.4 Chlorophyll analysis

The DMSO extraction technique of Hiscox & Israelstam (1979) was used for chlorophyll extraction. Samples were incubated at 65 °C until leaf disks were completely colorless. The absorbance of the DMSO-chlorophyll extractions and blank (pure DMSO) was measured at 645 nm and 663 nm using a spectrophotometer (Spectrophotometer uv-vis Genesys 10s). And finally, chlorophyll a, b, and total chlorophyll were calculated by Arnon's equations (Equations 1-3).

$$\text{Chl a (mg/g)} = \frac{12,7 (A663) - 2,69(A645) \times V}{100XW} \quad (1)$$

$$\text{Chl b (mg/g)} = \frac{22,9 (A645) - 4,68(A663) \times V}{1000XW} \quad (2)$$

$$\text{Chl Total (mg/g)} = \frac{20,2 (A645) + 8,02(A663) \times V}{1000XW} \quad (3)$$

2.5 Pasting properties analysis

The measurement of the pasting properties of MOLP was conducted using a Rapid Visco Analyzer (RVA, Perten TecMaster). About 25.0 ± 0.1 mL of distilled water (corrected to compensate for 14% moisture basis correction of the sample) was weighed into a new canister. The sample of 3.50 ± 0.01 g MOLP (14% moisture basis) was put into a weighing vessel and transferred onto the water surface. A paddle was placed into the canister, and they jogged vigorously through the sample 10 times, up and down. Then, the canister was put into the instrument. The analysis was started at 50 °C for 1 min. At 4 min, the temperature was raised to 95 °C and held for about 7 min. The temperature decreased to 50 °C, 11 min after the analysis started. The measurement was run for 13 min, with a 4-second interval between readings.

2.6 Microstructure analysis

The surface morphology of MOLP was analyzed with a scanning electron microscope (SEM) instrument (FEI Inspect-S50 model). The film specimen was placed on a 10 mm-diameter stub and subsequently coated with a Au-Pd layer to enhance the sample's conductivity.

2.7 Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean $\pm$ standard deviation. Statistical differences among treatment groups were evaluated using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. When significant differences were observed, Duncan's multiple range test (DMRT) was applied for post hoc comparisons. All statistical analyses were performed using appropriate statistical software.

3. RESULTS AND DISCUSSION

3.1 Bioactive Compound of *Guazuma ulmifolia* Leaf Powder (GULP)

The main phenolic compound groups include flavonoids, phenolic acids, tannins, stilbenes, and lignans. *G. ulmifolia* leaf contains many phenolic compounds comprising flavonoids, phenolic acids, and their glycosides (Hassan et al., 2021). The presence of phenolic compounds in MO leaves is associated with environmental stress. The analysis revealed that *Guazuma ulmifolia* leaf powder possesses a total phenolic content (TPC) of 105.00 mg GAE/100 g. This substantial concentration underscores the flour's potential as a reservoir of bioactive molecules. As established in the literature, these secondary metabolites play a pivotal role in scavenging free radicals by donating electrons or hydrogen atoms, thereby serving as potent antioxidant agents. Given this high phenolic density, *G. ulmifolia* flour emerges as a promising functional ingredient capable of mitigating oxidative stress-

related pathologies (Shahidi & Ambigaipalan, 2015). Variations in phenolic levels are typically determined by a confluence of factors, ranging from the plant's physiological maturity and environmental stressors to post-harvest protocols such as dehydration and pulverization. While thermal processing and oxidative exposure can often compromise the stability and antioxidant efficacy of plant phenolics (Shahidi & Ambigaipalan, 2015), this study indicates that *G. ulmifolia* maintains a robust bioactive profile despite the rigors of flour processing. Beyond their antioxidant capacity, the phenolic constituents in *G. ulmifolia* are linked to a broad spectrum of therapeutic benefits, including anti-inflammatory, antimicrobial, and metabolic regulatory effects specifically antidiabetic and anti-obesity properties. Such multifaceted biological activities reinforce the viability of integrating this leaf flour into halal-certified food systems. Consequently, utilizing this botanical resource not only addresses the rising consumer preference for plant-derived nutraceuticals but also enhances the nutritional and medicinal landscape of modern food formulations (Dzah et al., 2021).

The quantitative analysis of *Guazuma ulmifolia* leaf flour yielded a total flavonoid content (TFC) of 85.65 mg QE/100 g. This significant concentration highlights the material's potential as a source of bioactive compounds with multifaceted therapeutic properties. As a prominent class of polyphenols, flavonoids are renowned for their potent antioxidant capacity, primarily through mechanisms such as free radical scavenging, lipid peroxidation inhibition, and mitigation of cellular oxidative stress. These biochemical actions are fundamental in the systemic prevention of various chronic pathologies (Panche et al., 2016). The biological significance of these compounds extends beyond simple antioxidant activity; flavonoids are documented to possess anti-inflammatory, antimicrobial, antidiabetic, and cardioprotective functionalities (Panche et al., 2016). Specifically for *G. ulmifolia*, the identified flavonoid levels may substantiate its traditional use as an anti-obesity agent, likely through the modulation of lipid metabolic pathways and the reduction of oxidative damage. Thus, the presence of these metabolites reinforces the candidacy of this leaf flour as a high-value bioactive ingredient for the development of functional foods. It is worth noting that the final flavonoid concentration is often influenced by various pre- and post-harvest factors, including environmental stressors, plant phenology, and processing parameters such as thermal drying and milling. Although such processes can degrade flavonoids, the substantial retention observed in this study confirms that *G. ulmifolia* remains a robust source of natural antioxidants. These results further validate the integration of *G. ulmifolia* leaf flour into the plant-based and halal food sectors, offering both nutritional enhancement and preventative health benefits.

Table 1. Bioactive Compound of *Guazuma ulmifolia* leaf flour

Bioactive Compound	Total Phenolic (mg GAE/100 g db)	Total flavonoid (mg QE/100 g db)	Chlorophyll a (%)	Chlorophyll b (%)	Total chlorophyll (%)
<i>Guazuma Ulmifolia</i> leaf powder	105.00 ± 0.10	85.65 ± 0.16	0.44 ± 0.01	0.36 ± 0.05	0.8 ± 0.15

Data are expressed as mean ± SD (n = 3).

3.2 Pigments

The chlorophyll analysis of *Guazuma ulmifolia* leaf flour revealed chlorophyll a, chlorophyll b, and total chlorophyll contents of 0.44 ± 0.01, 0.36 ± 0.05, and 0.80 ± 0.15, respectively. The results showed that chlorophyll a content was higher than chlorophyll b, which is consistent with the natural composition of green plants where chlorophyll a serves as the primary photosynthetic pigment responsible for capturing light energy during photosynthesis. In contrast, chlorophyll b functions as an accessory pigment, broadening the spectrum of light absorption and transferring energy to chlorophyll a (Taiz et al., 2018). Leaves play a crucial role in capturing solar energy for normal photosynthetic processes, and broad leaf surface areas generally allow more efficient light interception. According to Tang (2022), chlorophyll accumulation in leaves is strongly influenced by light intensity, where optimal light exposure enhances chlorophyll biosynthesis and photosynthetic efficiency. Leaves with lower chlorophyll concentrations may compensate by increasing their surface area to maximize light absorption. In addition to leaf area, leaf thickness also contributes to photosynthetic performance because thicker leaves often contain more mesophyll tissue and chloroplasts, thereby increasing photosynthetic capacity. The presence of chlorophyll compounds in *Guazuma ulmifolia* leaf flour may also provide additional functional benefits beyond photosynthesis. Chlorophyll has been reported to possess

antioxidant and detoxification properties, which may contribute to health-promoting effects when incorporated into functional food products. Therefore, the chlorophyll content observed in this study supports the potential utilization of *G. ulmifolia* leaf flour as a natural plant-based ingredient for functional and halal food applications.

3.3 Pasting Properties

Pasting properties are important characteristics of starchy materials, reflecting the transition of starch granules from swelling to breakdown and the melting of the crystalline structure during cooking (Zhu et al., 2020).

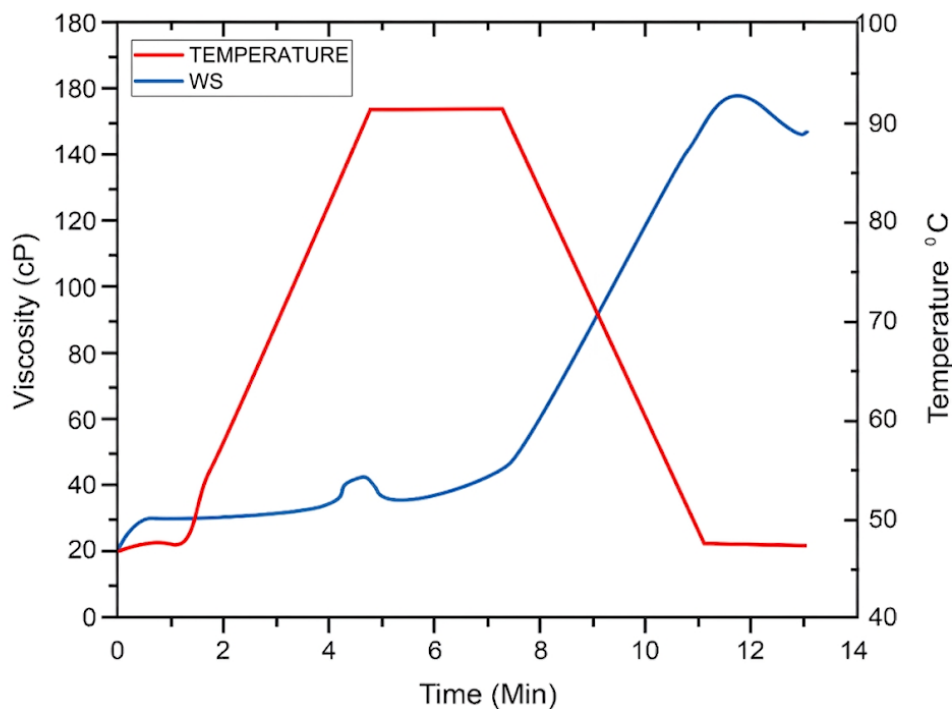


Figure 1. Pasting properties of *G. ulmifolia* leaf flour

The recorded profile shows a progressive increase in viscosity with increasing temperature (indicated by the red curve); however, the peak viscosity remains notably lower than that of pure cereal starches. This phenomenon suggests that the high fiber content and bioactive constituents in *G. ulmifolia* may act as physical barriers, preventing excessive swelling of starch granules. The relatively moderate peak viscosity indicates that this flour possesses high stability against mechanical shear. According to Widyasanti et al. (2023), incorporating fiber-rich botanical sources into food matrices can modify hydrocolloid interactions, thereby stabilizing the paste structure during thermal processing. The graph shows minimal breakdown viscosity, the difference between the peak and the minimum viscosity during the holding period at high temperatures. This suggests that the *G. ulmifolia* leaf paste is highly heat and shear-resistant, a desirable trait for products requiring consistent texture during high-temperature processing. Upon cooling (between minutes 10 and 12), a rise in viscosity, known as setback viscosity, occurs due to the recrystallization of amylose (retrogradation). However, the presence of indigenous polyphenols and flavonoids in the flour likely interferes with amylose chain reassociation, potentially minimizing syneresis. Suhaili et al. (2024) noted that using local plant resources with specific functional profiles can yield halal-certified functional foods with superior textural stability compared to those prepared with isolated starches. The stable viscosity profile at elevated temperatures and the controlled thickening during cooling suggest that *G. ulmifolia* leaf flour is an excellent candidate for use as a natural thickening or stabilizing agent. Such properties are particularly beneficial for halal-certified sauces, functional beverages, or gel-based products. This innovation aligns with the global shift toward replacing synthetic food additives with bioactive-rich, plant-derived alternatives from local biodiversity.

3.4 Microstructure Analysis

The morphological characteristics of *Guazuma ulmifolia* leaf powder (GULP), as illustrated in Figure 2, reveal significant structural heterogeneity. As noted by Huang (2020), the microstructure of botanical powders typically comprises coarse, asymmetrical particles, and superfine grinding often results in a reduction in particle dimensions. The diverse microstructural landscape observed in GULP is likely a reflection of its intricate chemical matrix, where dietary fiber, proteins, and starch granules interact to define the flour's overall architecture. In this matrix, fibrous fractions appear as elongated, band-like structures, whereas the more irregular formations are attributed to protein bodies. Concurrently, starch granules are identified as small, elliptical entities dispersed stochastically alongside the protein and fiber components. Analysis across different GULP samples showed a consistent microstructural pattern, with the primary distinction being the dimensions of the starch granules.

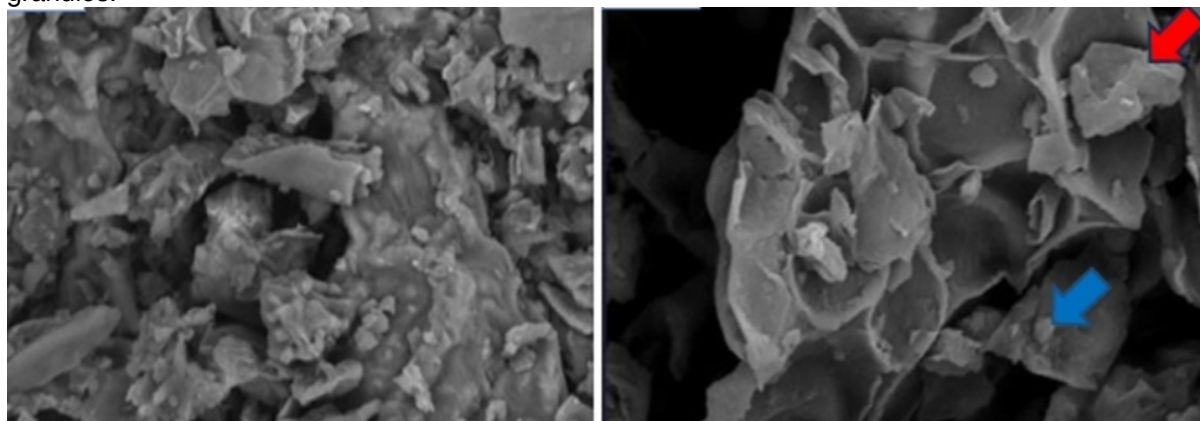


Figure 2. Microstructure by Scanning Electron Microscope of *G. ulmifolia* leaf flour. The red arrow indicates the protein body, and the blue arrow indicates the starch granule.

These granules were notably minute, measuring between 3.00–3.50 μm . According to Fuentes et al. (2019), the size of starch granules is inherently linked to their botanical source and plays a decisive role in determining the functional and physicochemical behavior of flour. While larger granules are characteristic of tubers, small granules (2–10 μm), similar to those found in GULP, are typically observed in cereals such as rice and oats. This small granule size is advantageous, as it may enhance hydration capacity, improve digestibility, and facilitate the seamless integration of GULP into various functional food systems. Furthermore, Li et al (2023) emphasize that granule size is a critical determinant of starch functionality, affecting parameters such as water absorption, gelatinization, and swelling behavior. The dense, heterogeneous microstructure of GULP underscores its viability as a functional ingredient, particularly for developing plant-based and halal-certified products enriched with bioactive compounds and dietary fiber. Additionally, the coexistence of protein bodies and fiber-rich structures is expected to impart positive nutritional and functional attributes to GULP-based food formulations.

4. CONCLUSION

The investigation into *Guazuma ulmifolia* leaf flour reveals its significant potential as a high-value, plant-based functional ingredient, underpinned by a robust profile of bioactive constituents and advantageous physicochemical attributes. The substantial concentrations of total phenolics and flavonoids identified in this study indicate a potent antioxidant capacity, essential for mitigating oxidative stress and promoting systemic health. Furthermore, the presence of natural chlorophyll enhances its functional appeal, offering additional therapeutic benefits that align with the requirements of clean-label, health-conscious food products. From a technological standpoint, the pasting properties of *G. ulmifolia* leaf flour demonstrate its versatility for integration into diverse food systems, particularly in the development of functional, flour-based matrices. The observed stability and hydration characteristics during hydrothermal processing suggest that the flour is well-suited for industrial food applications. This is further supported by microstructural evidence, which shows a complex assembly of dietary fiber, protein bodies, and small-scale starch granules 3.00–3.50 μm . Such a minute granule size is a critical advantage, likely facilitating superior water absorption, enhanced digestibility, and overall structural integrity in finished food products. Crucially, the findings underscore that *Guazuma*

ulmifolia leaf flour is a prime candidate for the global halal food industry. As a purely plant-based resource, it inherently satisfies the stringent requirements for halal integrity, offering a sustainable and permissible alternative to synthetic or questionable additives. Its rich nutritional profile and functional resilience directly address the burgeoning international demand for halal-certified functional foods that do not compromise on health benefits or ethical standards. Consequently, *G. ulmifolia* stands as a promising solution for developing healthy, sustainable, and Sharia-compliant food formulations. Future research should prioritize comprehensive safety evaluations, sensory profiling, and the optimization of its application across a broader spectrum of halal-certified nutraceutical products. a sustainable raw material for the production of functional vinegar. Furthermore, the near-complete conversion of ethanol into acetic acid supports the development of a halal-compliant fermentation process, as the final product contains minimal alcohol residue and utilizes food-grade microorganisms. Therefore, banana peel vinegar produced through co-culture fermentation can be considered a promising candidate for functional and halal-oriented food products.

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