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α-Glucosidase Inhibition-Guided Fractionation of Kalincung (*Uncaria gambir* Roxb. By-Product) using Diaion HP-20 Resin

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Abstract: Kalincung is a semi-solid by-product generated during the traditional processing of Uncaria gambir Roxb. and may undergo spontaneous fermentation during post-processing storage. However, it remains underexplored despite its potential as a polyphenol-rich resource. This study compared methanolic extracts of kalincung and dried gambir leaves and evaluated α-glucosidase inhibition-guided fractionation using Diaion HP-20 resin. An assessment of phytochemical constituents was carried out, including the determination of total phenolic (TPC), total flavonoid content (TFC), and vanillin–HCl–reactive flavan-3-ol equivalent profile (VAE; expressed as catechin equivalents, CAE), followed by antioxidant assessment using the DPPH assay (IC_{50}) and α-glucosidase inhibition (IC_{50}). Kalincung exhibited higher TPC (40.10 ± 0.07% GAE) and TFC (35.92 ± 1.12% RAE) than leaves (30.94 ± 0.71% GAE and 21.30 ± 0.71% RAE; $p < 0.05$), while leaves showed higher VAE values (27.46 ± 0.06% CAE) than kalincung (9.84 ± 0.36% CAE; $p < 0.05$), reflecting differences in vanillin-reactive flavan-3-ol units rather than absolute proanthocyanidin content. Kalincung demonstrated strong DPPH scavenging activity (IC_{50} = 9.13 ± 0.05 μg/mL) and α-glucosidase inhibition (IC_{50} = 32.32 ± 1.20 μg/mL), comparable to leaves. Solvent fractionation enriched bioactivity, with the butanol fraction showing improved α-glucosidase inhibition. Diaion HP-20 fractionation yielded a selected active fraction with increased VAE compared with the crude kalincung extract (48.33 ± 0.39% CAE), accompanied by enhanced functional potency (DPPH IC_{50} = 8.03 ± 0.07 μg/mL; α-glucosidase IC_{50} = 17.07 ± 0.42 μg/mL), showing activity within the same order of magnitude as (+)-catechin and showing lower IC_{50} than acarbose under the present in vitro assay conditions. Taken together, these findings position kalincung as a largely overlooked yet functionally relevant source of polar polyphenolic constituents. Its α-glucosidase inhibitory effect, supported by antioxidant performance, suggests meaningful prospects for early-stage functional evaluation.

Keywords: α-glucosidase inhibition; diaion hp-20 resin; kalincung; Uncaria gambir roxb.

1. INTRODUCTION

Diabetes mellitus is characterized by chronic dysregulation of glucose homeostasis resulting from impaired insulin secretion, defective insulin action, or a combination of both, leading to sustained hyperglycemia [1]. This chronic hyperglycemic condition is not only associated with hormonal dysregulation but also closely linked to increased free radical production and oxidative stress in the body. Chronic hyperglycemia promotes excessive formation of reactive oxygen species. This oxidative burden damages β-cell integrity, interferes with insulin signaling, and contributes to the progression of microvascular complications. Therefore, strategies for diabetes management should not only focus on lowering blood glucose levels but also on mitigating oxidative stress through antioxidant activity [2].

One effective therapeutic approach for controlling postprandial hyperglycemia involves inhibiting the intestinal enzyme α-glucosidase. The intestinal α-glucosidase enzyme mediates the terminal hydrolysis of dietary oligosaccharides, thereby directly influencing postprandial glycemic excursions. α-Glucosidase inhibitors can delay glucose absorption, thereby preventing postprandial

spikes in blood glucose [3]. Synthetic drugs such as acarbose, miglitol, and voglibose act through this mechanism; their implementation in therapy is, however, limited by unwanted gastrointestinal effects such as flatulence and diarrhea [4]. Consequently, natural products containing α-glucosidase inhibitory compounds with low toxicity have attracted growing attention as safer alternatives for diabetes management.

Polyphenol-derived antioxidants have attracted attention due to their dual capacity to neutralize oxidative mediators and modulate glucose-regulating enzymatic targets implicated in metabolic disorders. Phenolic and flavonoid compounds with strong antioxidant properties have been reported to attenuate oxidative stress, thereby improving insulin sensitivity and preserving pancreatic β-cell function [5], [6]. Moreover, numerous studies have shown that compounds with potent antioxidant activity often exhibit α-glucosidase inhibitory effects as well. This relationship suggests a biological link between antioxidant potential and α-glucosidase inhibition, indicating a synergistic mechanism in attenuating the progression of diabetes mellitus [3].

Gambir leaves (Uncaria gambir Roxb.) are a natural source rich in various polyphenolic constituents, notably catechin, epicatechin, and proanthocyanidins, recognized for their antioxidant capacity and antidiabetic potential [7]. A previous study by Soleh Ismail et al. (2021) successfully identified 27 bioactive compounds in the liquid by-product of gambir processing in West Sumatra, Indonesia. This finding indicates that by-products generated during gambir processing still contain abundant phenolic and flavonoid components [8].

Although the bioactive compounds of Uncaria gambir Roxb. have been extensively studied, scientific information regarding its solid by-product, known as kalincung, remains limited. Kalincung represents a viscous or semi-solid residue derived from the traditional extraction of gambir [8], [9]. In gambir-producing regions of West Sumatra, particularly Kabupaten Lima Puluh Kota and Kabupaten Pesisir Selatan, the cultivation and traditional utilization of Uncaria gambir Roxb. and its associated by-products have been documented for decades. Several local reports indicate that kalincung is often not discarded and may have potential for further utilization [9]. While ethnographic records describing its traditional medicinal use are limited, the existence of this by-product and its association with local gambir production are well documented [8].

During storage, kalincung may undergo spontaneous secondary fermentation or moist post-processing transformation, which can alter its chemical composition through hydrolysis of glycosidic bonds and related reactions affecting the solubility, molecular size, and availability of polyphenolic constituents. Similar transformation processes in plant-derived materials have been reported to influence the distribution and chemical forms of phenolic compounds, including flavan-3-ols and proanthocyanidin-related structures, without necessarily generating identifiable low-molecular-weight metabolites [10]. However, because microbial isolation or community profiling was not performed, the present study does not identify the specific microorganisms potentially involved in kalincung formation. Therefore, fermentation-related changes are discussed only as possible post-processing transformations based on reported patterns in plant-derived matrices, rather than as microbiologically confirmed events. These transformations may modify the polyphenolic profile of kalincung and, consequently, affect its antioxidant activity and α-glucosidase inhibitory potential [11]. Nevertheless, systematic comparative data evaluating the antioxidant capacity and α-glucosidase inhibition activity of kalincung, particularly through α-glucosidase inhibition-guided fractionation approaches, remain scarce.

Accordingly, this study adopts an α-glucosidase inhibition-oriented design, emphasizing comparative evaluation of crude extracts, solvent fractions, a selected Diaion HP-20 fraction, and the monomeric reference compound (+)-catechin. Kalincung and gambir leaves were investigated through an integrated phytochemical and biological approach, including phytochemical profiling, quantification of total phenolics, flavonoids, and assessment of the vanillin–HCl–reactive flavan-3-ol equivalent profile, as well as evaluation of antioxidant and α-glucosidase inhibitory activities [12], [13]. To further enrich the active constituents associated with α-glucosidase inhibition, the most active solvent fraction was subjected to Diaion HP-20 column chromatography [14], [15]. In this study, the selected Diaion HP-20 fraction refers to the subfraction obtained from the most active n-butanol fraction and selected based primarily on α-glucosidase inhibitory activity. Vanillin–HCl reactivity

was used only as supporting phytochemical information rather than as definitive structural identification. Comparative evaluation against (+)-catechin was incorporated as a functional reference to contextualize the antioxidant and α-glucosidase inhibitory activities of the selected Diaion HP-20 fraction. The α-glucosidase inhibitory activities of crude extracts, the Diaion HP-20-derived fraction, and the catechin standard were compared with the synthetic inhibitor acarbose [16], [17].

Based on currently available literature, no prior study has comprehensively evaluated kalincung, a semi-solid by-product of traditional gambir processing, as a bioactive polyphenolic resource using integrated phytochemical, antioxidant, α-glucosidase inhibitory, and α-glucosidase inhibition-guided fractionation approaches. Previous studies have mainly addressed U. gambir leaf extracts, plant-part comparisons, drying-related variation, isolated (+)-catechin, and proanthocyanidin fractions derived from U. gambir leaves [18], [19]. Therefore, the present study provides a distinct functional perspective on the bioactive potential of kalincung as an underexplored gambir-processing residue. The findings are expected to provide scientific support for the utilization of Indonesian natural sustainable sources of antioxidants and α-glucosidase inhibitors for further functional evaluation.

2. MATERIALS AND METHODS

2.1. Plant Sample Collection

A qualified botanist confirmed the taxonomic identity of the plant sample at the Herbarium of Andalas University. Freshly harvested leaves of Uncaria gambir Roxb. were collected from the Medicinal Plant Garden (KTOF), Sumatran Biota Laboratory, Andalas University, Indonesia. The collected leaves were subsequently processed into two sample types: (1) kalincung, a traditional by-product of gambir processing, and (2) dried gambir leaves.

2.2. Preparation of Kalincung

The preparation of kalincung followed a traditional gambir-processing practice commonly applied in West Sumatra, Indonesia, with minor modifications to ensure laboratory reproducibility. Fresh Uncaria gambir Roxb. leaves were boiled in water (approximately 100 °C) for 1.5 h, filtered, and the solid residues were pressed to recover the remaining extract. The combined filtrate was kept undisturbed at room temperature (approximately 25 °C) for 24 h to facilitate catechin (gambir) precipitation, consistent with reported traditional workflows and recent compositional studies on gambir by-products [20]. The non-precipitated liquid together with the pressed leaf residues was subsequently maintained at 25–28 °C for approximately 2–3 days, during which spontaneous fermentation may occur, yielding a viscous or semi-solid material known as kalincung.

However, active fermentation during this holding period was not microbiologically confirmed in the present study. Although inspired by traditional processing practices, all steps in this study were conducted under controlled and standardized laboratory conditions to ensure reproducibility across batches [21], [22].

2.3. Sample Treatment and Drying Process

All leaf samples were dried using a greenhouse solar dryer at approximately 60 °C for 24 hours. After drying, leaf samples were ground using a grinder to obtain fine powders. For the kalincung samples, the drying procedure was identical to that of the leaf samples and continued until no further change in weight was observed. The dried kalincung, which exhibited a resinous and partially cohesive texture, was gently crushed using a mortar and pestle to reduce particle size. The material was then additionally dried at 40 °C in a drying oven and subsequently pulverized with an electric grinder to yield a homogeneous coarse powder. All resulting materials were immediately sealed in airtight containers and stored in the dark until extraction and analysis.

2.4. *Preparation of Methanol Extract and Fractions*

All dried samples (leaf powders and coarse kalincung powder) were extracted using 70% methanol by maceration following the method described by Harborne (1973) [23]. Maceration was conducted at ambient temperature for a period of nine days, with solvent replacement every three days. All extractions were conducted under identical conditions to ensure comparability among samples. After extraction, the methanolic solutions were filtered through Whatman No.1 paper and evaporated under vacuum at 60 °C using a rotary evaporator.

The percentage yields obtained from the extraction process were 31.02% for kalincung and 21.30% for gambir leaves. Subsequently, an equal mass (5 g) of each methanolic extract underwent liquid–liquid partitioning with n-hexane, ethyl acetate, and n-butanol to obtain the corresponding solvent fractions. The fractionation yields, expressed as percentages relative to the initial extract weight, were as follows: ethyl acetate fraction of kalincung (40.6%), butanol fraction of kalincung (8.6%), ethyl acetate fraction of leaves (52.8%), and butanol fraction of leaves (12.6%).

2.5. Diaion HP-20 Column Chromatography of the n-Butanol Fraction

Among the solvent fractions derived from kalincung, the n-butanol fraction exhibited the strongest α-glucosidase inhibitory activity and was therefore selected for further fractionation using Diaion HP-20 column chromatography [14], [15]. A pooled n-butanol fraction (10 g) was subjected to Diaion HP-20 column chromatography purchased from Sigma-Aldrich (Singapore) with dimensions of 9 cm internal diameter x 60 cm length. The column was eluted sequentially with water followed by stepwise increasing concentrations of methanol. Eluates were collected into multiple subfractions. One representative subfraction (S38) was obtained as a brown-colored fraction and selected for further evaluation primarily based on α-glucosidase inhibitory activity, supported by vanillin–HCl reactivity and antioxidant assessment. All Diaion HP-20 subfractions were stored in amber bottles protected from light until further use.

2.6. Chemicals

α-Glucosidase enzyme obtained from Saccharomyces cerevisiae, the synthetic substrate p-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose as a reference inhibitor, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and (+)-catechin were used in this study. α-Glucosidase, pNPG, acarbose, DPPH, and gallic acid were procured from Sigma-Aldrich (Singapore). The reagents potassium dihydrogen phosphate (KH_2PO_4) and sodium carbonate solution (Na_2CO_3, 200 mM) were purchased from Merck (Germany). The remaining reagents and solvents were analytical grade and were used directly without additional treatment.

2.7. Phytochemical Screening

Concentrated, semi-solid extract (50 mg) was dissolved in methanol, and subsequently subjected to qualitative phytochemical screening for phenolics, alkaloids, saponins, steroids, terpenoids, and flavonoids according to the methods described by Harborne [23].

2.8. Quantification of Total Phenolic Content (TPC)

Total phenolic levels of the methanol extract were measured using the Folin–Ciocalteu colorimetric method, as described by Slinkard and Singleton [24]. This assay relies on the reduction of the Folin–Ciocalteu reagent by phenolic compounds, resulting in the formation of a blue -colored complex with absorbance proportional to the phenolic content. Higher phenolic levels yield a deeper blue color and correspondingly higher absorbance values. For the analysis, 50 µL of each extract was mixed with 50 µL of distilled water and 50 µL of 7.5% Folin–Ciocalteu reagent. The mixture was incubated at 37 °C for 8 minutes, followed by the addition of 50 µL of 1% sodium hydroxide. The reaction mixture was then incubated for another 60 minutes at 37 °C. The absorbance was recorded at 750 nm. TPC was calculated using a gallic acid calibration curve and expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry sample.

2.9. Quantification of Total Flavonoid Content (TFC)

The total flavonoid levels in the methanolic extracts of all samples were estimated using a spectrophotometric method according to the procedure previously described [25]. This method is based on the complex formation between flavonoid hydroxyl groups and aluminum chloride, yielding a stable colored complex suitable for spectrophotometric measurement. A 0.5 mL aliquot of the extract stock solution was blended with 1.5 mL of ethanol (P), together with 0.1 mL of 10% aluminum chloride, and 0.1 mL of 1 M sodium acetate. Distilled water was added to make up the reaction mixture to a final volume of 5 ml. Following a 30-minute incubation at room temperature, the absorbance was read at a wavelength of 415 nm. The TFC was determined using a calibration curve constructed from rutin equivalents (% w/w) of the dry extract.

2.10. Assessment of Vanillin–HCl–Reactive Flavan-3-ol Equivalent Profile (VAE)

Vanillin–HCl–reactive flavan-3-ol equivalent profile (VAE) of the methanolic extracts was assessed using the vanillin–HCl colorimetric assay, as described in recent methodological evaluations of flavanol-reactive polyphenolic assays [26]. The principle of this method involves the interaction between vanillin and the meta-substituted aromatic ring of flavanols, generating a red chromogenic complex.

Briefly, 100 μL of each extract or methanol (used as a control) was mixed with 2.5 mL of 8% hydrochloric acid prepared in glacial acetic acid, followed by 2.4 mL of 1% vanillin solution in glacial acetic acid. The solution was maintained under incubation at room temperature for 5 min, and absorbance was recorded at 510 nm. The VAE values were calculated by reference to a (+)-catechin calibration curve and expressed as percent catechin equivalents (% CAE) of dry extract. The assay reflects vanillin-reactive flavan-3-ol units, including monomeric catechins and terminal units of oligomeric proanthocyanidins, and should therefore be interpreted as an index of flavan-3-ol reactivity rather than absolute proanthocyanidin content.

2.11. Determination of DPPH Radical Scavenging Activity

The antioxidant capacity of the methanolic extracts from all samples was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [27]. This method is founded on the ability of antioxidants to reduce DPPH radicals, resulting in a decrease in absorbance proportional to the antioxidant capacity of the extract. A series of extract solutions at the following concentrations: 62.5, 31.25, 15.62, and 7.81 μg/mL were prepared. Each sample (200 μL) was pipetted into a cuvette containing 3.8 mL of DPPH solution in analytical-grade methanol, while methanol (200 μL) was used as the blank. The reaction mixture was incubated at room temperature for 30 minutes and absorbance was recorded at 517 nm. Gallic acid was included as the reference compound for comparison. The percent DPPH radical scavenging activity was determined in accordance with the decrease in absorbance relative to the blank, following a widely used spectrophotometric method as described in recent antioxidant assay guidelines [28], [29].

2.12. Determination of α-Glucosidase Inhibitory Activity (AGI)

The inhibitory effect on α-glucosidase, which reflects antidiabetic-related potential in vitro, was evaluated in accordance with the method outlined by Elya (2015), with minor modifications (Elya et al., 2015). In this enzymatic assay, 36 μL of phosphate buffer was combined with 30 μL of extract to obtain final concentrations of 0.312, 0.156, and 0.078 mg/mL, followed by the addition of 17 μL of 5 mM of p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate in a 96-well microplate. The reaction mixture was pre-incubated at 37 °C for a duration of 15 minutes. Subsequently, a 17 μL aliquot of α-glucosidase enzyme solution (0.15 U/mL) was introduced into each well to obtain a total reaction volume of 100 μL, followed by incubation for another 15 minutes at 37 °C. The reaction was terminated by adding 100 μL of 200 mM sodium carbonate (Na_2CO_3). Absorbance was measured at 405 nm using a microplate reader. All assays were conducted in triplicate. Acarbose was used as a positive control. The percentage of AGI was calculated based on the reduction of absorbance relative to the control, following the method of Elya (2015).

2.13. Determination of IC_{50} Value

The IC_{50} was estimated using linear regression of percentage inhibition against sample concentration, corresponding to the concentration producing 50% inhibition. Lower IC_{50} values reflect stronger inhibitory activity.

2.14. Statistical Evaluation

All experiments were conducted in triplicate, and the data were presented as mean ± standard error (SE) of three measurements. Prior to statistical evaluation, the data were tested for normality and homogeneity of variances. For parametric datasets, one-way ANOVA with Tukey's post hoc test was performed to evaluate differences among groups. Whenever the assumptions required for parametric analysis were violated, a non-parametric approach using the Kruskal-Wallis test was adopted. Results yielding p-values below 0.05 were interpreted as statistically meaningful.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Profile

Qualitative phytochemical screening (Table 1) showed that both kalincung and gambir leaves tested positive for major secondary metabolite classes, including phenolics, flavonoids, alkaloids, and terpenoids, confirming the polyphenolic character of Uncaria gambir Roxb. [31]. Notably, kalincung exhibited a broader phytochemical profile, with additional detection of saponins and steroids, whereas these classes were not observed in gambir leaves. This broader profile indicates that the solid by-product of gambir processing retains diverse bioactive constituents, consistent with previous reports demonstrating substantial phenolic and flavonoid retention in gambir processing residues [8]. The persistence of multiple metabolite classes in kalincung suggests potential synergistic contributions to its biological activities.

Table 1. Qualitative Detection of Secondary Metabolites

Samples	Phenolics	Alkaloids	Saponins	Steroids	Terpenoids	Flavonoids
Kalincung	+	+	+	+	+	+
Leaves	+	+	-	-	+	+

[1]Symbols: + = detected; − = not detected

3.2. Comparative Phenolics and Flavonoid Contents, VAE Profile and Antioxidant Activity

Quantitative analysis (Table 2) revealed that kalincung contained significantly higher total phenolic content (TPC, 40.10 ± 0.07% GAE) and total flavonoid content (TFC, 35.92 ± 1.12% RAE) than gambir leaves (30.94 ± 0.71 % GAE and 21.30 ± 0.71% RAE, respectively; $p < 0.05$). These findings indicate that phenolic and flavonoid compounds are substantially retained in kalincung despite undergoing traditional extraction and moist post-processing storage, supporting the concept that processing residues can remain relatively rich reservoirs of polyphenols [8]. Similar trends have been observed in processed or fermentation-associated plant matrices, where polyphenolic compounds and antioxidant activity may be retained despite compositional changes during processing [32], [33].

Table 2. Total Phenolic and Flavonoid Contents, Vanillin-HCl-Reactive Flavan-3-ol Equivalent Profile, and DPPH Radical Scavenging Activity

Samples	TPC (% GAE)	TFC (% RAE)	VAE (% CAE)	DPPH IC_{50} (μg/mL)
Kalincung	40.10 ± 0.07	35.92 ± 1.12	9.84 ± 0.36	9.13 ± 0.05
Leaves	30.94 ± 0.71	21.30 ± 0.71	27.46 ± 0.06	10.09 ± 0.02
Selected Diaion HP-20 fraction	-	-	48.33 ± 0.39	8.03 ± 0.07
(+)-Catechin	-	-	-	5.77 ± 0.05
Gallic Acid	-	-	-	3.06 ± 0.02

[2]Data represent mean ± SE of three replicates (n = 3). –: Not determined for the respective sample type. TPC: total phenolic content; TFC: total flavonoid content; VAE: vanillin–HCl-reactive flavan-3-ol equivalent profile, expressed as catechin equivalents (% CAE); DPPH IC_{50}: concentration required to inhibit 50% of DPPH radical activity; GAE: gallic acid equivalents; RAE: rutin equivalents; CAE: catechin equivalents. VAE represents a colorimetric reactivity index of vanillin-reactive flavan-3-ol structures and does not provide definitive molecular identification of the enriched constituents.

In contrast, gambir leaves exhibited significantly higher VAE values (27.46 ± 0.06% CAE) than kalincung (9.84 ± 0.36% CAE; $p < 0.05$), indicating stronger vanillin–HCl reactivity toward flavan-3-ol-type structures in the leaf extract. However, the vanillin–HCl assay is not specific to polymeric proanthocyanidins, as it also responds to monomeric catechins and terminal flavan-3-ol units in short oligomers (Mannino et al., 2021; Racine et al., 2019). Therefore, the higher VAE value observed in the leaf extract should be interpreted as greater vanillin-reactive flavan-3-ol reactivity rather than higher absolute proanthocyanidin content. During kalincung formation, oxidative and fermentative transformations may alter vanillin–HCl reactivity while preserving or redistributing polyphenolic functionality, which may explain the retained antioxidant activity observed despite lower VAE values [34], [35]. This suggests that the biological activity of kalincung may not depend solely on total vanillin-reactive units, but also on the overall composition and activity of retained polar phenolics. α-Glucosidase inhibition-guided fractionation yielded a selected Diaion HP-20 fraction characterized by a higher VAE value (48.33 ± 0.39% CAE) than the corresponding crude extract, supporting enrichment of vanillin–HCl-reactive polar polyphenolic constituents through Diaion HP-20 fractionation. However, because VAE is a reactivity-based index, the increased VAE value should be interpreted as supporting evidence of polyphenolic enrichment rather than direct proof of a specific compound class. Similar enrichment strategies using adsorption resins have also been reported for polyphenol-rich plant materials, supporting the use of resin-based fractionation to concentrate bioactive polar constituents [36], [37].

Antioxidant activity, assessed by the DPPH assay, showed that kalincung exhibited strong radical-scavenging activity (IC_{50} = 9.13 ± 0.05 μg/mL) compared with gambir leaves (10.09 ± 0.02 μg/mL; $p < 0.05$). Despite achieving statistical significance ($p < 0.05$), the magnitude of the difference was relatively small, suggesting comparable antioxidant potency between kalincung and gambir leaves at the extract level. Similar observations have been reported in processed or fermentation-associated polyphenol-rich plant matrices, where antioxidant activity may be retained despite compositional changes during processing [36], [37]. Following extract-level comparisons, further fractionation resulted in enhanced antioxidant potency in the selected Diaion HP-20 fraction (IC_{50} = 8.03 ± 0.07 μg/mL), showing activity within the same order of magnitude as the flavan-3-ol reference compound (+)-catechin (IC_{50} = 5.77 ± 0.05 μg/mL), while gallic acid exhibited the strongest activity (IC_{50} = 3.06 ± 0.02 μg/mL). The antioxidant behavior observed in the selected Diaion HP-20 fraction, with an IC_{50} value comparable to that of the standard flavan-3-ol (+)-catechin and only slightly higher than gallic acid, aligns with previous investigations showing that pure phenolic antioxidants such as catechin and gallic acid exhibit a notably free radical scavenging effect in the DPPH assay in vitro, with low IC_{50} values indicative of potent radical-quenching capacity [38], [39]. Overall, lower IC_{50} values tended to accompany higher phenolic, flavonoid, or VAE-related responses, suggesting that polyphenolic constituents contribute substantially to antioxidant capacity, although VAE alone did not fully explain the antioxidant pattern [40].

3.3. α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity results are presented in Table 3. Among crude extracts, kalincung (IC_{50} = 32.32 ± 1.20 μg/mL) and gambir leaves (IC_{50} = 34.73 ± 0.99 μg/mL) showed comparable α-glucosidase inhibitory activity, with no statistically significant difference between the two crude extracts ($p \geq 0.05$). This indicates that inhibitory constituents are retained in the solid by-product at a level similar to the leaf extract under the present assay conditions. Comparable trends have been observed in other fermented plant products, in which α-glucosidase inhibitory activity

remains detectable after fermentation, suggesting that inhibitory polyphenolic constituents are not completely removed during processing [38], [39].

Table 3. IC_{50} Values for α-Glucosidase Inhibition

Samples	AGI (IC_{50} μg/mL)
Kalincung	32.32 ± 1.20
Ethyl Acetate- Derived Fraction	23.31 ± 0.94
n-Butanol-Derived Fraction	21.13 ± 1.29
Leaves	34.73 ± 0.99
Ethyl Acetate-Derived Fraction	26.39 ± 0.50
n-Butanol-Derived Fraction	22.48 ± 1.79
Selected Diaion HP-20 Fraction	17.07 ± 0.42
(+)-Catechin	14.53 ± 0.37
Acarbose	46.42 ± 1.35

[3]Data represent mean ± SE of three replicates (n = 3). AGI: α-glucosidase Inhibitory Activity.

Solvent fractionation further enhanced inhibitory potency, with butanol fractions consistently exhibiting lower IC_{50} values than their corresponding extracts ($p < 0.05$). The butanol fraction of kalincung showed the strongest inhibition among solvent fractions (IC_{50} = 21.13 ± 1.29 μg/mL), followed closely by the butanol fraction of leaves (22.48 ± 1.79 μg/mL). This pattern aligns with previous reports indicating that α-glucosidase inhibitors are preferentially associated with polar fractions such as n-butanol [16]. Collectively, the findings indicated that the n-butanol fraction of kalincung was selected for further α-glucosidase inhibition-guided fractionation using Diaion HP-20 resin.

Notably, the selected Diaion HP-20 fraction demonstrated further enhancement of α-glucosidase inhibitory activity (IC_{50} = 17.07 ± 0.42 μg/mL), approaching that of (+)-catechin (IC_{50} = 14.53 ± 0.37 μg/mL) and exhibiting significantly stronger inhibition than the synthetic inhibitor acarbose (IC_{50} = 46.42 ± 1.35 μg/mL; $p < 0.05$). Although the selected fraction was not chemically defined in the present study, comparable α-glucosidase inhibitory potency has been reported for flavan-3-ol-related polyphenolic systems, including (+)-catechin and oligomeric procyanidins, depending on composition and degree of polymerization [41], [42]. These results suggest that polar polyphenolic constituents retained in the selected Diaion HP-20 fraction may contribute to enzyme inhibition and that fractionation guided by bioactivity outcomes effectively sharpens functional potency relative to unfractionated samples. Moreover, previous mechanistic studies have attributed the pronounced α-glucosidase inhibitory effects of flavan-3-ol-related polyphenol system to strong polyphenol–enzyme interactions including hydrogen bonding, hydrophobic interaction and stable inhibitor–enzyme complex formation, as evidenced by spectroscopic and molecular docking analyses [41]. In addition, previous studies have demonstrated that flavan-3-ols such as (+)-catechin show considerable enzyme-suppressing properties against α-glucosidase, with inhibitory effects in some studies exceeding those of acarbose under in vitro conditions [43], [44].

Beyond fractionation-driven enrichment, the retained α-glucosidase inhibitory activity observed in kalincung-derived fractions may also be related to compositional changes occurring during moist post-processing storage. In phenolic and flavan-3-ol-related systems, changes in phenolic composition may alter vanillin–HCl reactivity while preserving or enhancing α-glucosidase inhibitory interactions [45], [46]. However, this interpretation remains inferential because microbial composition and definitive structural changes were not examined in the present study.

3.4. Functional Relevance of the Selected Diaion HP-20 Fraction

The selected Diaion HP-20 fraction showed increased VAE value together with improved antioxidant and α-glucosidase inhibitory activities, indicating enrichment of functionally active polar polyphenolic constituents. In this study, the selected fraction is interpreted primarily as a bioactivity-enriched fraction rather than as a chemically defined flavan-3-ol or proanthocyanidin fraction. The

vanillin–HCl assay was used as supporting phytochemical information because it reflects vanillin-reactive flavan-3-ol units, including monomeric catechins and terminal units of oligomeric proanthocyanidins [13], [47]. Therefore, detailed molecular confirmation of the enriched constituents using validated chromatographic or mass spectrometric approaches remains necessary in future studies.

4. CONCLUSION

This study demonstrates that kalincung, a semi-solid by-product of traditional gambir processing, retains a diverse phytochemical profile and exhibits pronounced antioxidant and α-glucosidase inhibitory activities, supporting its functional relevance as a potential source of antioxidant and α-glucosidase inhibitory constituents. At the extract level, kalincung contained higher total phenolic and flavonoid contents than dried gambir leaves, while the leaf extract exhibited higher VAE values, reflecting stronger vanillin-HCl reactivity toward flavan-3-ol-type structures. Importantly, these differences reflect distinct polyphenolic profiles rather than diminished functional potential, as kalincung retained comparable antioxidant and α-glucosidase inhibitory activities despite lower vanillin–HCl reactivity.

Selective fractionation enhanced bioactivity outcomes, indicating that polar polyphenolic constituents play a dominant role in both free radical scavenging and α-glucosidase inhibition. The selected Diaion HP-20 fraction exhibited an increased VAE value relative to the crude kalincung extract and improved antioxidant and α-glucosidase inhibitory activities compared with the corresponding unfractionated extracts. Comparative evaluation against (+)-catechin, used as a well-defined functional reference, further showed that the selected fraction approached catechin-level potency and demonstrated greater inhibitory activity than acarbose under the present in vitro assay conditions. These findings support the use of α-glucosidase inhibition-guided fractionation as a straightforward strategy for early-stage pharmacological evaluation of plant-derived bioactive fractions.

Nevertheless, the present findings should be interpreted within the scope of an in vitro, α-glucosidase inhibition-guided study. Definitive molecular identification of the enriched constituents using validated chromatographic or mass spectrometric approaches, microbial characterization potentially involved in kalincung formation, and in vivo antidiabetic evaluation were not performed. Therefore, the current findings provide an early functional basis for further investigation of kalincung-derived fractions as potential plant-based α-glucosidase inhibitory agents, with antioxidant activity as supporting functional evidence.

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