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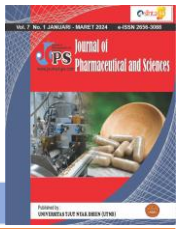

Effect of Vacuum Liquid Chromatography Fractionation on the Phenolic and Flavonoid Contents and α-Glucosidase Inhibitory Activity of Jackfruit (*Artocarpus heterophyllus* Lam.) Leaves

Pengaruh Fraksinasi Kromatografi Cair Vakum terhadap Kandungan Fenolik, Flavonoid, dan Aktivitas Penghambatan α-Glukosidase Daun Nangka (*Artocarpus heterophyllus* Lam.)

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Abstract

Artocarpus heterophyllus Lam leaves are recognized as a promising source of bioactive compounds with potential antidiabetic activity. This study aimed to evaluate the effects of vacuum liquid chromatography (VLC) fractionation on the distribution of phenolic and flavonoid compounds and their α-glucosidase inhibitory activity. Crude ethanolic extract was fractionated by VLC into polar, semipolar, and nonpolar fractions. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using the Folin–Ciocalteu and aluminum chloride colorimetric assays, respectively, while α-glucosidase inhibitory activity was evaluated using a p-nitrophenyl-α-D-glucopyranoside-based assay. The crude extract exhibited the highest TPC (4.70 ± 0.37 mg GAE/g extract) and TFC (5.63 ± 0.11 mg QE/g extract), whereas both parameters decreased markedly after fractionation. Consistently, the crude extract showed the strongest α-glucosidase inhibitory activity, as indicated by the lowest IC_{50} value, while the nonpolar fraction exhibited the weakest activity. These findings demonstrate that VLC fractionation redistributed rather than enhanced the bioactive constituents responsible for α-glucosidase inhibition. The crude extract therefore represents a promising natural source of α-glucosidase inhibitors and supports the potential utilization of *Artocarpus heterophyllus* Lam for the development of plant-derived antidiabetic agents.

Keywords: α-glucosidase inhibition; chromatographic fractionation; phenolic compounds; phytochemicals; vacuum liquid chromatography.

Abstrak

Daun Artocarpus heterophyllus Lam dikenal sebagai sumber senyawa bioaktif yang menjanjikan dengan potensi aktivitas antidiabetes. Studi ini bertujuan untuk mengevaluasi efek fraksinasi kromatografi cair vakum (KCV) pada distribusi senyawa fenolik dan flavonoid serta aktivitas penghambatan α-glukosidase. Ekstrak etanol kasar difraksinasi dengan VLC menjadi fraksi polar, semipolar, dan nonpolar. Kandungan fenolik total (TPC) dan kandungan flavonoid total (TFC) ditentukan menggunakan uji kolorimetri Folin–Ciocalteu dan aluminium klorida, sedangkan aktivitas penghambatan α-glukosidase dievaluasi menggunakan uji berbasis p-nitrophenyl-α-D-glucopyranoside. Ekstrak kasar menunjukkan TPC tertinggi (4,70 ± 0,37 mg GAE/g ekstrak) dan TFC (5,63 ± 0,11 mg QE/g ekstrak), sedangkan kedua parameter tersebut menurun secara signifikan setelah fraksinasi. Secara konsisten, ekstrak kasar menunjukkan aktivitas penghambatan α-glukosidase terkuat, seperti yang ditunjukkan oleh nilai IC_{50} terendah, sedangkan fraksi nonpolar menunjukkan aktivitas terlemah. Temuan ini menunjukkan bahwa fraksinasi KCV mendistribusikan kembali, bukan meningkatkan, konstituen bioaktif yang bertanggung jawab atas penghambatan α-glukosidase. Oleh karena itu, ekstrak kasar merupakan sumber alami penghambat α-glukosidase yang menjanjikan dan mendukung potensi pemanfaatan Artocarpus heterophyllus Lam untuk pengembangan agen antidiabetes yang berasal dari tumbuhan.

Kata Kunci: Penghambatan α-glukosidase; fraksinasi kromatografi; senyawa fenolik; fitokimia; kromatografi cair vakum.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. Despite advances in therapeutic management, the global burden of diabetes continues to increase, particularly for type 2 diabetes mellitus (T2DM), which accounts for approximately 90–95% of all diabetes cases. According to the International Diabetes Federation, the number of adults living with diabetes reached 463 million in 2019 and is projected to increase to 700 million by 2045, emphasizing the urgent need for effective preventive and therapeutic strategies [1], [2]. One of the established therapeutic approaches for controlling postprandial hyperglycemia is the inhibition of intestinal α-glucosidase. This enzyme catalyzes the final step of carbohydrate digestion by hydrolyzing oligosaccharides into absorbable glucose. Consequently, inhibition of α-glucosidase delays glucose absorption and reduces postprandial blood glucose excursions, making this enzyme an important pharmacological target in the management of T2DM [1]. However, currently available α-glucosidase inhibitors, such as acarbose, miglitol, and voglibose, are frequently associated with gastrointestinal adverse effects, encouraging the search for safer bioactive compounds from natural sources.

Among plant-derived bioactive compounds, phenolic compounds and flavonoids have attracted considerable attention because of their ability to inhibit carbohydrate-hydrolyzing enzymes through multiple molecular interactions, including hydrogen bonding and hydrophobic interactions with catalytic amino acid residues [3], [4]. Recent evidence suggests that the α-glucosidase inhibitory activity of many medicinal plants is closely associated with their phenolic and flavonoid composition, although the inhibitory potency depends on the chemical structure and concentration of individual constituents [5], [6]. Therefore, the determination of total phenolic content (TPC) and total flavonoid content (TFC) is not only useful for phytochemical characterization but also provides important information for understanding the contribution of secondary metabolites to α-glucosidase inhibitory activity [7], [8].

Artocarpus heterophyllus Lam. (Moraceae) has been widely recognized as a rich source of phenolic compounds, flavonoids, prenylated flavonoids, stilbenoids, xanthones, and other secondary metabolites with diverse pharmacological properties. Phytochemical investigations have identified bioactive constituents such as artocarpin, morin, quercetin, and related phenolic derivatives in different parts of the plant, including the leaves [9], [10]. Several studies have demonstrated the antidiabetic potential of *Artocarpus heterophyllus* Lam., while Abdulhaniff et al., 2024), further reported that leaf extracts inhibited mammalian maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI), two major α-glucosidase complexes involved in intestinal carbohydrate digestion. These findings suggest that *Artocarpus heterophyllus* Lam. leaves represent a promising source of natural α-glucosidase inhibitors.

The phytochemical composition of plant extracts is strongly influenced by extraction and fractionation procedures. Chromatographic fractionation separates metabolites according to their polarity, leading to differences in the distribution and enrichment of phenolic and flavonoid compounds among fractions [12]–[14]. Such differences may subsequently influence their biological activity, including α-glucosidase inhibition. Although numerous studies have reported either the phytochemical composition or the antidiabetic activity of *Artocarpus heterophyllus* Lam., comparative information regarding total phenolic content, total flavonoid content, and α-glucosidase inhibitory activity among chromatographic fractions of its leaves remains scarce. [10], [13], [15]. Therefore, this study aimed to determine the total phenolic content, total flavonoid content, and α-glucosidase inhibitory activity of the crude extract and chromatographic fractions of *Artocarpus heterophyllus* Lam. leaves. The findings are expected to provide insight into the distribution of bioactive

secondary metabolites following chromatographic fractionation and to identify metabolite-enriched fractions with promising α-glucosidase inhibitory activity, thereby supporting the future development of plant-derived antidiabetic agents.

Materials and Methods

Fresh leaves of Artocarpus heterophyllus Lam. were collected from Semin, Gunungkidul Regency, Special Region of Yogyakarta, Indonesia. Ethanol (70%), methanol, n-hexane, chloroform, ethyl acetate, aluminum chloride, sodium acetate, Folin–Ciocalteu reagent, sodium carbonate, gallic acid, quercetin, acarbose, and a commercial α-glucosidase assay kit (BC2555, Solarbio Science & Technology Co., Ltd., Beijing, China), containing p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate and assay buffers, were used in this study. Crude α-glucosidase enzyme was prepared from rat intestinal mucosa as described below. Absorbance was measured using a microplate reader (BioTek Instruments Inc., Winooski, VT, USA). Solvent evaporation was performed using a rotary vacuum evaporator (DLAB Scientific Co., Beijing, China), while plant materials were dried in a hot-air oven (Memmert GmbH, Schwabach, Germany). Vacuum liquid chromatography (VLC) was carried out using silica gel with a laboratory vacuum manifold. Standard laboratory equipment was used throughout the study.

Preparation of Crude α-Glucosidase Enzyme

Crude α-glucosidase enzyme was prepared from rat intestinal mucosa. The intestinal mucosa was homogenized in cold phosphate buffer under chilled conditions and processed to obtain the crude enzyme extract. The enzymatic activity of the crude extract was initially evaluated by measuring the absorbance of the enzyme control reaction. The same crude enzyme preparation was subsequently used throughout all α-glucosidase inhibitory assays to minimize variability among experiments.

Preparation of Material

Fresh leaves of *Artocarpus heterophyllus* Lam. were collected from Semin, Gunungkidul Regency, Indonesia. The leaves were washed, oven-dried at 55°C to a moisture content below 10%, ground into powder, and stored in airtight containers until extraction.

Extraction of *Artocarpus heterophyllus* Leaves

Powdered leaves were extracted by maceration with 70% ethanol (10:1, solvent-to-solid ratio) for 48 h at room temperature with occasional stirring with slight modifications. The filtrate was concentrated under reduced pressure to obtain the crude extract.

Vacuum Liquid Chromatography Fractionation

The crude ethanolic extract was fractionated by vacuum liquid chromatography (VLC) following the procedure described by [16] with minor modifications. The crude extract (25 g) was fractionated by vacuum liquid chromatography (VLC) according to [16] with minor modifications. The extract was dry-loaded onto silica gel G60 (30–70 mesh) at a 1:2 (w/w) ratio and fractionated using a gradient solvent system optimized by TLC consisting of *n*-hexane, *n*-hexane:ethyl acetate (9:1), *n*-hexane:chloroform (8:2), ethyl acetate:chloroform (1:1), chloroform:methanol (1:1), and methanol. Fractions (200 mL) were monitored by TLC, pooled according to similar chromatographic profiles, and concentrated under reduced pressure for subsequent analyses.

Determination of Total Flavonoid Content

The total flavonoid content (TFC) of the crude extract and chromatographic fractions was determined using the aluminum chloride colorimetric method described by Chatatikun and Chiabchalard with modifications by [17]. Quercetin (5–50 µg/mL) was used for calibration. Sample or standard solution (25 µL) was mixed with 10% $AlCl_3$, absolute ethanol, and sodium acetate, incubated for 40 min, and the absorbance was measured at 415 nm. TFC was calculated from the calibration curve using Equation (1) and expressed as mg quercetin equivalent per gram of extract (mg QE/g extract).

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$$\text{TFC} = \frac{C \times V \times DF}{m}$$

where:
C = concentration obtained from the quercetin calibration curve (mg/mL)
V = volume of extract solution (mL)
DF = dilution factor (if applicable)
m = weight of the extract used in the assay (g)

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric assay according to [17] with minor modifications. The total flavonoid content was determined using the aluminum chloride colorimetric assay according to [17]. Quercetin (5–50 µg/mL) was used for calibration. Sample or standard solution (25 µL) was mixed with 10% $AlCl_3$, absolute ethanol, and sodium acetate, incubated for 40 min, and the absorbance was measured at 415 nm. TFC was calculated from the calibration curve using Equation (1) and expressed as mg quercetin equivalent per gram of extract (mg QE/g extract).

$$\text{TPC} = \frac{C \times V \times DF}{m}$$

where:
C = concentration obtained from the quercetin calibration curve (mg/mL)
V = volume of extract solution (mL)
DF = dilution factor (if applicable)
m = weight of the extract used in the assay (g)

Preparation of Crude α-Glucosidase Enzyme

Crude α-glucosidase enzyme was prepared from the small intestinal mucosa of male Wistar rats following the procedure described [6] with slight modifications. The mucosa was homogenized in sodium phosphate buffer (100 mM, pH 6.8), stored at 4°C for 24 h, centrifuged (13,500 rpm, 25 min, 4°C), and the supernatant was collected as the enzyme source.

α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory activity of the crude extract and VLC fractions was evaluated using a commercial assay kit (BC2555, Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions with slight modifications. Serial dilutions of the extract and fractions (31.25–500 ppm) and acarbose (0.625–10 ppm) were prepared. Following incubation with crude α-glucosidase and *p*-nitrophenyl-α-D-glucopyranoside (pNPG), absorbance was measured at 400 nm using a microplate reader. Percentage inhibition was calculated using Equation (3), and IC_{50} values were determined by nonlinear regression analysis of the concentration–response curves. All measurements were performed in triplicate.

$$\%\text{Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

where Acontrol is the absorbance of the control reaction without inhibitor, and Asample is the absorbance of the reaction in the presence of the sample or acarbose [18], [19].

Results and Discussion

The crude extract and vacuum liquid chromatography (VLC) fractions of Artocarpus heterophyllus Lam. leaves were comparatively evaluated to determine how fractionation influences the distribution of phenolic and flavonoid compounds and their α-glucosidase inhibitory activity. Quantitative determination of total phenolic content (TPC), total flavonoid content (TFC), and enzyme inhibition was performed using validated analytical methods. The findings provide insight into the relationship between phytochemical composition and the antidiabetic potential of different fractions.

Determination of Total Phenolic and Total Flavonoid Contents

Reliable quantification of total phenolic and flavonoid contents depends on the linearity of the analytical method. Therefore, calibration curves were established using gallic acid and quercetin as reference standards prior to sample analysis. The calibration curves exhibited excellent linearity over the investigated concentration range, with coefficients of determination (R^2) exceeding 0.95 for both gallic acid and quercetin

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(Figure 1). These results indicate that the analytical method was suitable for the quantitative determination of TPC and TFC in the crude extract and VLC fractions.

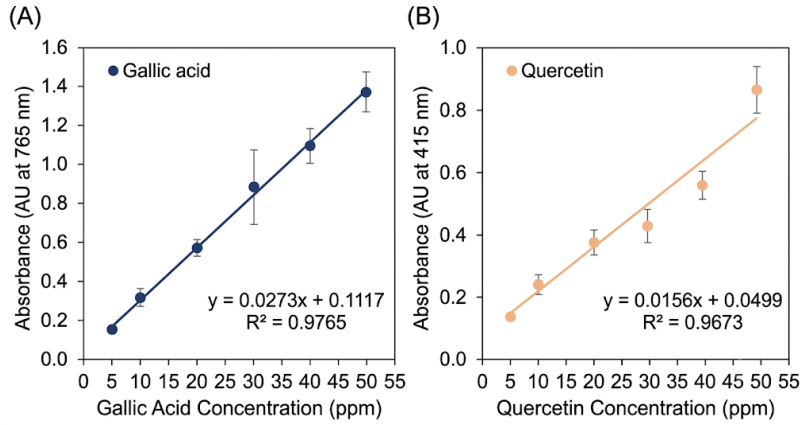

Figure 1. Calibration curves of (A) gallic acid for total phenolic content determination and (B) quercetin for total flavonoid content determination.

Calibration curves constructed using gallic acid and quercetin standards demonstrated satisfactory linear relationships within the working concentration range of 5–50 ppm (Figure 1). The gallic acid standard produced the regression equation $y = 0.0273x + 0.1117$ ($R^2 = 0.9765$), while the quercetin standard yielded $y = 0.0156x + 0.0499$ ($R^2 = 0.9673$). These calibration curves were subsequently employed for the quantification of total phenolic content (TPC) and total flavonoid content (TFC) in the crude extract and VLC fractions of *Artocarpus heterophyllus* Lam.

Following validation of the calibration curves, the total phenolic content (TPC) and total flavonoid content (TFC) of the crude extract and vacuum liquid chromatography (VLC) fractions of Artocarpus heterophyllus Lam. leaves were quantitatively determined using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. The quantitative results are summarized in Table 1, while the distribution patterns of both phytochemical groups among the crude extract and VLC fractions are illustrated in Figure 2.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of the crude extract and VLC fractions of *Artocarpus heterophyllus* Lam. leaves.

Sample	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Crude extract	4.70 ± 0.37	5.63 ± 0.11
Polar fraction	4.36 ± 0.33	2.30 ± 0.22
Semipolar fraction	0.92 ± 0.12	0.38 ± 0.11
Nonpolar fraction	0.63 ± 0.03	0.18 ± 0.04

Values are presented as mean ± SD (n = 3).

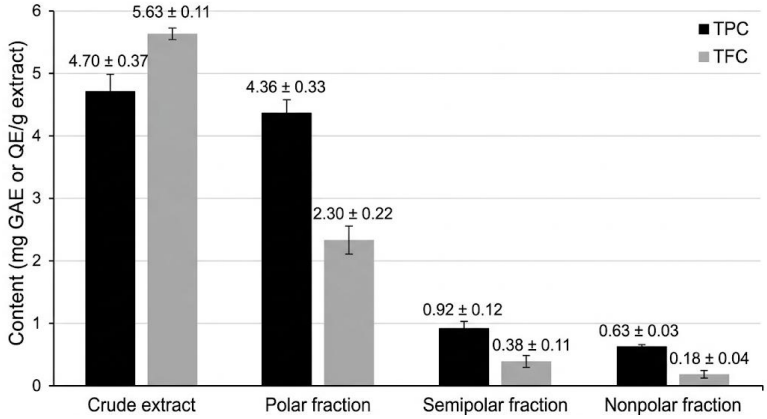

Figure 3. Comparison of total phenolic content (TPC) and total flavonoid content (TFC) in the crude extract and VLC fractions of *Artocarpus heterophyllus* Lam. leaves (mean ± SD, n = 3).

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As shown in **Table 1** and **Figure 2**, the crude extract exhibited the highest total flavonoid content (5.63 ± 0.11 mg QE/g extract) and total phenolic content (4.70 ± 0.37 mg GAE/g extract). Following vacuum liquid chromatography (VLC) fractionation, both phytochemical groups showed a marked decrease in all fractions. Among the obtained fractions, the polar fraction retained relatively high levels of phenolics (4.36 ± 0.33 mg GAE/g extract) and flavonoids (2.30 ± 0.22 mg QE/g extract), whereas the semipolar and nonpolar fractions contained considerably lower amounts of both compounds.

The higher TPC and TFC observed in the crude extract indicate that ethanol extraction was able to recover a broad spectrum of phytochemicals with different polarities. In contrast, VLC fractionation redistributed these metabolites according to their affinity toward the stationary phase and solvent polarity [20], [21]. Consequently, phenolic and flavonoid compounds were concentrated predominantly in the polar fraction, whereas substantially lower levels were detected in the semipolar and nonpolar fractions [22]. This finding is consistent with the predominantly polar nature of phenolic hydroxyl groups, which exhibit greater solubility in polar solvents [23], [24].

Similar distribution patterns have been reported in previous phytochemical studies, where polar fractions generally contain higher concentrations of phenolic and flavonoid compounds than less polar fractions [25]. The predominance of these metabolites in the polar fraction observed in the present study can be attributed to the presence of multiple hydroxyl groups within phenolic and flavonoid structures, which increase their polarity and affinity toward polar extraction systems through hydrogen-bond interactions [26]. Consequently, the polar fraction retained considerably higher TPC and TFC than the semipolar and nonpolar fractions, although both remained lower than those of the crude extract.

The quantification of flavonoids in this study was performed using the aluminum chloride colorimetric assay, in which aluminum ions form stable complexes with the carbonyl and hydroxyl groups of flavonoid molecules, producing a yellow chromophore whose absorbance is proportional to flavonoid concentration [8]. Likewise, total phenolic content was determined using the Folin–Ciocalteu assay, which is based on the reduction of phosphomolybdic–phosphotungstic complexes by phenolic compounds under alkaline conditions, resulting in the formation of a blue-colored complex that can be quantified spectrophotometrically [27]–[29]. These complementary colorimetric methods are widely applied for comparative phytochemical evaluation because they provide rapid and reproducible estimation of total flavonoid and phenolic contents in plant extracts.

The present findings are generally consistent with previous phytochemical studies demonstrating that *Artocarpus heterophyllus* leaves are a rich source of phenolic and flavonoid compounds. Previous studies have also shown that higher total phenolic and flavonoid contents are associated with stronger enzyme inhibitory activity, indicating that these phytochemicals contribute substantially to the biological potential of the plant [30]. Furthermore, considerable variation in total phenolic content (TPC) and total flavonoid content (TFC) has been reported among jackfruit leaf extracts, reflecting the influence of geographical origin, extraction procedures, and experimental conditions on phytochemical composition [31]. In addition, phytochemical investigations have identified flavonoids, phenolic acids, prenylated flavonoids, and other polyphenolic constituents as the major secondary metabolites responsible for the diverse pharmacological properties of *Artocarpus heterophyllus* [32]. In the present study, although vacuum liquid chromatography redistributed these metabolites into fractions of different polarity, the crude extract retained the highest TPC and TFC, suggesting that the fractionation process separated rather than selectively concentrated these phytochemical classes.

α-Glucosidase Inhibitory Activity

The *α*-glucosidase inhibitory activity of the crude extract and VLC fractions of *Artocarpus heterophyllus* Lam. leaves was evaluated using *p*-nitrophenyl-*α*-D-glucopyranoside (pNPG) as the substrate. Prior to the inhibition assay, a *p*-nitrophenol (pNP) calibration curve was established to quantify the reaction product generated during enzymatic hydrolysis and to enable the calculation of percentage inhibition.

The *α*-glucosidase inhibitory activity was determined using *p*-nitrophenyl-*α*-D-glucopyranoside (pNPG) as the substrate. Prior to the inhibition assay, a *p*-nitrophenol (pNP) calibration curve was established to convert absorbance values into the amount of pNP released during the enzymatic reaction, thereby enabling quantitative determination of enzyme inhibition (Figure 4) [33], [34]. The pNP calibration curve exhibited a linear relationship between absorbance and pNP concentration over the tested range, indicating that the analytical method was suitable for quantitative determination of *α*-glucosidase activity. The regression

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equation obtained from this calibration curve was subsequently applied to calculate pNP formation and the percentage inhibition of each sample.

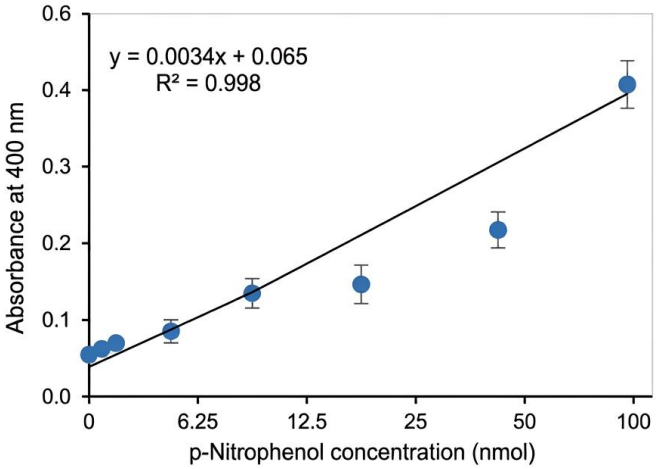

Figure 4. Calibration curve of *p*-nitrophenol (pNP) used for quantification of α-glucosidase activity.

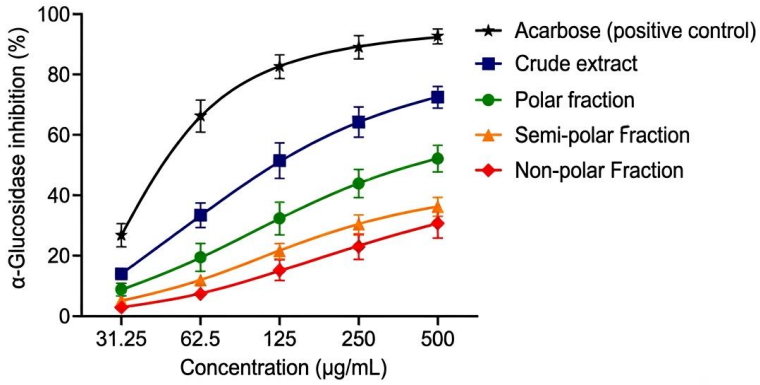

Figure 5. Concentration-dependent α-glucosidase inhibitory activity of the crude extract, VLC fractions, and acarbose. Data are presented as mean ± SD (n = 3).

The concentration–response curves of the crude extract, VLC fractions, and acarbose are presented in **Figure 5**. All samples exhibited concentration-dependent α-glucosidase inhibition, with increasing concentrations resulting in higher percentages of inhibition. However, the inhibitory responses varied among the tested samples, indicating differences in their biological potency. This trend suggests that higher concentrations of bioactive constituents provide greater inhibition of α-glucosidase, which is consistent with previous reports demonstrating the inhibitory potential of phenolic-rich plant extracts against carbohydrate-hydrolyzing enzymes [35]. Furthermore, the observed activity supports the antidiabetic potential of *Artocarpus heterophyllus* leaves, which have previously been reported to possess significant α-glucosidase inhibitory activity attributable to their phytochemical constituents [36]. To further compare the inhibitory potency among the tested samples, the IC_{50} values were determined from the concentration–response curves and are presented in Figure 6.

As shown in Figure 6, the crude extract exhibited the lowest IC_{50} value among the tested samples, indicating the strongest α-glucosidase inhibitory activity, whereas the non-polar fraction showed the highest IC_{50} value and therefore the weakest inhibitory potency. The polar and semi-polar fractions displayed intermediate activities, suggesting that VLC fractionation redistributed the bioactive constituents without improving the inhibitory potency of the crude extract. This finding suggests that VLC fractionation redistributed rather than concentrated the bioactive constituents responsible for enzyme inhibition. The superior activity of the crude extract may be attributed to the preservation of a broader spectrum of phytochemicals and the synergistic interactions among multiple metabolites, which can enhance biological activity beyond that of individual isolated fractions [37], [38]. Similar observations have also been reported for phenolic-rich fractions exhibiting α-glucosidase inhibitory activity, where enzyme inhibition depends not

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only on the abundance of phenolic compounds but also on their chemical composition and interactions [39]. Furthermore, solvent extraction has been reported to markedly influence phytochemical recovery and consequently the biological activity of plant extracts, supporting the superior inhibitory activity observed in the crude extract compared with the VLC fractions [40]. Overall, the results indicate that maintaining the natural phytochemical composition of the crude extract provides greater α-glucosidase inhibitory activity than VLC fractionation, highlighting the contribution of metabolite diversity and synergistic interactions to the observed bioactivity [38], [40].

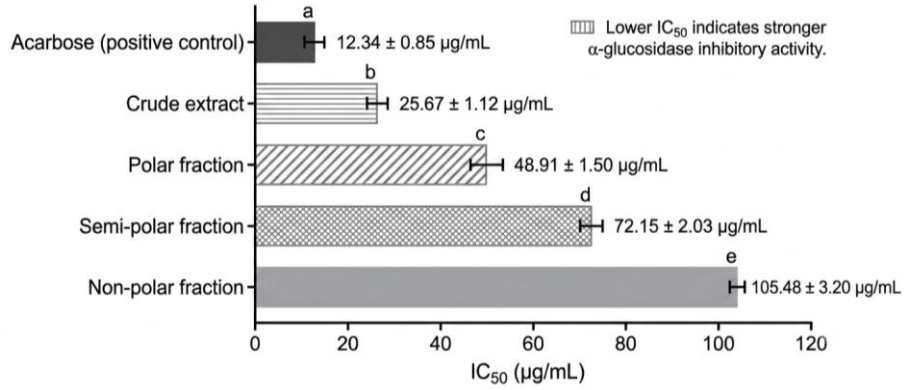

Figure 6. IC_{50} values of the crude extract, VLC fractions, and acarbose for α-glucosidase inhibition. Data are presented as mean ± SD (n = 3).

Conclusions

Vacuum liquid chromatography fractionation influenced the phytochemical composition and α-glucosidase inhibitory activity of Artocarpus heterophyllus Lam. leaves. The crude extract showed the highest phenolic and flavonoid contents and exhibited the strongest α-glucosidase inhibitory activity, indicating that fractionation redistributed rather than enhanced the bioactive constituents. These findings support the potential of the crude extract as a promising natural source of α-glucosidase inhibitors.

Conflict of Interest

The authors declare that there are no financial or non-financial conflicts of interest regarding the publication of this study.

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