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Research Article

# PURIFICATION OF A PROTEIN-BASED α-GLUCOSIDASE INHIBITOR FROM *Aspergillus niger* VTCC 031

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## ABSTRACT

Alpha-glucosidase inhibitors, such as acarbose, voglibose, and miglitol, constitute a prevalent class of pharmaceuticals employed in the clinical management of diabetes. These inhibitors originate mostly from microorganisms, including *Aspergillus* sp., *Bacillus* sp., and *Actinoplanes* sp., representing a promising natural resource for identifying α-glucosidase inhibitors (AGIs) that offer enhanced therapeutic efficacy with reduced adverse effects. *Aspergillus* sp. is a fungus that has a rich array of secondary metabolites and produces several AGIs. In this work, the *A. niger* VTCC 031 demonstrated the capacity to manufacture compounds with significant α-glucosidase inhibitory activity, achieving a rate of 67.24%. The aqueous phase, obtained after ethyl acetate extraction, was purified using a Sephadex G100 column followed by a 30 kDa cut-off column, resulting in the purification of the α-glucosidase inhibitor characterized by low molecular weight protein properties. The SDS-PAGE chromatogram indicates that the active ingredient had a molecular weight of 14 kDa, with α-glucosidase inhibitory activity of 80.2% and a recovery efficiency of 0.02%. This study provides the scientific foundation for ongoing research into optimal conditions for the extraction and purification of the α-glucosidase inhibitor derived from the *A. niger* strain, a metabolically active fungus found in Vietnam.

**Keywords:** *Aspergillus niger* VTCC 031, α-glucosidase inhibitor, chromatography.

## INTRODUCTION

In recent years, the incidence of diabetes mellitus, a chronic metabolic disorder, has increased rapidly, making this disease a global health problem and an economic burden for many countries around the world. The International Diabetes Federation (IDF)

report estimates that 537 million adults worldwide had diabetes in 2021, with a projected increase to 783 million by 2045 (International Diabetes Federation, 2021; American Diabetes Association, 2020; Holt *et al.,* 2004). Diabetes has been considered a non-communicable disease pandemic with the third highest mortality rate after cancer and cardiovascular diseases. According to recent studies, diabetes is considered one of the factors that not only increases the risk of sepsis but also promotes rapid progression and a poor prognosis in COVID-19 patients.

Clinical practitioners widely use α-glucosidase inhibitors (AGIs) to treat type 2 diabetes, aiming to reduce postprandial blood glucose, protect patients from cardiovascular complications, and thereby reduce mortality in diabetic patients due to this dangerous complication. While traditional AGIs such as acarbose and voglibose effectively regulate postprandial blood glucose levels, their adverse effects slightly restrict their clinical use. Therefore, in recent decades, scientists have intensified their efforts to discover new AGIs with both beneficial therapeutic effects and minimal unwanted side effects. In recent years, there has been a global trend, including in Vietnam, toward developing biologically derived hypoglycemic agents, especially those biosynthesized from fungi and bacteria. Among potential microorganisms, *Aspergillus* fungus is one of the microorganisms being investigated for the manufacture of α-glucosidase enzyme inhibitors for diabetes therapy (Dewi *et al.,* 2016). Nonetheless, research on α-glucosidase enzyme inhibitors derived from *Aspergillus* strains in Vietnam remains rather scarce. This study investigates the extraction and purification of a low molecular weight α-glucosidase inhibitory peptide from *A. niger* VTCC 031, a fungal strain isolated in Vietnam, aiming to identify potential AGI candidates for future antidiabetic drug development. Despite promising AGI-producing *Aspergillus* strains reported in other countries, no previous study has examined the potential of *A. niger* VTCC 031 in Vietnam. Our work contributes new data on this strain and supports the discovery of safer, biologically derived antidiabetic agents.

Endophytic fungi, particularly *Aspergillus* spp., are well-documented sources of bioactive secondary metabolites, including potential AGIs. Over the past decades, several AGI compounds have been isolated from *Aspergillus* species, exhibiting diverse chemical structures such as butenolides, peptides, and alkaloids. For example, Dewi *et al.* (2015) reported that butyrolactone I, isolated from *Aspergillus terreus*, exhibited α-glucosidase inhibitory activity with an $IC_{50}$ of 52.17 ± 5.68 μmol/L (Dewi *et al.,* 2015). In 2013, an α-glucosidase inhibitor was generated from *Aspergillus oryzae* N159-1, isolated from traditional Korean fermented foods. After cultivating the fungus in soybean broth for 5 days at 27°C, the inhibitor concentration reached its highest level. The inhibitor underwent purification using ultrafiltration, Sephadex G25 gel permeation chromatography, strong cation exchange solid phase extraction, reversed phase HPLC, and size exclusion chromatography. The cell-free extract from *A. oryzae* N159-1 exhibited the greatest AGI activity at 48.3%. The final recovery of the purifying procedure was 1.9% (Kang *et al.,* 2013). Singh and Kaur *et al.* (2016) from the *Aspergillus awamori* strain found a peptide that could prevent both α-amylase (81%) and α-glucosidase (80%) from working in 2016. The inhibitor was purified via a Sephadex

LH20 column and HPLC. The $IC_{50}$ values of 3.75 and 5.625 µg/mL were used to measure the inhibitory activity against α-amylase and α-glucosidase. Although this peptide had a low $IC_{50}$ value, it was stable under low pH and extreme temperature conditions and was non-reactive. As a result, it could be stable at low stomach pH, which had the potential to be effective in the treatment of diabetes and enhanced its commercial potential (Singh and Kaur, 2016). In 2019, the endophytic fungus *Aspergillus* sp. 16-5c from mangrove forests identified six novel diketopiperazine alkaloids, named aspergiamide A-F, along with 10 established counterparts. The test findings suggested that the chemical aspergiamide A-F inhibited α-glucosidase with $IC_{50}$ values of 18.2 and 7.6 µM (Ye *et al.*, 2021). These studies highlight the potential of *Aspergillus* species as AGI producers. However, there is still a lack of data on native fungal strains in Vietnam. This study addresses this gap by investigating the AGI production capacity of *A. niger* VTCC 031, aiming to purify and evaluate a protein-based inhibitor with potential clinical relevance. In 2020, *Aspergillus fumigatus* SWZ01 was isolated from marine sediments in Shenzhen, Guangdong, China. Three compounds were isolated from fermentation and purification. A new compound, 2,6'-dihydroxy-2,4'dimethoxy-8'-methyl-6-methoxy-acyl-ethyl-diphenylmethanone (1), and two known compounds, fumigaclavine C (2) and alternarosin A (3), were obtained, which were characterized by UV, IR, 1D, 2D-NMR, and MS data. The α-glucosidase inhibitory activities of these compounds were evaluated to be stronger than acarbose (Liu *et al.,* 2021).

While several AGIs have been isolated from *Aspergillus* species worldwide, studies on local fungal resources in Vietnam remain limited. In particular, the strain *A. niger* VTCC 031 has not been previously investigated for its potential to produce bioactive AGIs. Therefore, this study aims to purify and characterize a protein-based α-glucosidase inhibitor by the extraction solvents to sellect with the best ability to separate the active ingredients of AGIs, and then the gel filtration chromatography and cut-off column methods, contributing novel insights into indigenous fungal strains and supporting the development of safer, biologically derived antidiabetic agents.

## MATERIALS AND METHODS

### Microorganism strain and culture media

The *Aspergillus niger* VTCC 031 was purchased from the Vietnam Type Culture Collection (VTCC). The strain was activated in PDA agar plate medium for 72 hours at 30°C and pH 7.0.

### Chemical reagent

Acarbose, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), and α-glucosidase (Intestinal acetone powders from rat) were purchased from Sigma (Louis, USA). Thin layer of silica gel F254 was procured from Merck. Sephadex G25, Sephadex G100, and Sephacryl S200 were purchased from Sweden. All other chemical is analytical grade, otherwise stated.

### Culture media

PDA activation medium: Potato starch 20%, D-glucose 2%, agar 2%, pH 7.0. Fungal culture medium: D-glucose 5%, malt extract 1%, pH 5, 37°C, shaking at 200 rpm, and culture for 7 days. Adjust the pH of the medium with 0.1 M HCl and 0.1 M NaOH.

## Methods

### *Culture and fermentation*

The *Aspergillus niger* VTCC 031 was activated in a PDA medium for 72 hours at 30˚C and pH 7. Then, this strain was inoculated from the agar plate into a conical flask containing 5% D-glucose, 1% malt extract, pH 5.0, 37˚C, shaken at 200 rpm, and cultured for 7 days. The fermentation medium experienced centrifugation at 8000 rpm for 10 minutes at 4°C, and the fermentation broth was obtained.

### *α-glucosidase inhibitory activity assay*

The α-glucosidase inhibitory activity was determined based on the method described by Kim *et al.* (2004), with slight modifications. Briefly, 10 µL of the test sample and 40 µL of 0.1 M sodium phosphate buffer (pH 6.9) were added to each well of a 96-well plate, followed by 100 µL of α-glucosidase solution (1–2 IU/mL in 0.1 M sodium phosphate buffer, pH 6.9). After incubation at 30°C for 10 minutes with shaking at 300 rpm, 50 µL of p-nitrophenyl-α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 6.9) was added to initiate the reaction. The mixture was incubated at 37°C for 5 minutes, and the absorbance was measured at 405 nm. Control wells received buffer or pre-culture medium instead of the test sample. All measurements were performed in triplicate, and inhibitory activity was expressed as the percentage reduction in absorbance compared to the control. The percentage of α-glucosidase inhibition is calculated using the following formula:

$$\%\,Inhibition\ activity = \frac{\Delta Ac - \Delta As}{\Delta Ac}\ x\ 100\%$$

In which:

$\Delta Ac$: the change in $OD_{405nm}$ value at 0 minutes and 5 minutes of the control.

$\Delta As$: the change in $OD_{405nm}$ value at 0 minutes and 5 minutes of the experimental sample.

For the solvent phase, when measuring $OD_{405nm}$, the solvent must be evaporated using a rotary evaporator at 60°C with a rotation speed of 65 rpm, the residue is collected, the residue is dissolved in DMSO, and the α-glucosidase inhibitory activity is determined.

### *Selection of extraction solvents to collect AGI active ingredients*

After obtaining the fermentation broth, the experiment was conducted to extract the extracellular fluid with organic solvents of different polarities to investigate and select solvents with the best ability to separate the active ingredients of AGIs.

The study used solvents with decreasing polarities, specifically n-butanol, ethyl acetate, chloroform, dichloromethane, and n-hexane, with a raw material solvent ratio of 1:1. These solvents were extracted three times at room temperature using a shaker for two hours, resulting in the extraction of the solvent phase and the aqueous phase. To extract active ingredients of a protein nature, the aqueous phase was used in this study to determine the α-glucosidase inhibitory activity (Figure 1).

***Purification of AGI active ingredient by gel filtration chromatography***

Gel filtration chromatography separates molecules based on differences in molecular size and mass (Duong-Ly and Gabelli, 2014). The aqueous phase obtained after extraction with ethyl acetate solvent was examined through 3 chromatographic columns: Sephacryl S200, Sephadex G100, and Sephadex G25. The columns were equilibrated with 20 mM sodium phosphate buffer, pH 7.5, and pushed with 50 mM sodium phosphate buffer, pH 7.5. Five mL of sample was applied to the columns Sephacryl S200, Sephadex G100, and Sephadex G25 (2.6 cm × 26 cm), the flow rate was about 25 mL/hour, the volume of each fraction was 1.5 mL, and 12 fractions were collected.

After purification via the aforementioned three columns, the fractions were analyzed spectrophotometrically to assess α-glucosidase inhibitory activity, and thin layer chromatography (TLC) was conducted to evaluate the purification efficacy of the fractions. Figure 1 illustrates the study's scheme.

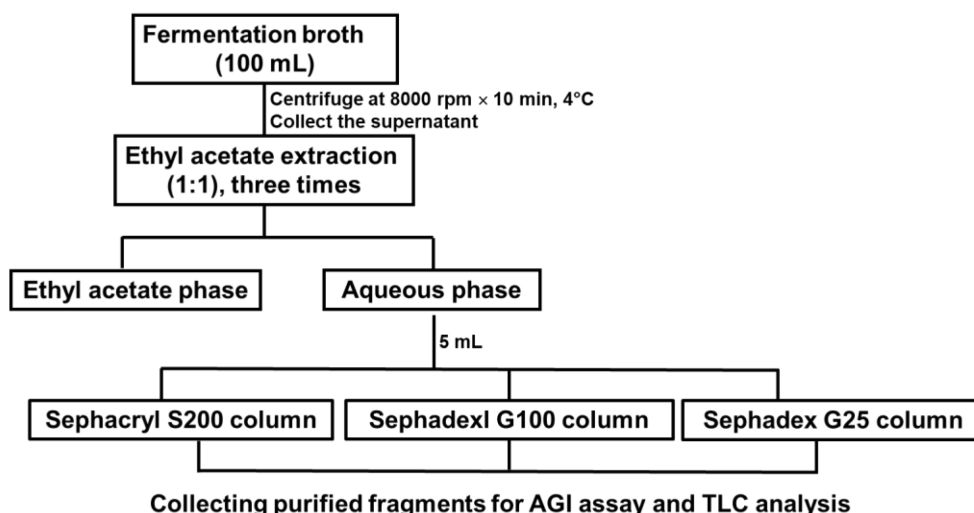


**Figure 1.** Investigation of AGIs' initial purification from the aqueous phase.

***Purity assessment by thin layer chromatography***

TLC is a solid-liquid separation technique. The thin layer plate is a stationary phase, consisting of a sheer layer of gel (usually silica gel) about 0.1 – 0.2 mm thick coated on a glass or aluminum surface. The solvent system is the mobile phase chosen to separate the sample injected into the gel (Santiago and Strobel, 2013).

Following the gel filtration chromatography column, the eluate fragments were subjected to TLC to determine their purity. The solvent used for this procedure was isopropanol : acetic acid : water = 4:1:1. Visualization was carried out by development with 2% ninhydrin.

***Purification by cut-off column***

Employ a 30 kDa cut-off column to isolate the active component, AGIs. Subsequent to elution from the Sephadex G100 column,

transfer the highly active fraction to the 30 kDa cut-off column, centrifuge at 4000 rpm for 15 minutes, and collect the solution passing through the filter membrane (molecular weight was under 30 kDa). The upper phase containing proteins with a molecular weight of greater than or equal to 30 kDa was eliminated from the combination. The product is then assessed for α-glucosidase inhibitory activity and analyzed by electrophoresis on an SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel.

***SDS-PAGE analysis***

The eluted protein was analyzed by SDS-PAGE. The fractions containing protein were pooled and concentrated by ultrafiltration. SDS–PAGE was performed in a 12.5% gel on a Mini-II apparatus (Bio-Rad Laboratories, Hercules, CA, USA) according to the method of Sambrook (Sambrook and Russell, 2006). After electrophoresis, the gel was stained and visualized by silver staining (Bassam *et al.*, 1991). Protein concentration was measured using the Bradford assay with crystalline bovine serum albumin as the standard (Bradford, 1976).

***Data analysis method***

The results were measured using an ELISA reader at a wavelength of 405 nm. The experiments were repeated three times independently, and the results were presented as average values; the results were processed and illustrated with Microsoft Excel 2016 software.

## RESULTS

### Cultivation and biosynthesis of AGIs from *A. niger* VTCC 031

The *A. niger* VTCC 031 was activated and cultured according to the method described above. After the culture period, the centrifuged fermentation broth was determined for α-glucosidase inhibitory activity, the results are shown in Figure 2.

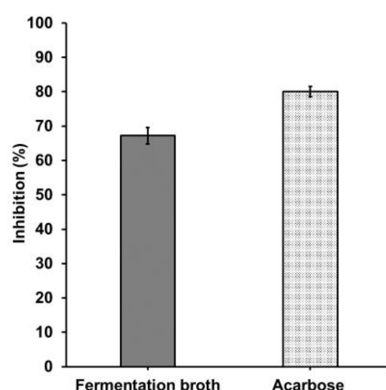


**Figure 2.** Results of determination of α-glucosidase inhibitory activity of fermentation broth and acarbose (Sigma) with concentration of 0.01 mg/mL.

Figure 2 indicated that under cultivated conditions, *A. niger* VTCC 031 produced active compounds that inhibit α-glucosidase, with the inhibitory activity reaching 67.24% compared with 80.03% of the standard acarbose control (10 μg/mL) (Figure 2). This outcome enables us to advance to the next extraction and purification procedures.

### Extraction of active AGIs from *A. niger* VTCC 031

Among the five solvents tested for liquid-liquid extraction of the fermentation broth, ethyl acetate yielded the highest α-glucosidase inhibitory activity in the aqueous phase, followed by n-butanol and chloroform, while dichloromethane and n-hexane showed minimal activity (Figure 3). This trend suggests that moderately polar solvents are more effective in extracting AGI-active compounds from the culture medium of *A. niger* VTCC 031. These results were used to select ethyl acetate as the optimal solvent for subsequent purification steps.

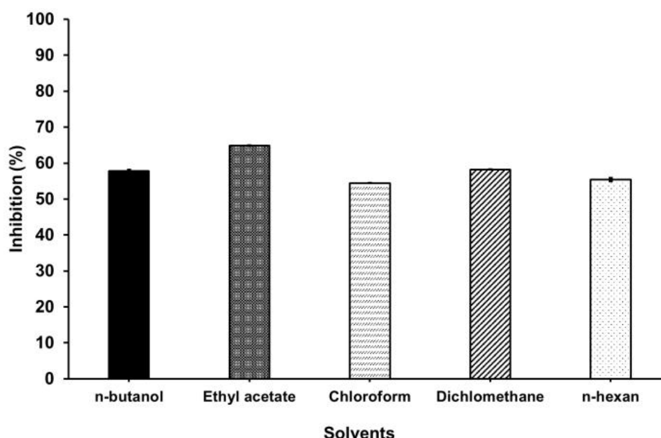


**Figure 3.** α-glucosidase inhibition results of aqueous phases after liquid-liquid extraction with different solvents.

Figure 3 illustrates that among the evaluated solvents, the aqueous phase after extraction with ethyl acetate had the highest efficiency, achieving an α-glucosidase inhibitory activity of 64.85%, whereas extractions with other solvents yielded inhibitory activities ranging from 54.44% to 58.18%. Based on the results of the solvent screening, ethyl acetate was selected as the extraction solvent for all subsequent steps, particularly for the aqueous phase. The process then proceeded to the purification stage to identify the active components—AGIs—produced by the *A. niger* strain.

### Purification of AGI active ingredients from *A. niger* VTCC 031

The fractions after passing through three columns of Sephacryl S200, Sephadex G100, and Sephadex G25 were measured spectrophotometrically to determine α-glucosidase inhibitory activity. The results are presented in Figure 4 to Figure 6.

The graphs in Figures 4A, 5A, and 6A demonstrate that fractions from 5 to 10 exhibited α-glucosidase inhibitory activity after examining three columns of varying sizes. The fractions showing significant activity were fractions 6 to 8.

Results from TLC analysis of the purified fraction revealed distinct spots after ninhydrin staining. Specifically, pink and purple spots were observed, suggesting that the α-glucosidase inhibitory compounds present in the purified fraction were likely peptides or low-molecular-weight proteins. These spots exhibited Rf values of 0.31 (purple spot) and 0.6 (pink spot),

respectively. Notably, these spots were most clearly separated after purification using a Sephadex G-100 column, demonstrating superior resolution compared to both Sephacryl S200 and Sephadex G25 columns.

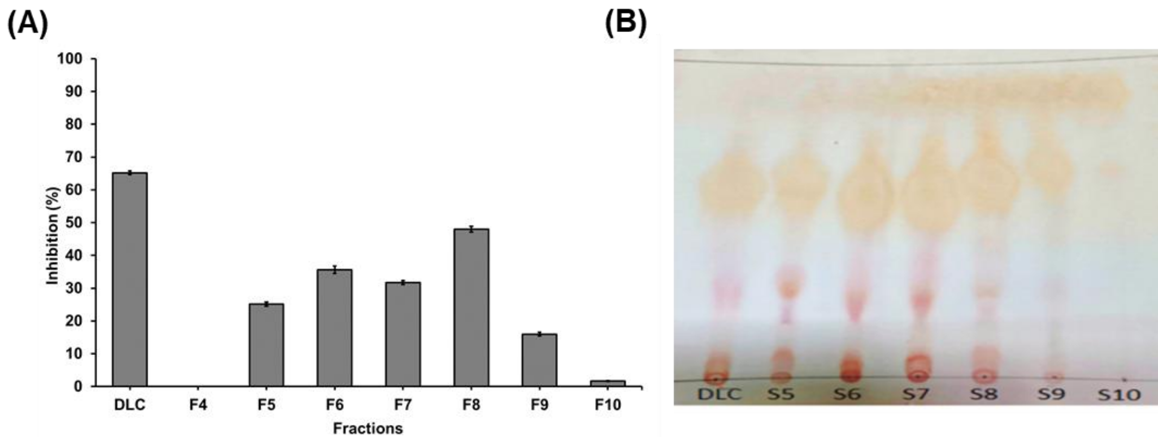


**Figure 4.** AGI activity of fractions 4-10 after being purified by the Sephacryl S200 column (A) and thin layer chromatography to evaluate the separation of the AGI-active fractions after passing through the Sephacryl S200 column (B). DLC: Aqueous phase; S5-S10: active fractions of Sephacryl S-200 column purification.

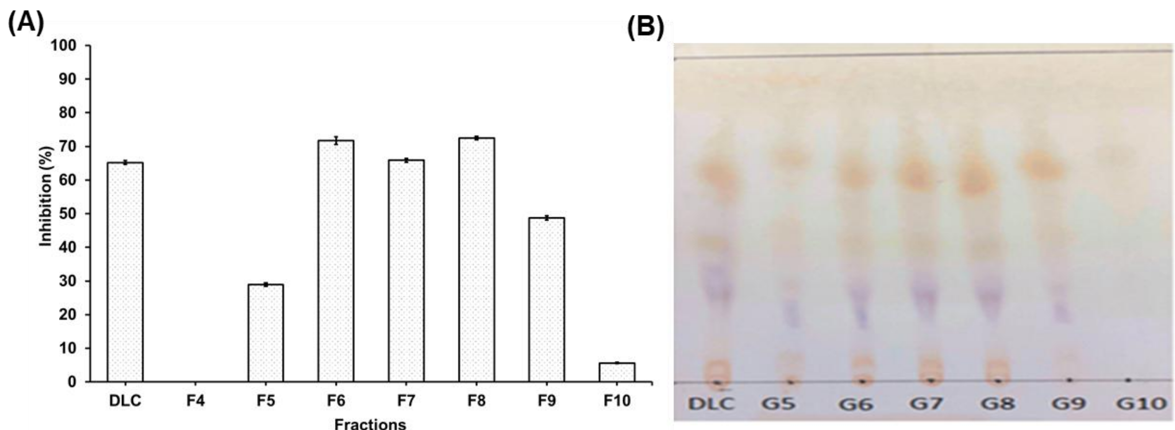


**Figure 5.** AGI activity of fractions 4-10 after being purified by the Sephadex G100 column (A) and thin layer chromatography to evaluate the separation of the AGI-active fractions after passing through the Sephadex G100 column (B). (DLC: Aqueous phase; G5-G10: active fractions of Sephadex G100 column purification.

The outcomes of TLC in conjunction with the activity measurement results of the fractions, following the examination of three columns of varying dimensions, indicated that the Sephadex G100 column effectively separated contaminants, resulting in more distinct and concentrated spots compared to the Sephacryl S200 and Sephadex G25 columns (Figure 4B, Figure 5B, and Figure 6B). The maximum inhibitory activity of α-glucosidase was observed, with a value exceeding 70% (Figure 5B).

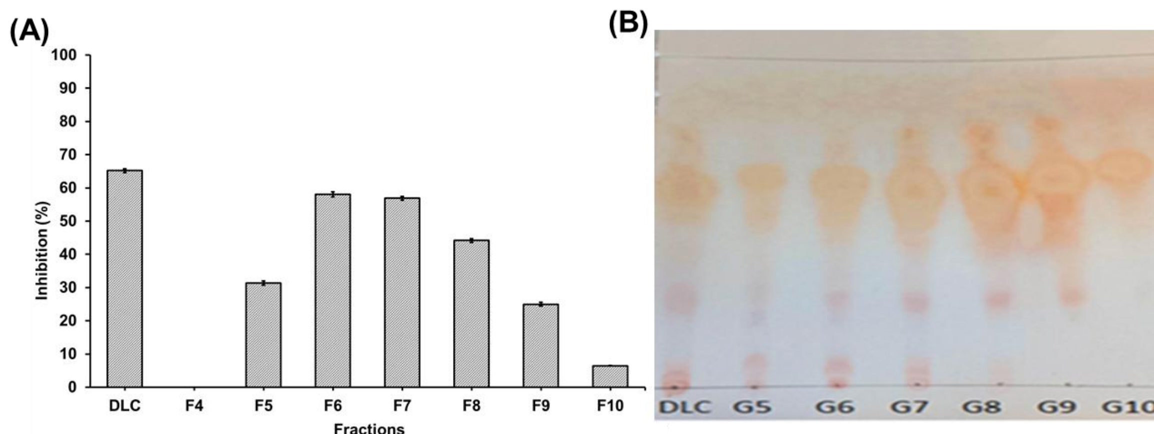


**Figure 6.** AGI activity of fractions 4-10 after being purified by the Sephadex G25 column (A) and thin layer chromatography to evaluate the separation of the AGI-active fractions after passing through the Sephadex G25 column (B). DLC: Aqueous phase; G5-G10: active fractions of Sephadex G25 column purification.

The fractions 6, 7, and 8 that were obtained via the Sephadex G100 column emerged for further purification using the 30 kDa cut-off column. The SDS-PAGE electrophoresis outcomes revealed a protein band with a molecular weight under 14 kDa (Figure 7). The refined compound presented an α-glucosidase inhibitory activity of 80.2% (at a concentration of 0.25 mg/mL), with a recovery efficiency of 0.02% (Table 1). The findings provide the foundational scientific evidence for acquiring a secondary active ingredient, specifically a protein exhibiting α-glucosidase inhibitory action, intended for use as a pharmaceutical component in the treatment of type 2 diabetes.

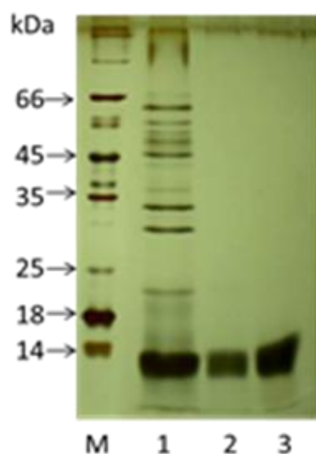


**Figure 7.** SDS-PAGE electrophoresis of samples after purification by a 30 kDa cut-off column. 1: sample through Sephadex G100 column (concentration of 25 μg), 2-3: purified fractions after 30 kDa cut-off column (concentration of 25 μg and 30 μg, respectively), M: molecular mass of standard proteins (Fermentas, Thermo Fisher Scientific Inc., Waltham, MA, USA).

**Table 1.** Summary of the initial purification steps of the protein-based α-glucosidase inhibitor from *Aspergillus niger* VTCC 031

| Fraction | Volume (mL) | Dry residue mass (mg) | Recovery efficiency (%) | α-Glucosidase inhibitory activity (%) |
|---|---|---|---|---|
| Fermentation broth | 100.0 | 2520.0 | 100.00 | 67.2 ± 0.6 |
| Ethyl acetate extract | 15.0 | 1230.0 | 48.80 | 64.8 ± 0.7 |
| Sephadex G100 fraction | 4.5 | 4.5 | 0.17 | 69.5 ± 0.9 |
| 30 kDa cut-off fraction | 4.0 | 0.5 | 0.02 | 80.2 ± 1.2 |

*Note.* Values of α-glucosidase inhibitory activity are presented as mean ± SD (n = 3). Recovery efficiency was calculated relative to the dry residue mass of the starting fermentation broth.

## DISCUSSION

In recent years, the economic expansion has been accompanied by an ongoing increase in the prevalence of diabetes among patients in several nations worldwide. This unfortunate situation presents obstacles for researchers in discovering novel pharmacological interventions for diabetes management. The pharmacological management of type 2 diabetes involves several major drug classes, including biguanides, sulfonylureas, DPP-4 inhibitors, SGLT2 inhibitors, thiazolidinediones, and AGIs (American Diabetes Association, 2015). Although AGIs are not considered first-line agents in type 2 diabetes treatment, they contribute significantly to glycemic control by targeting postprandial hyperglycemia without inducing hypoglycemia or weight gain (Johnston *et al.,* 1998). However, AGIs currently used clinically also cause unwanted effects on the digestive tract, such as bloating, flatulence, discomfort, diarrhea, etc. There have even been some reports of liver damage and increased liver transaminases (AST and ALT) associated with the use of AGIs in patients with type 2 diabetes (Carrascosa *et al.,* 1997; Coniff *et al.,* 1995). To mitigate these adverse implications, the pursuit of novel AGIs has consistently captivated scientists.

Among various production methods, microbial biosynthesis of AGIs has gained growing attention due to its economic feasibility and scalability, as microorganisms rapidly produce bioactive compounds in large quantities. Multiple studies have identified AGIs biosynthesized from the genus *Aspergillus*, including the strains *A. flavus*, *A. niger, A. terreus, A. awamori, A. fumigatus,* and *A. oryzae.* In accordance with this tendency, our research group has undertaken many investigations on the isolation, selection, and purification of novel secondary active compounds that inhibit α-glucosidase for potential use in the treatment of type 2 diabetes, derived from microbial strains obtained in Vietnam. We published an article on the ideal conditions for synthesizing AGI inhibitors from the *Oceanimonas* sp. strain (Nguyen *et al.,* 2021b), which, in detail, is the *Oceanimonas sminovir* strain isolated from the coast of Bac Lieu, Khanh Hoa, and registered on GenBank with code number MN611430.1. In another study, we extracted and purified the active ingredient DNJ from the *Bacillus subtilis* YT20 strain with high efficiency (Nguyen *et al.,* 2021a) and selective isolation of endophytic *Streptomyces* sp.

strains on Cao Phong orange trees grown in Hoa Binh, Vietnam (Nguyen *et al.,* 2022). In line with previous efforts, this study focused on extracting and preliminarily purifying AGI-active compounds from the *A. niger* VTCC 031 strain. The extract exhibited promising inhibitory activity, reinforcing the potential of native Vietnamese micro-organisms as sources of novel AGIs.

Solvent extraction experiments using n-butanol, ethyl acetate, chloroform, and n-hexane revealed that ethyl acetate yielded the highest AGI activity in the aqueous phase (64.85%). This result aligns with findings by Singh and Kaur (2016), who also identified ethyl acetate as the most effective solvent for extracting AGIs from *A. awamori*. In contrast, n-butanol demonstrated superior activity in the organic phase, differing from Dewi *et al.*'s observations, highlighting the strain-dependent nature of AGI biosynthesis and solvent partitioning behavior (Dewi *et al.,* 2016). These findings informed the selection of ethyl acetate for subsequent purification.

However, each strain will have different conditions for the biosynthesis of AGIs. We examined the fermentation extraction solvent for the *A. niger* strain, using 4 solvents with different polarities: n-butanol, ethyl acetate, chloroform, and n-hexane, and measured the activity of each extract in both phases, detecting the distribution of AGIs in both the aqueous and solvent phases. The results revealed that extracting with ethyl acetate produced the highest activity in the aqueous phase, which was completely consistent with the publication from Singh and Kaur (2016). However, extracting with n-butanol produced a different highest activity in the solvent phase compared to Dewi's 2016 publication, indicating that each strain will have a unique biosynthesis of AGIs and a different extraction efficiency for each solvent (Dewi *et al.,* 2016). From there, we made initial predictions about the polarity and separation ability of the investigated AGIs in solvents as a premise for the next purification step. In the aqueous phase, the AGIs are polar substances and have a fairly high α-glucosidase inhibitory activity of 64.85%. Given the complexity of the aqueous extract and the presence of polar impurities such as sugars, amino acids, and proteins, gel filtration chromatography was employed using columns with different pore sizes (Sephacryl S200, Sephadex G100, and Sephadex G25) to fractionate the AGI-active components. Active fractions (No. 4–10) were identified based on α-glucosidase inhibition assays.

It is imperative to select the appropriate method and conditions in order to purify AGIs from the aqueous phase, as there are numerous impurities that are soluble in water, including carbohydrates, amino acids, and proteins. A peptide isolated from the aqueous phase of the *A. oryzae* N159-1 strain has been identified as the AGI inhibitor, according to Kang *et al.* (2013). Singh and Kaur (2016) reported a peptide with a mass of 22 kDa that may simultaneously block two enzymes, α-amylase and α-glucosidase, derived from the *Awamori* sp.. Consequently, we anticipate that AGIs in the aqueous phase could be composed of low molecular weight peptides and proteins. This is the rationale for using gel filtration chromatography with varying sieve sizes to purify AGIs from the aqueous phase.

The fractions produced from three gel filtration chromatography columns, Sephacryl S200, Sephadex G100, and Sephadex G25, were evaluated for α-

glucosidase inhibitory activity, identifying fractions 4–10 as active. TLC coupled with ninhydrin staining revealed pink and purple spots, indicating the presence of peptides or low molecular weight proteins. The active fractions (>70% inhibition) were further purified using a 30 kDa cut-off filter. SDS-PAGE analysis identified a dominant band at 14 kDa, suggesting the AGI is a low molecular weight protein or peptide. However, the recovery yield was relatively low (0.02%) despite retaining strong inhibitory activity (80.2%).

Many studies have been published on the extraction and purification of AGIs from *Aspergillus* sp. strains such as *Aspergillus insulicola* (Dewi *et al.,* 2016; Sun *et al.,* 2022; Zhao *et al.,* 2016; Zhao *et al.,* 2023), *A. terreus* RCC1 (Dewi *et al.,* 2015), *Aspergillus* sp. 16-5c (Ye *et al.,* 2021), and *A. niger* ITV-01 (del Moral *et al.,* 2018). Most of these publications did not mention the purification efficiency of the obtained AGIs. Kang *et al.* (2013) reported the purification efficiency from *A. oryzae* N159-1 strain with an efficiency of 1.9%. The purification efficiency in our study was only 0.02%, lower than the study of Kang *et al.* (2013). It is possible that due to the study of purification of secondary compounds from the aqueous phase after extracting the fermentation broth with solvent, a large part of the α-glucosidase inhibitory was dissolved in the solvent phase (secondary compounds), while the substances that are essentially low molecular weight proteins were dissolved in the aqueous phase. That was one of the reasons for the low purification efficiency compared to the original fermentation broth. In addition, the purification yield is also highly dependent on the purification methods employed. To enhance the purification efficiency in future studies, we propose several strategies: utilizing smaller cut-off columns for sample concentration, employing specific purification columns designed for low molecular weight proteins, combining various chromatographic techniques such as gel filtration, ion-exchange, and HPLC chromatography, etc. These approaches are expected to significantly improve the recovery and purity of the AGIs.

## CONCLUSION

*Aspergillus niger* VTCC 031 was demonstrated to produce a protein-based α-glucosidase inhibitor with notable inhibitory activity. Under the selected culture conditions (5% D-glucose, 1% malt extract, pH 5, 37°C, 200 rpm, 7 days), the culture supernatant exhibited 67.24% α-glucosidase inhibition. Purification by Sephadex G100 chromatography and a 30 kDa cut-off column yielded an active protein fraction showing 80.2% inhibitory activity. SDS-PAGE analysis indicated a protein band of less than 14 kDa, suggesting that the inhibitor is a low-molecular-weight protein. Although the recovery efficiency remained limited (0.02%), the present study provides a preliminary foundation for further optimization of purification procedures and for subsequent structural and functional characterization of peptide-based AGIs from *A. niger* VTCC 031.

## ACKNOWLEDGMENTS

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glucosidase inhibitors producing from *Aspergillus, Oceanimonas* and *Streptomyces* isolates in Vietnam” 2024–2026.

## CONFLICT OF INTEREST

The authors declare no competing interests.

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