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# Evaluation of the *alpha*-glucosidase and *alpha*-amylase inhibitory potential of *Costus speciosus* (J. Koenig) Sm. aerial parts from Vietnam

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***Abstract:***

**This study aimed to evaluate the inhibitory activity of *α*-glucosidase and *α*-amylase enzymes using aqueous and 40% ethanol extracts from the aerial parts of *Costus speciosus* harvested in Binh Phuoc province, tested *in vitro*. The enzyme inhibition activity was measured using *α*-amylase from porcine pancreas and *α*-glucosidase from *Saccharomyces cerevisiae*. Results showed that both the aqueous extract and the ethanol extract from the aerial parts of *Costus speciosus* showed significant *α*-glucosidase inhibition, with $IC_{50}$ values of 18.51 and 77.25 µg/ml, respectively. Both extracts demonstrated lower $IC_{50}$ values compared to the positive control, acarbose ($IC_{50}$=122.20 µg/ml). For *α*-amylase inhibition, the aqueous extract and ethanol extract exhibited relatively low inhibition rates of 3.79 and 3.12%, respectively, compared to acarbose, which had an $IC_{50}$ of 50 µg/ml for *α*-amylase inhibition. The study demonstrated that the aqueous and ethanol extracts of the *Costus speciosus* aerial parts exhibited *in vitro* inhibitory effects on *α*-glucosidase. However, the extracts showed poor inhibitory effects on *α*-amylase activity. These findings provide additional scientific evidence supporting its potential use in Vietnam for its hypoglycaemic properties.**

***Keywords*: aqueous extract, *Costus speciosus*, *in vitro*, 40% ethanol extract.**

***Classification numbers:*** **3.3, 3.5**

## 1. Introduction

Diabetes mellitus is a metabolic disorder characterised by elevated blood sugar levels, insulin resistance, and dyslipidaemia (abnormal lipid levels). Today, diabetes is one of the most widespread health conditions globally, affecting millions of people and potentially leading to serious complications such as cardiovascular disease, kidney failure, and nerve damage [1]. Diabetes mellitus arises from a relative or absolute deficiency in insulin secretion, resistance to insulin action in body tissues, or both. The disease is broadly classified into Type I and Type II diabetes, and its prevalence is influenced by several contributing factors, including obesity, stress, poor diet, and lack of physical activity [2].

There is a strong correlation between inflammation and diabetes. In particular, inflammatory responses within adipose tissue play a key role in the onset of Type II diabetes by promoting insulin resistance. In turn, persistent hyperglycaemia contributes to the development of long-term diabetic complications [3]. Chronic inflammation is a common feature in individuals with Type II diabetes, characterised by elevated levels of circulating cytokines such as tumour necrosis factor-alpha (TNF-α), a pro-inflammatory marker closely associated with diabetes. Additionally, levels of adiponectin - an anti-inflammatory cytokine produced by adipose tissue - are reduced in diabetic patients, which further diminishes insulin sensitivity [4]. An increasing body of evidence suggests that inflammation may be a primary driver of diabetic pathology. Therefore, therapeutic approaches that simultaneously target hyperglycaemia - by inhibiting carbohydrate-hydrolysing enzymes such as *α*-amylase and *α*-glucosidase - and inflammatory pathways may represent a promising strategy for both the prevention and management of diabetes and its associated complications [2, 4]. Celecoxib, a selective COX-2 inhibitor, may reduce insulin resistance, making it a potential anti-diabetic agent with anti-inflammatory properties [2, 5].

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Carbohydrates serve as a major source of glucose in the human body. These macronutrients are initially hydrolysed into oligosaccharides by the enzyme $\alpha$-amylase, which is secreted by the pancreas. Subsequently, at the intestinal membrane, the enzyme $\alpha$-glucosidase hydrolyses oligosaccharides into glucose, allowing it to be absorbed into the bloodstream. Inhibiting these two key enzymes can significantly reduce blood glucose levels, contributing to more effective diabetes management. According to H. Kashtoh, et al. (2022) [6], $\alpha$-amylase (*1,4-α-D-glucan-glucanohydrolase, EC 3.2.1.1*) and $\alpha$-glucosidase (*EC 3.2.1.20*) are the main enzymes involved in the digestion of carbohydrates, catalysing the breakdown of complex carbohydrates into simpler sugars such as glucose. Inhibitors of $\alpha$-amylase and $\alpha$-glucosidase delay the final stage of carbohydrate digestion, helping to prevent glucose from entering the bloodstream too rapidly and thereby providing a viable preventive treatment for hyperglycaemia - a condition commonly associated with type II diabetes.

While synthetic and chemical inhibitors of these enzymes are available and effective, they are often accompanied by several side effects, including flatulence, diarrhoea, and abdominal discomfort. As noted by H. Kashtoh, et al. (2022) [6], these side effects have led to growing interest in natural glucosidase inhibitors derived from plants. Natural inhibitors from plant sources are gaining attention due to their lower incidence of side effects, effective glycaemic control, biocompatibility, and favourable safety profiles. These benefits make them a promising component in the development of natural therapeutics for managing Type II diabetes.

$\alpha$-glucosidase and $\alpha$-amylase are key enzymes involved in the digestion of carbohydrates. By inhibiting these enzymes, the breakdown of complex carbohydrates into glucose is delayed, leading to a slower rise in blood sugar levels after eating [6]. This approach is particularly relevant for Type II diabetes management in Vietnam, where rice is a staple food and carbohydrate intake constitutes a significant part of the daily diet.

As the prevalence of diabetes continues to rise, particularly in developing countries, finding effective strategies to control blood glucose levels is critical. Inhibitors of $\alpha$-glucosidase and $\alpha$-amylase have emerged as a promising area of research and application in both natural medicine and pharmaceuticals. In recent years, natural compounds derived from medicinal plants that inhibit the enzyme $\alpha$-glucosidase have garnered significant attention for their potential in diabetes management. Research by M.L. Le, et al. (2023) [7] demonstrated the effectiveness of *Mangifera indica*, *Gynostemma pentaphyllum*, and *Gymnema sylvestre* extracts in inhibiting $\alpha$-glucosidase, highlighting their potential as therapeutic agents for controlling blood glucose levels. S. Poovitha, et al. (2016) [8] studied bitter melon (*Momordica charantia*), revealing its capacity to inhibit both $\alpha$-amylase and $\alpha$-glucosidase enzymes, supporting its traditional use for blood sugar regulation. T.S. Vo, et al. (2024) [9] found that *Psidium guajava*, *Mangifera indica*, *Physalis angulata*, *Pandanus amaryllifolius*, *Ficus glomerata*, *Artocarpus altilis*, and *Gomphrena celosioides* extracts possess $\alpha$-amylase inhibitory effects, further expanding the scope of plant-based interventions for diabetes management. M. Taher, et al. (2016) [10] identified the $\alpha$-glucosidase inhibitory activity of mangosteen (*Garcinia mangostana*) extracts, which could contribute to developing plant-based treatments for diabetes mellitus.

*Costus speciosus*, also known as the insulin plant, contains the active compound diosgenin. It belongs to the Costaceae family and thrives in humid and shaded environments, particularly in the tropical and subtropical regions of Asia, including India, Indonesia, and Vietnam. This medicinal plant has long been recognised for its therapeutic properties in treating various ailments, including inflammation, skin diseases, and especially in the management of diabetes [11]. Research has shown that diosgenin may have insulin-like effects, helping to regulate blood sugar levels and improve glucose metabolism, making it a promising natural remedy for people with type II diabetes. Additionally, diosgenin has been studied for its anti-inflammatory and anti-cancer properties [12].

In Vietnam, the rhizome of *Costus speciosus* is known for its medicinal properties, including the treatment of fever, painful urination, yellowish urine, and bladder inflammation. Additionally, the branches of *Costus speciosus* are grilled and then squeezed to extract juice used to treat eye and ear pain [13]. In the Ba Vi region, the Dao ethnic group traditionally uses *Costus speciosus* to manage diabetes mellitus. However, there is currently limited scientific evidence to confirm the true therapeutic effectiveness of *Costus speciosus* in treating diabetes in Vietnam. This gap in scientific knowledge has led to the research: "Evaluation of the $\alpha$-glucosidase and $\alpha$-amylase inhibitory potential of the *Costus speciosus* (J. Koenig) Sm. aerial part".

## 2. Materials and methods

### *2.1. Materials*

The study focused on the aerial parts of *Costus speciosus* (Koen.) Sm., which were freshly harvested in Binh Phuoc province in December 2023. The plant material was thoroughly cleaned, air-dried, and stored at room temperature in a laboratory environment (25°C). DNA identification of the plant samples was performed by Phu Sa Genomics. The DNA identification results indicated that the sample labelled M01.23 shares 99.82% sequence similarity with *Hellenia speciosa* (synonym: *Costus speciosus* (J. Koenig) Sm., voucher IBSC:005).

Automatic gene sequencing read DNA sequences in the 5'-3' direction:

TTAAGATTACAAATTGAATTATTATACTCCTGAC
TACGAAGTCAAAGATACGGATATCTTGGCAGCATT
TCGAGTAACTCCTCAACCTGGAGTTCCGCCCGAA
GAAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTA
CTGGTACATGGACAACTGTGTGGACTGATGGACT
TACCAGCCTTGATCGTTACAAAGGGCGATGCTAC
CACATCGAGGCCGTTGTTGGGGAGGATAATCAAT
ATATTGCTTATGTAGCTTATCCTTTAGACCTTTTTG
AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTG
TGGGTAATGTATTTGGTTTCAAAGCCTTACGGGCT
CTACGTCTGGAGGATCTGCGAATTCCCACTTCTTAT
TCCAAAACTTTCCAAGGCCCGCCTCACGGCATTC
AGGTTGAAAGAGATAAGTTGAACAAGTATGGTCG
TCCCCTGTTGGGATGTACTATTAAACCAAAATTGG
GATTATCCGCAAAAAACTACGGTAGAGCGGTTTA
TGAATGTCTACGCGGTGGACTTGATTTTACAA.

The positive control: acarbose (≥95 % HPLC, Sigma).

Chemical reagents: $\alpha$-amylase enzyme (0.5 U/ml, Sigma), $\alpha$-glucosidase enzyme (from *Saccharomyces cerevisiae,* 0.2 U/ml, Sigma), *p*-nitrophenyl-$\alpha$-*D*-glucopyranoside (p-NPG, Sigma), iodine reagent solution (0.1%), and starch solution (1 mg/ml).

The following solvents and chemicals were used in the study: Distilled water, $Na_2CO_3$ (0.2 M), and HCl solution (1 N).

### *2.2. Methods*

*2.2.1. Extraction and quality assessment of herbal extract*

*Extraction process:* The *Costus speciosus* extracts were prepared using two main solvents: water and 40% ethanol. Each extract underwent a specific process to maximise yield and preserve active compounds:

*Water extraction:* The herb was washed twice with clean water to remove impurities. After washing, the herbs were placed into a decoction pot, and 7 litres of purified water were added, ensuring the liquid level just covered the surface of the herbs. The mixture was simmered on low heat for 45 minutes, counting from the moment it began to boil. The extract was decanted and then filtered through two layers of gauze to remove plant residue and ensure clarity. Three litres of purified water were then added to the remaining herbal mass in the pot, and the mixture was simmered again on low heat for 30 minutes. The second extract was collected and filtered in the same way. Both extracts were combined and concentrated using a water bath to avoid degrading the active compounds. The resulting thick aqueous herbal extract was stored in a refrigerator at 4-8°C to maintain stability and preserve bioactive constituents.

*Ethanol extraction:* The herbal material was coarsely ground into a powder and moistened with 40% ethanol for 30 minutes to allow better solvent penetration. The moistened herb was packed into a percolator, and an additional 40% ethanol was added at a herb-to-solvent ratio of 1:10. The mixture was steeped for 24 hours. The extract was then allowed to percolate at a flow rate of 50-60 drops per minute. Solvent was replenished as needed until the percolate ran nearly colourless. The collected extract was concentrated by rotary evaporation at 50°C to recover the ethanol. The concentrate was further thickened using a water bath. The final extract was stored at 4-8°C to preserve its quality.

*Quality evaluation criteria:* Both extracts were subjected to quality assessments based on the standards of the Vietnamese Pharmacopoeia V. Key criteria included sensory characteristics (appearance, odour, and texture), extraction yield, and moisture content (not exceeding 20% to ensure the extract's stability and longevity).

*2.2.2. Study on α-glucosidase inhibition activity*

The $\alpha$-glucosidase inhibition activity was evaluated following the method outlined by M.N. Qaisar, et al. (2014) [14], with minor modifications as described below: 60 µl of the sample solution was combined with 50 µl of 0.1 M phosphate buffer (pH 6.8) containing the $\alpha$-glucosidase enzyme (0.2 U/ml) in the wells of a 96-well plate. The mixture was incubated at room temperature for 20 minutes. After the pre-incubation period, 50 µl of p-NPG (*p*-nitrophenyl-$\alpha$-D-glucopyranoside) solution prepared in 0.1 M phosphate buffer (pH 6.8) was added to each well, followed by an additional 10 minutes of incubation. The reaction was terminated by adding 160 µl of 0.2 M $Na_2CO_3$.

Absorbance was then recorded at a wavelength of 405 nm using a microplate reader (Biotek, USA). A control well was prepared by replacing the sample with 60 µl of phosphate buffer.

This method measures the enzyme inhibition effect of the extract based on the change in absorbance, which is compared with the control to determine the extract's inhibitory activity against α-glucosidase. The percentage inhibition of α-glucosidase activity was calculated as follows:

$$(\%)\ \alpha\text{-glucosidase inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$, $A_{sample}$ are the absorbance values of the control (without sample) and the test samples, respectively. In this assay, acarbose (Sigma, USA) is used as a positive control, serving as a reference standard for comparison of α-glucosidase inhibition effectiveness.

*2.2.3. Investigation of α-amylase inhibition activity*

To assess the inhibition of starch hydrolysis by the plant extracts, the protocol follows a modified version of T.X.T. Dai, et al. (2012) [15] with adjustments detailed below:

A mixture of 100 µl of phosphate buffer (pH 7) with 100 µl of the plant extract and 50 µl of α-amylase enzyme (0.5 U/ml) was created. The mixture was incubated at 37°C for 10 minutes. Then, 250 µl of starch solution (1 mg/ml) was added to the mixture. The incubation was continued at 37°C for an additional 10 minutes. After that, 100 µl of 1 N HCl was added to stop the reaction, and 300 µl of 0.1% iodine solution was further added to detect residual starch by forming a characteristic blue complex. Then, 1.6 ml of distilled water was added to bring the total volume to 2.5 ml. Finally, the absorbance of the starch-iodine complex was measured at 660 nm using a spectrophotometer.

Acarbose was used as the positive control to compare the inhibitory effects on α-amylase activity. This protocol helps determine the inhibitory potential of the extracts on α-amylase, contributing to insights into their potential antidiabetic properties by slowing carbohydrate digestion.

The percentage of α-amylase inhibition activity is calculated using the following formula:

$$(\%)\ \alpha\text{-amylase inhibition} = ((A_0 - A_1)/A_0) \times 100$$

where $A_0$, $A_1$ are the absorbance values of the control solution (without plant extract) and the solution after the reaction (with plant extract), respectively. This formula measures the enzyme inhibitory potential by comparing the absorbance values before and after adding the plant extract.

## 3. Results and discussion

### *3.1. Extraction and quality assessment of herbal extract*

After harvesting and drying, 1,000 g of raw material was finely ground, yielding 500 g of herbal powder, which was then used for preparing the 40% ethanol extract. Similarly, 1,000 g of dried raw material was used to obtain the water extract. Following the extraction steps outlined in Section 2.2.1, two types of concentrated extracts were obtained, each presenting a uniform brownish-yellow colour and a distinctive aroma. The results of the quality parameters for these extracts were as follows:

The extraction yield for the water extract was 6.4%, while the 40% ethanol extract achieved a yield of 14.25%. The moisture content for these extracts was 5.44 and 8.57%, respectively, both well within the acceptable limit of not more than 20% as specified in Appendix PL.1 of the Vietnamese Pharmacopoeia V for concentrated extracts.

These results confirm that the moisture levels of both extracts meet the quality standards for concentrated herbal extracts, ensuring stability and compliance with pharmaceutical guidelines. This standardised moisture content supports the long-term preservation and consistency of the extracts' medicinal properties.

### *3.2. Screening for α-glucosidase inhibition activity*

The experiment to assess the α-glucosidase inhibitory activity of acarbose was conducted following the method established by M.N. Qaisar et al. (2014) [14]. The obtained results are presented in Table 1 below:

**Table 1. Results of α-glucosidase inhibitory activity of acarbose.**

| Initial concentration (µg/ml) | Reaction concentration (µg/ml) | $A_{sample}$ average | % inhibition |
|---|---|---|---|
| 50 | 18.75 | 1.637 | 21.62 |
| 100 | 37.50 | 1.396 | 33.18 |
| 250 | 93.75 | 1.158 | 44.56 |
| 500 | 187.50 | 0.881 | 57.80 |
| 1,000 | 375.00 | 0.704 | 66.31 |

From Table 1, the following logarithmic equation is established:

$$\text{Logarithm} = 14.974\ln(x) - 21.96;\ R^2 = 0.996$$

From this, we can calculate the $IC_{50}$ for acarbose, which is 122.20 µg/ml. This $IC_{50}$ value indicates the concentration of acarbose required to inhibit 50% of α-glucosidase activity and serves as a reference standard for comparing the inhibitory potency of the other samples tested.

At a concentration of 1,000 µg/ml, acarbose showed high α-glucosidase inhibitory activity, with an inhibition rate of 66.31%. At progressively lower concentrations - 500, 250, 100, and 50 µg/ml, the α-glucosidase inhibition levels of acarbose were 57.80, 44.56, 33.18, and 21.62%, respectively.

These data underscore acarbose's concentration-dependent inhibitory effect on α-glucosidase, providing useful insights into its efficacy as an inhibitor at various dosage levels.

### *3.3. Results from the α-glucosidase inhibition test on aqueous extract of Costus speciosus*

Table 2 presents the results of the α-glucosidase inhibition activity of the aqueous extract from *Costus speciosus*.

**Table 2. Results of α-glucosidase inhibitory activity of *C. speciosus* water extract.**

| Initial concentration (µg/ml) | Reaction concentration (µg/ml) | $A_{sample}$ average | % inhibition activity |
|---|---|---|---|
| 500 | 93.75 | 0.144 | 94.07 |
| 250 | 46.88 | 0.367 | 84.87 |
| 100 | 18.75 | 1.035 | 57.28 |
| 50 | 9.38 | 1.874 | 22.67 |
| 25 | 4.69 | 2.253 | 7.00 |

Using Table 2 data, the logarithmic equation was established as follows:

Logarithm=31.077ln(x)-40.688; $R^2$=0.9719

From this, the $IC_{50}$ value was determined to be 18.51 µg/ml.

At a concentration of 500 µg/ml, the aqueous extract of *C. speciosus* demonstrated high α-glucosidase inhibition activity, with an inhibition percentage of 94.07%. At concentrations of 250, 100, 50, and 25 µg/ml, the inhibitory effects of the *C. speciosus* aqueous extract on α-glucosidase were recorded as 84.87, 57.28, 22.67, and 7.00%, respectively.

### *3.4. Results from the α-glucosidase inhibition test on ethanol extract of Costus speciosus*

The α-glucosidase inhibition activity of the ethanol extract from *C. speciosus* was evaluated using the method described by M.N. Qaisar, et al. (2014) [14]. The results are presented in Table 3.

**Table 3. Results of α-glucosidase inhibition activity of *C. speciosus* ethanol extract.**

| Initial concentration (µg/ml) | Reaction concentration (µg/ml) | $A_{sample}$ average | % inhibition |
|---|---|---|---|
| 2,000 | 375.00 | 0.235 | 90.30 |
| 1,000 | 187.50 | 0.691 | 71.48 |
| 500 | 93.75 | 1.164 | 51.97 |
| 250 | 46.88 | 1.432 | 40.89 |
| 100 | 18.75 | 2.086 | 13.89 |

Based on Table 3, the inhibition equation was obtained:

Logarithm=24.897ln(x)−58.229; $R^2$=0.9938

Thus, the $IC_{50}$ value was calculated to be 77.25 µg/ml.

At a concentration of 2,000 µg/ml, the ethanol extract from *C. speciosus* exhibited a high level of α-glucosidase inhibition, achieving an inhibitory capacity of 90.3%. At progressively lower concentrations - specifically 1,000, 500, 250, and 100 µg/ml - the ethanol extract displayed α-glucosidase inhibition rates of 71.48, 51.97, 40.89, and 13.89%, respectively. These results indicate that the ethanol extract possesses significant α-glucosidase inhibitory activity, particularly at higher concentrations, with an $IC_{50}$ of 77.25 µg/ml, suggesting its potential as a moderate α-glucosidase inhibitor.

### *3.5. Results of α-amylase inhibitory activity testing for acarbose*

The α-amylase enzyme inhibitory capacity of acarbose was evaluated using the method described by T.X.T. Dai, et al. (2012) [15]. The results are presented in Table 4.

**Table 4. Results of α-amylase inhibitory activity testing for acarbose.**

| Initial concentration (µg/ml) | Reaction concentration (µg/ml) | $A_1$ average | % inhibition |
|---|---|---|---|
| 300 | 60 | 0.251 | 68.41 |
| 250 | 50 | 0.370 | 53.40 |
| 200 | 40 | 0.464 | 41.65 |
| 150 | 30 | 0.566 | 28.78 |
| 100 | 20 | 0.633 | 20.39 |

From Table 4, the following logarithmic equation was derived:

Logarithm=1206.8ln(x)-5.7466; $R^2$=0.9918

From this equation, $IC_{50}$=50 µg/ml.

At a concentration of 300 µg/ml, acarbose showed α-amylase enzyme inhibitory activity with an inhibition rate

of 68.41%. At concentrations of 250, 200, 150, and 100 µg/ml, acarbose inhibited the α-amylase enzyme with inhibition rates of 53.40, 41.65, 28.78, and 20.39%, respectively.

### *3.6. Results of α-amylase inhibition activity of C. speciosus extracts*

The inhibitory activity of the α-amylase enzyme by the aqueous extract and ethanol extract of *C. speciosus* was evaluated following the method described by T.X.T. Dai, et al. (2012) [15]. The obtained results are presented in Table 5.

**Table 5. Results of α-amylase enzyme inhibition activity for *C. speciosus* extracts.**

| Samples | Initial concentration (µg/ml) | $A_1$ average | % inhibition |
|---|---|---|---|
| *C. speciosus* water extract | 2,000 | 1.142 | 3.79 |
| *C. speciosus* ethanol extract | 2,000 | 1.150 | 3.12 |

At a concentration of 2,000 µg/ml, the *C. speciosus* aqueous extract showed an α-amylase enzyme inhibition rate of 3.79%, while the ethanol extract showed an inhibition rate of 3.12%. Due to the very low inhibitory activity, it was not possible to calculate the $IC_{50}$ value for comparison with acarbose's α-amylase inhibitory activity.

### *3.7. Discussion*

*Costus speciosus* is a plant with multiple usable parts, including the rhizomes, stems, and leaves. According to H.K. Perera, et al. (2016) [11], most studies conducted on *C. speciosus* rhizomes and leaves have shown promising results for its potential in developing medicinal products, particularly for hypoglycaemic and cholesterol-lowering effects.

In the recent literature, there are no reports on the effects of *C. speciosus* harvested in Vietnam on hypoglycaemic activity. This study highlights the inhibitory effects of the aerial parts of *C. speciosus* on α-glucosidase and α-amylase enzymes, demonstrating its potential role in blood sugar regulation.

#### *3.7.1. Extraction of C. speciosus using water and 40% ethanol solvents*

This study used water and 40% ethanol as solvents to extract natural compounds for testing. The extraction process was carried out under the standards of the Vietnamese Pharmacopoeia V. Water was selected as it is the most common solvent for preparing traditional decoctions, while 40% ethanol was chosen due to its ability to dissolve a wide range of active compounds, including both polar and non-polar substances present in the medicinal materials.

In this study, the yield of the aqueous extract was 6.40%, with a moisture content of 5.44%. For the ethanol extract, the yield was 14.25%, with a moisture content of 8.57%. Thus, the moisture content of both extracts was below 20%, meeting the moisture standards for thick extracts as specified in Appendix PL.1 of the Vietnamese Pharmacopoeia V. The lower moisture content in the aqueous extract compared to the 40% ethanol extract may be due to differences in solvent properties and compound composition, resulting in varying moisture levels.

The extraction yield of *C. speciosus* in this study is consistent with the research conducted by H.K. Perera, et al. (2016) [11], in which the methanol extraction yield from *C. speciosus* leaf powder was reported as 15.8%. The slight difference observed between the yield in this study and that reported by H.K. Perera, et al. (2016) [11] may be attributed to several factors, including differences in soil conditions, variation in plant parts used, and the use of different extraction solvents.

#### *3.7.2. Inhibitory potential of C. speciosus extracts on α-glucosidase and α-amylase enzymes*

α-Glucosidase inhibitors work through competitive and reversible inhibition of the α-glucosidase hydrolase enzyme system located in the small intestinal mucosa. By inhibiting the activity of α-glucosidase, these agents slow the absorption of monosaccharides in the small intestine, thereby helping to reduce postprandial blood glucose levels [1]. Acarbose, a clinically used drug known for its α-glucosidase inhibitory effect, was chosen as the positive control for this study.

Based on the $IC_{50}$ results obtained, the aqueous extract from the aerial parts of *C. speciosus* exhibited α-glucosidase inhibitory activity with an $IC_{50}$ value of 18.51 µg/ml, which was lower than that of the ethanol extract ($IC_{50}$=77.25 µg/ml) and the positive control acarbose ($IC_{50}$=122.20 µg/ml). These findings indicate that extraction solvents can significantly influence the α-glucosidase inhibitory capacity in *in vitro* experiments.

This result aligns with the findings reported by H.K. Perera, et al. (2016) [11], who investigated the α-glucosidase inhibitory activity of *C. speciosus* leaf extracts using an *in vitro* method, with acarbose also employed as a positive

control. Their study demonstrated that the methanol extract of *C. speciosus* leaves exhibited *α*-glucosidase inhibitory activity with an $IC_{50}$ value of 67.5 µg/ml, which was lower than that of acarbose ($IC_{50}$=208.53 µg/ml).

*3.7.3. Inhibitory potential of C. speciosus extracts on α-amylase enzyme*

The *α*-amylase inhibitory activity is assessed by measuring the difference between the initial amount of starch and the remaining starch after the hydrolysis reaction, to evaluate the extent of *α*-amylase enzymatic activity. The greater the amount of remaining starch post-reaction, the stronger the inhibitory effect [1].

The experimental results of this research indicated that at a concentration of 2,000 µg/ml, the aqueous extract and ethanol extract of *C. speciosus* exhibited inhibition rates of 3.79 and 3.12%, respectively. Compared to acarbose, which has an $IC_{50}$ value of 50 µg/ml for *α*-amylase inhibition, the extracts from *C. speciosus* collected in Binh Phuoc province displayed significantly lower *α*-amylase inhibitory activity.

A study conducted by H.K. Perera, et al. (2016) [11] investigated the *α*-amylase inhibitory activity of a methanol extract from *C. speciosus* leaves collected in Moratuwa, Sri Lanka. Their findings showed that the methanol extract exhibited *α*-amylase inhibition with an $IC_{50}$ value of 5.88 µg/ml, which was significantly lower than the $IC_{50}$ of acarbose (262.54 µg/ml).

The results of this study do not align with those of H.K. Perera, et al. (2016) [11]. These discrepancies may stem from factors such as the solvents used, the plant parts extracted, differences in the chemical compositions of the extracts, and environmental factors such as soil conditions.

One of the most widely recognised strategies for the prevention and management of diabetes involves reducing postprandial blood glucose levels, which rise due to dietary carbohydrate intake. This can be achieved by slowing the digestion of carbohydrates in the intestine through the inhibition of digestive enzymes, particularly *α*-amylase and *α*-glucosidase. *α*-Amylase plays a central role in the initial hydrolysis of dietary starch and polysaccharides. It breaks the glycosidic bonds present in these carbohydrates, converting them into smaller units such as oligosaccharides and absorbable monosaccharides. There are two primary sources of *α*-amylase: Salivary amylase, produced by the salivary glands, which initiates carbohydrate digestion in the mouth; and pancreatic amylase, secreted by the pancreas into the duodenum, which continues the breakdown of starch as it enters the small intestine [16].

Recent research has evaluated the inhibitory effects of 96% ethanol extracts from various medicinal plants traditionally used in herbal medicine. The study examined *Psidium guajava* (guava leaves), *Mangifera indica* (mango leaves), *Physalis angulata* (ground cherry), *Pandanus amaryllifolia* (pandan leaves), *Ficus glomerata* (cluster fig leaves), *Artocarpus altilis* (breadfruit leaves), and *Gomphrena celosioides*. Their inhibitory strength was assessed using the $IC_{50}$ value, with a lower $IC_{50}$ indicating stronger inhibitory activity. The results indicated that the ethanol extracts of these medicinal plants exhibited *α*-amylase inhibitory activity with $IC_{50}$ values of 136.8, 184.7, 228.3, 159.1, 264.5, 152.4, and 204.4 µg/ml, respectively [9].

*α*-Glucosidase inhibitors are a class of oral antidiabetic drugs that act through competitive and reversible inhibition of the *α*-glucosidase hydrolase enzyme system located in the mucosa of the small intestine. By inhibiting the activity of the *α*-glucosidase enzymes, these drugs slow the absorption of monosaccharides in the small intestine. This results in a reduction of postprandial (after-meal) blood glucose levels, offering a valuable mechanism in the treatment of type 2 diabetes mellitus (T2DM). *α*-glucosidase inhibitors are particularly effective in T2DM patients who consume diets high in carbohydrates. This makes them especially suitable for Asian populations, including Vietnam, where rice and other carbohydrate-rich foods form a large part of daily meals [17].

A study was conducted to evaluate the *α*-glucosidase inhibitory activity of 50% ethanol extracts from three medicinal plants (*Mangifera indica, Gynostemma pentaphyllum,* and *Gymnema sylvestre*) and compare them with the synthetic drug acarbose. The extracts of the three herbs and acarbose inhibited *α*-glucosidase with $IC_{50}$ values of 39.68±0.27µg/ml, 126.94±4.58µg/ml, 92.32±0.97µg/ml, and 176.09±0.26µg/ml, respectively [7].

Based on the results of this study, both aqueous and 40% ethanol extracts from the stems and leaves of *Costus speciosus* exhibited weak *α*-amylase inhibitory activity but showed significant inhibition of *α*-glucosidase, a key

enzyme involved in postprandial glucose regulation. Both extracts outperformed acarbose in inhibiting $\alpha$-glucosidase, with $IC_{50}$ values of 18.51 (aqueous extract), 77.25 µg/ml (ethanol extract), and 122.20 µg/ml (acarbose), respectively. Notably, the aqueous extract demonstrated the lowest $IC_{50}$ value. The low $IC_{50}$ values of both extracts indicate strong inhibitory potential, which is especially relevant for managing T2DM by delaying carbohydrate digestion and reducing postprandial blood glucose spikes.

Further studies are needed to investigate the specific bioactive constituents present in both the aqueous and ethanolic extracts of *Costus speciosus* to elucidate their inhibitory effects on $\alpha$-amylase and $\alpha$-glucosidase enzymes. Additionally, *in vivo* research on the hypoglycaemic effects of this medicinal plant should be conducted to confirm its efficacy and safety in practical applications.

*Costus speciosus*, commonly known for its wide range of traditional medicinal uses, contains a diverse array of bioactive phytochemicals distributed across various parts of the plant. These compounds are responsible for its therapeutic properties and pharmacological effects. The plant is rich in numerous phytochemical groups, including alkaloids, glycosides, steroids, phenolics, flavonoids, tannins, terpenoids, and saponins. These constituents contribute significantly to the plant's anti-inflammatory, antidiabetic, antioxidant, and hepatoprotective activities. Extensive phytochemical studies have identified several bioactive molecules in *C. speciosus*, such as diosgenin, dioscin, costusosides, eremanthin, prosapogenins A and B (of dioscin), gracillin, β-sitosterol, β-carotene, and β-*D*-glucoside [18].

Diosgenin, a naturally occurring steroidal sapogenin, has been reported to exert antidiabetic activity through multiple biological mechanisms [12]. According to S. Ghosh, et al. (2014) [19], diosgenin exhibits inhibitory effects on both $\alpha$-amylase and $\alpha$-glucosidase, which are crucial enzymes in carbohydrate digestion. Their inhibition helps reduce glucose absorption in the intestines, thereby lowering postprandial blood glucose levels. As described by Q. Gan, et al. (2020) [12], diosgenin also inhibits sodium-glucose co-transporter 1 (SGLT-1) and reduces $Na^+/K^+$-ATPase activity in the intestines. These effects further contribute to limiting glucose uptake, making diosgenin a promising compound for managing hyperglycaemia.

## 4. Conclusions

This study successfully evaluated the $\alpha$-glucosidase and $\alpha$-amylase inhibitory activities of *Costus speciosus* aerial part extracts. The results demonstrated strong $\alpha$-glucosidase inhibition by both the aqueous and ethanol extracts compared with acarbose, while highlighting their limited effectiveness in inhibiting $\alpha$-amylase and the important role of solvent choice in enhancing inhibitory potency. These findings contribute valuable insights into the potential use of *Costus speciosus* harvested in Vietnam for managing blood glucose levels.

## CRediT author statement

Oanh Hoang Hua: Conceptualisation, Methodology, Writing - Reviewing, Editing, Supervision, Visualisation; Nu Thi: Writing - Original draft preparation, Data analysis.

## COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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