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α-AMYLASE AND α-GLUCOSIDASE INHIBITORY ACTIVITIES OF THE FLOWER EXTRACTS FROM *OCHNA INTEGERRIMA*

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Summary

α-Amylase and α-Glucosidase Inhibitory Activities of the Flower Extracts from *Ochna integerrima*

This research aimed to examine the inhibitory effects of *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), aqueous, and methanol (MeOH) extracts of *Ochna integerrima* on α-amylase and α-glucosidase enzymes for the first time. Out of the five extracts tested, the aqueous extract exhibited remarkable anti-glucosidase and anti-amylase activities with IC_{50} values of 8.78 ± 0.26 and 7.16 ± 0.13 μg/mL, respectively, followed by *n*-hexane extract with IC_{50} values of 71.82 ± 1.43 and 42.45 ± 0.69 μg/mL. Then, compounds were isolated from the active extracts. Stigmasterol (**1**) and maslinic acid (**2**), extracted from *n*-hexane, exhibited inhibitory effects against α-amylase with IC_{50} values of 71.65 ± 1.34 μM and 48.06 ± 1.6 μM, respectively. However, the isolated compounds 6-(γ,γ-dimethylallyl)taxifolin 7-*O*-β-D-glucoside (**3**) and dimethylallyl-dihydrokaempferol 7-*O*-β-D-glucoside (**4**) from aqueous extract did not support the observed effect. Further investigation of the bioactive compounds from the aqueous extract is necessary to support its potent activity.

Keywords: *α-Amylase; α-Glucosidase; Extracts; Ochna integerrima flowers.*

1. Background

Diabetes mellitus is a metabolic disorder caused by the dysfunction of carbohydrate metabolism, where the hormone insulin from the pancreas is either deficient or has reduced effectiveness in the body [1]. α-Amylase breaks down complex carbohydrates into smaller oligosaccharides, while α-glucosidase hydrolyzes these oligosaccharides into glucose, which is then absorbed into the bloodstream. The primary source of glucose in the body is carbohydrates, which are broken down by α-amylase and released into the bloodstream [2]. In the small intestine, the α-glucosidase enzyme hydrolyzes the oligosaccharides into glucose, which is then absorbed into the bloodstream. In diabetes, insulin deficiency or resistance leads to impaired glucose uptake. It can result in a variety of complications, including cardiovascular diseases, kidney damage, nerve damage, and poor wound healing [3]. Therefore, α-amylase and α-glucosidase inhibitors have been explored as potential therapeutic agents for diabetes management. These inhibitors are particularly beneficial in type 2 diabetes, where insulin resistance is common. Given the rising interest in natural remedies, medicinal plants have been widely studied for their potential to act as α-amylase and α-glucosidase inhibitors in diabetes treatment.

Ochna integerrima (Loureiro) Merrill is commonly called the "lucky flower" in Vietnam. This plant is widely used for both medicinal and decorative purposes. It is predominantly found in Southeast and South Asian regions [4]. Key compounds in the *Ochna* genus include flavonoids, anthranoids, triterpenes, steroids, and fatty acids. They all exhibit significant pharmacological properties, such as anti-inflammatory and antioxidant effects. Traditionally, its bark and roots are employed as digestive aids and antibiotics, while the leaves serve as treatments for fever and dysentery [5],[6],[7]. Despite its widespread use in traditional medicine, no studies have focused on its effects on glucose metabolism. For the first time, this study aims to examine the potential hypoglycemic properties of *Ochna integerrima* by focusing on its ability to inhibit key enzymes involved in glucose metabolism, namely α-amylase and α-glucosidase.

2. Materials and methods

2.1. Plant material and extraction

Flowers of *O. integerrima* were gathered in Can Tho City, Vietnam, in January 2023 and identified by Associate Professor Dang Van Son, a botanist at the Institute of Life Sciences, Vietnam Academy of Science and Technology, Ho Chi Minh City. The voucher specimen (CT-OIF) is stored at the Faculty of Pharmacy, Ton Duc Thang University, and the Organic Chemistry Lab 1, College of Natural Sciences, Can Tho University, Vietnam.

Extraction: Air-dried flowers of *O. integerrima* (11.5 kg) were subjected to maceration in methanol (15 L × 3) for 48 hours, after which the solvent was evaporated under reduced pressure to produce 850 g of crude methanol extract. This extract was then partitioned using liquid-liquid extraction with *n*-hexane (10 L × 3), DCM (7 L × 3), and EtOAc (9 L × 3), yielding the following extracts: 147.8 g of *n*-hexane, 14.5 g of DCM, 237.4 g of EtOAc, and 350.5 g of the aqueous extracts.

Isolation: The *n*-hexane extract was separated using normal phase column chromatography (NPCC) with an increasing polarity gradient of *n*-hexane-$CHCl_3$, $CHCl_3$-MeOH, resulting in four fractions (FrH1-FrH4). Compound **1** (102.5 mg) was purified from FrH2 (24.1 g) by *silica gel* column chromatography (CC) using an *n*-hexane-$CHCl_3$ (8:2). FrH4 was chromatographed by *silica gel* CC using $CHCl_3$-MeOH (9:1) to yield **2**. Out of five subfractions (FrA1-FrA6), reversed phase CC) was used to isolate **3** (4.1 mg) and **4** (10.3 mg) from FrA1 of the aqueous extract. MeOH-water (35:65) was used for elution, and then normal phase preparative TLC with $CHCl_3$-MeOH-water (65:35:10) was performed.

2.2. Methods

2.2.1. Chemicals and instruments:

Nuclear Magnetic Resonance (NMR) spectra were acquired using Bruker Avance NEO spectrometers (Bruker, USA), operating at 500 MHz for ^1H and 125 MHz for ^{13}C. The optical density (OD) of *α*-amylase and *α*-glucosidase inhibitory activities were measured using a Thermo Fisher Scientific UV-Vis spectrophotometer. These enzymes, *p*-nitrophenyl-*α*-D-glucopyranoside (*p*NPG), and acarbose, were supplied by Sigma Aldrich. Iodine and starch were provided by Fisher Scientific.

2.2.2. *α*-Amylase and *α*-glucosidase inhibitory assays:

The *α*-amylase inhibitory assay was evaluated using the method described by Moein et al., with minor modifications [8]. The *n*-hexane, DCM, EtOAc, and MeOH extracts (31.25 to 500 µg/mL) and aqueous extract (1.95 to 31.25 µg/mL) were mixed with 150 µL enzyme solution (2.5 units/mL). Then, 300 µL of 0.01 M phosphate buffer (pH 6.9) was incubated at 37°C for 10 minutes. After the incubation, 750 µL of starch 0.01% was added and incubated at 37°C for 10 minutes. 300 µL of 1.0 M HCl was added to stop the reaction. After the reaction, 90 µL of 10% iodine was used to detect the remaining starch. Each assay was performed in triplicate. The absorbance was recorded at 660 nm. Acarbose was used as a positive control, and the IC_{50} values were calculated as the mean ± SD of the three experiments. The enzyme inhibitory rates were calculated as follows:

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

Where $A_{control}$ and A_{sample} are the absorbances of control and extracts

The *α*-glucosidase inhibitory assay was evaluated using the method outlined by Guo et al., with slight changes [9]. 50 µL of the extracts (6.25 to 100 µg/mL) and compounds (6.25 to 100 µM) were mixed with 10 µL enzyme solution (0.5 units/mL) and 60 µL of 0.01 M phosphate buffer (pH 6.9) in a 96-well plate. This mixture was incubated at 37°C for 15 minutes. Following the incubation, 20 µL of *p*NPG (5 mM) prepared in the same buffer was added and incubated at 37°C for 15 minutes. 80 µL of 0.2 M sodium carbonate solution was added to stop the reaction. Each assay was performed in triplicate. The hydrolysis of *p*NPG was monitored at a 405 nm reader. The control sample consists of no extract. The IC_{50} values were determined as the mean ± SD of the three tests, with acarbose as a positive control. The following formula was used to get the enzyme inhibitory rates:

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

Where $A_{control}$ and A_{sample} are the absorbances of control and extracts

2.5. Statistical analysis

The data are presented as mean ± standard deviation (n = 3). Statistical analyses were performed using GraphPad Prism version 9.3.1 (San Diego, CA, USA).

3. Results and discussion

3.1. Qualitative analysis of phytochemicals

Flavonoids, steroids, polysaccharides, saponin glycosides, and tannins were detected in the MeOH extract by preliminary phytochemical screening. Alkaloids, however, were not found in this extract.

Table 1. Preliminary phytochemical screening of MeOH extract from flower

Phytoconstituents	Reagents	MeOH extract
Alkaloids	Dragendorff and Mayer	-
Flavonoids	Shinoda	+
Steroids	Liebermann-Burchard and Salkowski	+
Carbohydrates	Fehling	+
Saponin glycosides	Foam test	+
Tannins	Ferric chloride	+

(-): negative; (+): positive.

3.2. In vitro α-amylase and α-glucosidase inhibition of the extracts

The antidiabetic and anti-*α*-amylase potentials of *O. integerrima* extracts were assessed through *α*-amylase and *α*-glucosidase inhibition assays. The findings were reported as IC_{50} values and compared to acarbose, a known antidiabetic medication.

Anti-α-amylase activity: The aqueous extract exhibited remarkable inhibitory activity, with the lowest IC_{50} value of 7.13 ± 0.13 µg/mL, suggesting it is the most potent among the extracts tested. The *n*-hexane extract followed with an IC_{50} of 42.45 ± 0.69 µg/mL. In contrast, the MeOH and EtOAc extracts showed higher IC_{50} values of 111.0 ± 0.86 µg/mL and 170.8 ± 5.44 µg/mL, respectively, indicating weaker inhibitory effects. Since aqueous extract exhibited strong anti-*α*-amylase activity with an IC_{50} value lower than 62.5 µg/mL, its concentration range was adjusted from 1.95 to 31.25 µg/mL. The positive control, acarbose, had an IC_{50} of 108.9 ± 2.52 µg/mL. This result suggested that the aqueous extract significantly inhibited the enzyme activity more than other extracts (Fig. 1 and Table 2).

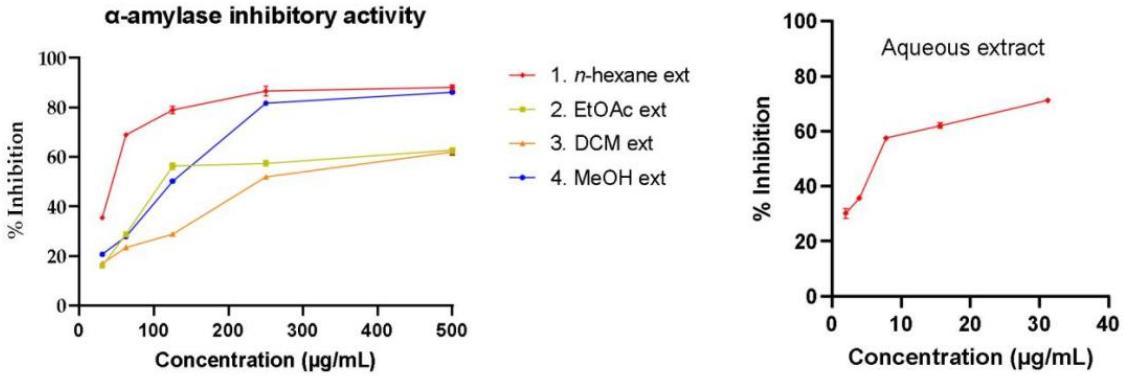

Fig. 1. Anti- *α*-amylase activity of *O. integerrima* flower extracts

Anti-α-glucosidase activity: Among the tested extracts, the aqueous extract exhibited the most effective *α*-glucosidase inhibitory activity, with the lowest IC_{50} value of 8.78 ± 0.26 µg/mL. The MeOH and EtOAc extracts demonstrated good inhibitory effects as well, as evidenced by their higher IC_{50} values of 14.84 ± 0.7 µg/mL and 16.45 ± 0.03 µg/mL, respectively. The *n*-hexane extract showed moderate activity, with an IC_{50} of 71.82 ± 1.43 µg/mL. The IC_{50} for acarbose, the positive control, was 108.9 ± 2.52 µg/mL (Fig. 2 and Table 1). These findings imply that the aqueous extract considerably reduced the target activity compared to the other extract. It was consistent with the observed *α*-amylase inhibitory activity.

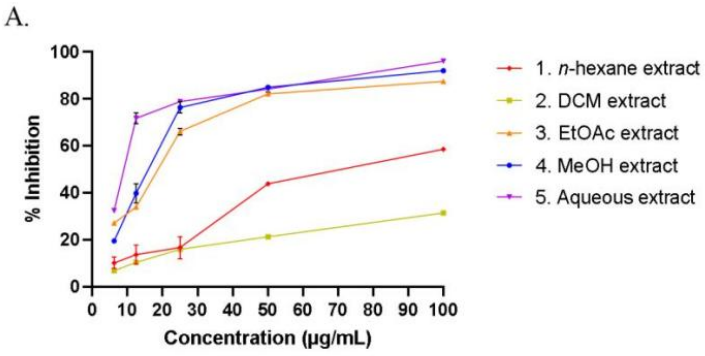

Fig. 2. Anti-α-glucosidase activity of *O. integerrima* flower extracts

Table 2. IC_{50} values for the inhibition of α-amylase and α-glucosidase of the extracts

Extracts	IC_{50} (µg/mL)	
	α-amylase inhibition	α-glucosidase inhibition
n-Hexane	42.45 ± 0.69	71.82 ± 1.43
DCM	271.0 ± 7.62	>100
EtOAc	170.8 ± 5.44	16.45 ± 0.03
MeOH	111.0 ± 0.86	14.84 ± 0.70
Aqueous	7.16 ± 0.13	8.78 ± 0.26
Positive control (Acarbose)	70.52 ± 0.58	108.9 ± 2.52

The significant α-amylase inhibition observed in the *n*-hexane and aqueous extracts of *O. integerrima* may be attributed to bioactive compounds that effectively block the activity of the α-amylase enzyme involved in carbohydrate digestion. The *n*-hexane extract primarily contains lipophilic compounds such as terpenoids, whereas the aqueous extract is rich in hydrophilic compounds, including flavonoids and polyphenols. These compounds likely interact with the enzyme's active site, preventing the breakdown of starch into sugars, which could reduce blood glucose levels [10]. The EtOAc and MeOH extracts exhibited potent α-glucosidase inhibition, although their effects were more moderate compared to the anti-amylase activity. It suggests that these enzymes operate at different stages of carbohydrate digestion, with both playing critical roles in converting dietary carbohydrates into glucose.

3.3. The isolation and elucidation of ***1-4***

Prioritizing α-amylase activity is considered an effective way to manage blood sugar levels because it is the first step in carbohydrate breakdown. Therefore, we conducted isolation due to the active inhibition of α-amylase activity. Based on these promising results, the aqueous and *n*-hexane extracts demonstrated the highest and second-highest α-amylase inhibitory activity. These extracts were selected for further isolation and characterization to identify the bioactive compounds responsible for their observed enzymatic inhibition. Therefore, compounds **1** and **2** were isolated from *n*-hexane extract. Compounds **3** and **4** were isolated from aqueous extract. Their structures were elucidated by ^{1}H and ^{13}C NMR data and compared with published literature (Fig. 3).

Compound **1**: White crystal; Two multiplets at δ 3.51 and δ 5.35, indicative of the protons at H-3 and H-6 of the steroidal nucleus, are visible in the ^{1}H-NMR spectra of stigmasterol. Furthermore, two olefinic protons are present at δ_H 5.14 (1H, dd) and δ_H 5.04 (1H, dd), corresponding to the protons at H-22 and H-23; methyl groups δ_H 0.69 (3H, s, H-18), 1.00 (3H, s, H-19), 0.92 (3H, d, J = 7.0 Hz, H-21), 0.78 (3H, d, J = 8.0 Hz, H-26), 0.83 (3H, d, J = 8.0 Hz, H-27), and 0.80 (3H, t, J = 9.0 Hz, H-29). It was named stigmasterol by comparing it with published data [10],[11] (Fig. 3).

Compound **2**: White powder; ^{1}H NMR (500 MHz, CD_3OD): typical signals at δ_H 0.81-1.01 ppm (3×7CH_3), 2.82 (1H, dd, J = 7.5; 8.0 Hz, H-

18), 1.95 (2H, s, H-16), 5.25 (1H, t, J = 3.2, H-12), 3,65 (1H, m, H-2), 3,42 (1H, m, H-3). ^{13}C NMR (125 MHz, CD_3OD): δ_C 47.5 (C-1), 68.1 (C-2), 83.1 (C-3), 39.1 (C-4), 55.3 (C-5), 18.2 (C-6), 32.2 (C-7), 39.2 (C-8), 48.2 (C-9), 38.1 (C-10), 23.3 (C-11), 122.0 (C-12), 144.0 (C-13), 41.5 (C-14), 27.9 (C-15), 22.6 (C-16), 46.2 (C-17), 41.3 (C-18), 46.2 (C-19), 30.4 (C-20), 33.8 (C-21), 32.8 (C-22), 27.9 (C-23), 15.7 (C-24), 15.9 (C-25), 16.7 (C-26), 25.0 (C-27), 180.5 (C-28), 32.2 (C-29), 22.6 (C-30). It was assigned as maslinic acid by comparison with published data [12],[13].

Compound **3**: Yellow powder; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 4.99 (1H, d, J = 9.5, H-2), 4.56 (1H, d, J = 9.5 Hz, H-3), 6.29 (1H, s, H-8), 6.97 (1H, brs, H-2′), 6.85 (1H, d, J = 6.5 Hz, H-5′), 6.80 (1H, d, J = 6.5, H-6′), 4.93 (1H, d, J = 9.5 Hz, H-1‴), 3.67-3.31 (6H, H-2‴-H-6‴), 1.78 (s, H-5″), 1.65 (m, H-4″), 5.22 (m, H-2″). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 84.0 (C-2), 73.4 (C-3), 198.1 (C4), 101.8 (C4a), 160.0 (C-5), 110.7 (C-6), 163.5 (C-7), 94.0 (C-8), 161.0 (C-8a), 128.4 (C-1’), 114.5 (C-2’), 145.0 (C-3′), 145.8 (C-4′), 114.6 (C-5′), 119.6 (C-6′), 20.7 (C-1″), 122.3 (C-2″), 130.6 (C-3″), 16.6 (C-4″), 24.5 (C-5″), 100.0 (C-1‴), 73.4 (C-2‴), 76.9 (C-3‴), 69.8 (C-4‴), 76.9 (C-5‴), 61.0 (C-6‴). It was identified as 6-(γ,γ-dimethylallyl)taxifolin 7-*O*-β-D-glucoside ($C_{26}H_{30}O_{12}$) based on a comparison with published data [5]

Compound **4**: Pale yellow powder; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 4.99 (1H, d, J = 10.0, H-2), 4.70 (1H, d, J = 10.0 Hz, H-3), 6.28 (1H, s, H-8), 7.36 (2H, brs, H-2′, H6′), 6.84 (2H, d, J = 8.0 Hz, H-3′, H-5′), 5.21 (1H, d, J = 9.5 Hz, H-1‴), 3.78-3.35 (6H, H-2‴-H-6‴), 1.80 (s, H-5″), 1.70 (m, H-4″), 5.31 (m, H-2″). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 85.0 (C-2), 73.8 (C-3), 198.9 (C4), 102.9 (C4a), 161.0 (C-5), 110.9 (C-6), 164.9 (C-7), 94.9 (C-8), 161.8 (C-8a), 128.9 (C-1’), 130.7 (C-2’), 116.2 (C-3′), 159.3 (C-4′), 116.2 (C-5′), 129.9 (C-6′), 22.1 (C-1″), 124.1 (C-2″), 132.2 (C-3″), 18.2 (C-4″), 26.1 (C-5″), 102.1 (C-1‴), 78.6-63.0 (5C, C-2‴- C-6‴).^{1}H and ^{13}C-NMR data of **4** was similar to that of **3**, lacking the -OH group at C-3'. This difference was confirmed by comparing it with the published data [4]. It was identified as 6-γ,γ-dimethylallyldihydrokaempferol 7-*O*-β-D-glucoside ($C_{26}H_{30}O_{11}$).

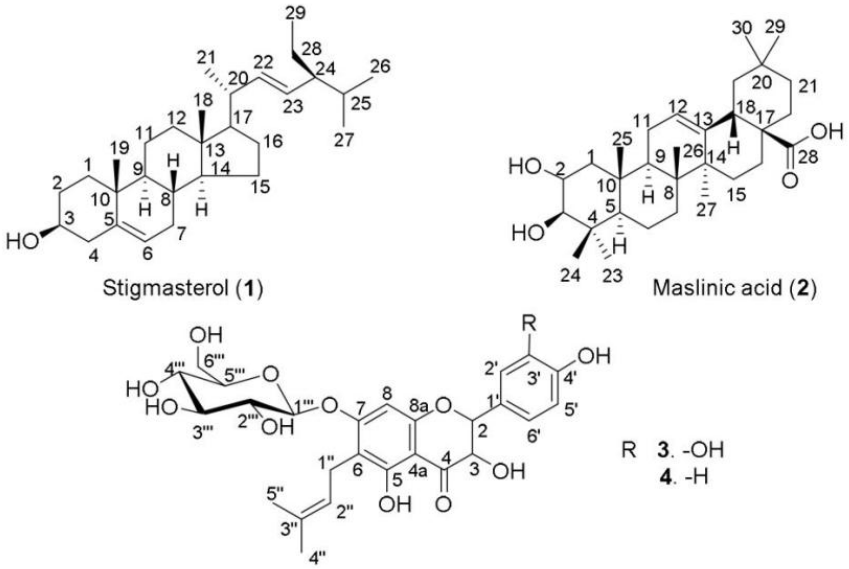

Fig. 3. Chemical structures of **1**–**4**.

*3.4. In vitro α-amylase inhibition of **1–4***

Compounds **1**–**4** were assessed to validate this activity of the extracts. As the results, compounds **1** and **2** showed significant α-amylase inhibitory activity with IC_{50} values of 71.65 ± 1.34 and 48.06 ± 1.6 (Fig. 4 and Table 3), respectively which could explain the potency of the *n*-hexane extract. Our results were consistent with those of other

publications. Previous studies indicated that **1** inhibited glucosidase and amylase enzymes due to its chemical structure and ability to interact with the active sites of these enzymes, which influences glucose metabolism [14],[15]. Hyperglycemia and dyslipidemia can arise from impaired insulin secretion caused by cholesterol accumulation. With its anti-atherosclerotic qualities, stigmasterol could help protect *β*-cell function (insulin-producing cells in the pancreas) [16]. Maslinic acid (**2**) plays a significant role in modulating glycogen metabolism by enhancing the insulin signaling pathway and inhibiting glycogen phosphorylase, which leads to improved glucose homeostasis and enhanced glycogen storage. It contributes to its potential as an antidiabetic treatment, offering promise for treating type 2 diabetes. In addition to its impact on glycogen metabolism, it demonstrates potential by improving insulin sensitivity and lowering blood glucose levels [17].

However, compounds **3** and **4** from the aqueous extract did not exhibit any anti-*α*-amylase activity with an IC_{50} value of more than 100 µM, contrasting with the potency observed in the extract. The difference may be explained by the formulation, as the extract contains many compounds that work together synergistically. Furthermore, the parent flavonoids exhibited a strong anti-*α*-glucosidase effect [18]. The presence of *O*-glycosylation at C-7 and prenylation at C-6 reduced their *α*-glucosidase inhibitory potency due to steric hindrance and solubility issues, as evidenced in many publications [19],[20]. Importantly, further isolation from other fractions of the aqueous extract is essential to identify the main bioactive compounds responsible for the antidiabetic effects.

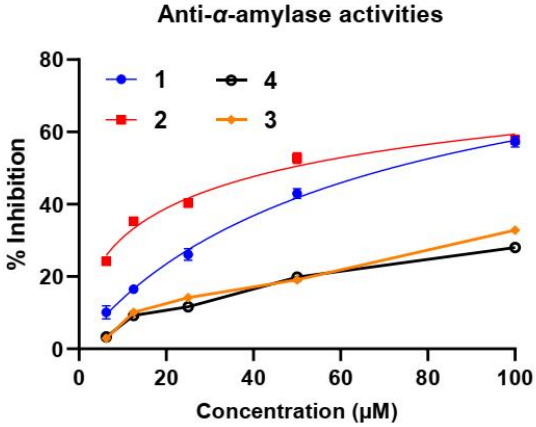

Fig. 4. Anti-*α*-amylase activity of **1-4**

Table 3. IC_{50} values for *α*-amylase inhibition of **1-4**

Compound	*α*-amylase inhibition IC_{50} (µM)
1	71.65 ± 1.34
2	48.06 ± 1.6
3	> 100
4	> 100

4. Conclusion

This research was the first to demonstrate the *α*-amylase and *α*-glucosidase inhibitory effects of extracts from the flower of *Ochna integerrima*. The *n*-hexane extract had good *α*-amylase and *α*-glucosidase inhibition, with **1** and **2** showing significant *α*-amylase inhibitory activity. The aqueous extract exhibited the most potent properties, with IC_{50} values of 7.16 ± 0.13 and 8.78 ± 0.26 µg/mL, respectively. Since compounds **3** and **4** from the aqueous extract did not show significant activity, with IC_{50} values

greater than 100 µM, the observed activity may be due to a synergistic effect. Hence, additional isolation steps are required to prove specific compounds contributing to the enzyme inhibitory effects of the extract. To sum up, this species suggests its potential for preventing and treating type 2 diabetes.

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