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α-Glucosidase inhibition of Vietnam's medicinal plants belonging to Zingiberaceae family and some flavonoids isolated from the rhizomes of *Zingiber zerumbet* Sm.

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Abstract. 23 extracts from the rhizomes of seven An Giang's medicinal plants belonging to the Zingiberaceae family were tested for *α*-glucosidase inhibitory activity. The screening results showed that 21/23 samples showed IC_{50} values less than 250 µg/mL, 17 samples with IC_{50} values below 100 µg/mL, and 6 samples displayed IC_{50} values below 10 µg/mL. *Zingiber zerumbet* is a perennial herb belonging to the Zingiberaceae family, and its rhizomes show various biological activities. A further phytochemical study on the $CHCl_3$ extract of this plant furnished the isolation of seven flavonoids, consisting of naringenin (**1**), aromadendrin (**2**), kaempferol (**3**), *iso*-kaempferide (**4**), afzelin (**5**), kaempferol-3-*O*-(4"-*O*-acetyl)-*α*-L-rhamnopyranoside (**6**), and kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl)-*α*-L-rhamnopyranoside (**7**). Their structures were determined based on extensive spectroscopic analysis and in comparison with previous works. Among them, compounds **1**, **2**, **5**, and **7** have been found for the first time in this plant. All compounds possessed significant *α*-glucosidase inhibitory activity and showed more potent inhibitory activity than that of a positive control, acarbose (IC_{50}, 214.5 µM or 138.2 µg/mL).

Keywords: *Zingiber zerumbet*, Zingiberaceae, flavonoids, *α*-glucosidase.

Classification numbers: 1.1.1.

1. INTRODUCTION

Enzyme *α*-glucosidase (EC 3.2.1.20) exists in most organisms. In humans, the enzyme *α*-glucosidase is found on the surface membranes of the intestinal tract and is involved in the final stage of carbohydrate metabolism [1]. When blood glucose rises, an increase in insulin secretion occurs to control blood glucose levels. After that, blood glucose continues to rise and the pancreas's ability to produce insulin will not respond to the increased level of blood glucose, leading to type II diabetes [1]. Thus, inhibiting the enzyme *α*-glucosidase and reducing the absorption of sugar will lower blood glucose levels to a normal state [2]. To search for natural

sources of *α*-glucosidase inhibition, we have screened the activity of 23 extracts from the rhizomes of An Giang's medicinal plants belonging to the Zingiberaceae family which are *Alpinia officinarum*, *Boesenbergia pandurata*, *Curcuma aromatica*, *C. zanthorrhiza*, *C. zedoaria*, *Zingiber montanum*, and *Z. zerumbert*. However, there are no reports on the *α*-glucosidase inhibitory activity of these plants from Viet Nam.

The *Z. zerumbet* is native to India and is mainly found in Southeast Asia [3, 4]. In Viet Nam, this plant is called "Gừng gió" and is widely grown in the northern mountainous provinces [3]. In the fall, its rhizomes can be used freshly soaked in wine or can be sliced and then dried as a food flavoring and appetizer [4]. The rhizome of *Z. zerumbet* has traditionally been used in herbal medicine to treat a variety of conditions, including inflammation, fever, toothache, indigestion, constipation, diarrhea, severe sprains, and to relieve pain, as well as antispasmodic, antirheumatic, and diuretic properties [3, 4]. According to reports, the plant has a plenty of high medicinal values because of the variety of its chemical constituents consisting of flavonoids and monoterpenes [5-8]. Some flavonoids have been previously reported to exhibit inhibitory effects against α-glucosidase enzyme, these flavonoids exhibit both hypoglycemic and antioxidant effects in diabetic animals [9, 10]. Besides, its pharmacological potentials have been reported to exhibit a wide range of biological activities, such as anti-inflammatory, antinociceptive, antiulcer, antioxidant, anticancer, antimicrobial, antihyperglycemic, antiallergic, antioxidant, and antiplatelet effects across various doses and concentrations [4].

As part of a research program aimed at identifying biologically active compounds capable of reducing glucose uptake, screening studies were conducted to assess natural product extracts for their *α*-glucosidase. Hence, we carried out the bioactivity-guided fraction of the $CHCl_3$-soluble extract of the rhizomes of *Z. zerumbet* which led to seven flavonoids. In this paper, we describe the screening *α*-glucosidase inhibitory activity of 23 extracts from seven Vietnamese Zingiberaceae plants. Additionally, the isolation and structural elucidation of seven flavonoids from *Z. zerumbet* together with their *α*-glucosidase inhibitory activity.

2. MATERIALS AND METHODS

2.1. General experimental procedures

UV spectra were recorded using a Shimadzu UV-1800 spectrophotometer (Shimadzu Pte. Ltd.). NMR experiments were performed on a Bruker Avance III 500 instrument (Bruker BioSpin AG, Bangkok, Thailand), and chemical shifts were reported in *δ* (ppm). High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data were acquired on a Bruker micrOTOF-QII mass spectrometer (Bruker Singapore Pte. Ltd., Singapore). Column chromatography was conducted using silica gel 60 (40–63 μm, 230–400 mesh ASTM) supplied by Scharlau (Barcelona, Spain). Analytical and preparative thin-layer chromatography (TLC) was carried out on precoated Kieselgel 60 F254 or RP-18 plates (0.25 or 0.5 mm thickness, Merck KGaA, Darmstadt, Germany). α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae* (750 UN) and *p*-nitrophenyl-*α*-D-glucopyranoside were obtained from Sigma Chemical Co. (St. Louis, MO, USA), while acarbose was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade or higher.

2.2. Plant materials

Seven Vietnamese species belonging to the Zingiberaceae family used in this study were collected in An Giang Province, Viet Nam, in April 2015 and were taxonomically identified by Ms. Hoang Viet (Faculty of Biology, University of Science, Vietnam National University–Ho Chi Minh City, VNU-HCM). Voucher specimens have been deposited at the Division of Medicinal Chemistry, Faculty of Chemistry, University of Science, VNU-HCM, where they were stored under cool, dry, and dark conditions to minimize exposure to light and heat. The rhizomes (100–200 g) of each plant were cut into small pieces and subjected to Soxhlet extraction using *n*-hexane, chloroform, ethyl acetate, methanol, and water for each sample.

The rhizomes of *Zingiber zerumbet* Sm. were collected in An Giang Province, Viet Nam, in March 2017 and were taxonomically identified by Dr. rer. nat. Anh Tuan Dang-Le (Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, Viet Nam). A voucher specimen (MCE0062) has been deposited at the Division of Medicinal Chemistry, Faculty of Chemistry, University of Science, Vietnam National University–Ho Chi Minh City (VNU-HCM).

2.3. Extraction and isolation

The dried powdered rhizomes of *Z. zerumbet* (5.3 kg) were refluxed with MeOH to yield the MeOH-soluble extract (620 g). This extract was suspended in H_2O and then successively partitioned with *n*-hexane, $CHCl_3$, and EtOAc to yield the corresponding fractions, including the *n*-hexane (150 g)-, $CHCl_3$ (60 g)-, EtOAc (20 g)-, and H_2O (360 g)-soluble extracts. A part of the $CHCl_3$-soluble extract (50 g) was subjected to silica gel column (6.5 × 120 cm) chromatography, eluted with MeOH–$CHCl_3$ (0–100 %) to yield ten fractions: fr.1 (6.2 g), fr.2 (8.0 g), fr.3 (11.5 g), fr.4 (2.7 g), fr.5 (4.9 g), fr.6 (5.0 g), fr.7 (4.8 g).

The air-dried rhizomes of *Zingiber zerumbet* were ground into powder (5.3 kg) and extracted under reflux with methanol to obtain a crude methanolic extract (620 g). The extract was subsequently suspended in water and partitioned sequentially with *n*-hexane, chloroform, and ethyl acetate, affording the respective fractions: *n*-hexane (150 g), chloroform (60 g), ethyl acetate (20 g), and aqueous (360 g) extracts. A portion of the chloroform fraction (50 g) was subjected to column chromatography over silica gel (6.5 × 120 cm), using a gradient elution system of methanol–chloroform (0–100%) to yield ten fractions (fr.1–fr.10), including fr.1 (6.2 g), fr.2 (8.0 g), fr.3 (11.5 g), fr.4 (2.7 g), fr.5 (4.9 g), fr.6 (5.0 g), and fr.7 (4.8 g).

Fraction 2 was further separated by silica gel column chromatography using a gradient elution of ethyl acetate–*n*-hexane to afford twelve subfractions (fr.2.1–fr.2.12). Subfraction 2.12 was subsequently chromatographed on silica gel with an ethyl acetate–*n*-hexane system (0–80%) to yield three subfractions (fr.2.12.1–fr.2.12.3). Subfraction 2.12.1 was further fractionated using an ethyl acetate–*n*-hexane gradient (0–50%) and finally purified by normal-phase preparative TLC employing an acetone–chloroform (5:95) system to afford compounds **1** (5.4 mg) and **4** (4.4 mg).

Fraction 3 was subjected to silica gel column chromatography using a gradient system of acetone–chloroform to afford twelve subfractions (fr.3.1–fr.3.12). Subfraction 3.7 was further separated on silica gel with an ethyl acetate–*n*-hexane gradient (0–80%) to yield nine subfractions (fr.3.7.1–fr.3.7.9). Subfractions 3.7.1 and 3.7.2 were subsequently purified by

normal-phase preparative TLC using an acetic acid–ethyl acetate–*n*-hexane (1:20:80) solvent system, affording compounds **2** (5.0 mg) and **3** (5.7 mg), respectively.

Fraction 6 was subjected to silica gel column chromatography and eluted with a gradient system of acetone–*n*-hexane to afford twelve subfractions (fr.6.1–fr.6.12). Both subfractions 6.1 and 6.3 were further purified by repeated silica gel column chromatography using an acetone–*n*-hexane gradient (0–50%), yielding subfractions fr.6.1.1–fr.6.1.2 and fr.6.3.1–fr.6.3.2, respectively. These were subsequently purified by normal-phase preparative TLC with an ethyl acetate–*n*-hexane (50:50) solvent system to afford compounds **7** (5.3 mg) from fr.6.1.1 and **6** (5.5 mg) from fr.6.3.1. Subfraction 6.8 was further rechromatographed on silica gel using an acetone–*n*-hexane gradient (0–80%) to give four subfractions (fr.6.8.1–fr.6.8.4), followed by purification *via* normal-phase preparative TLC with ethyl acetate–*n*-hexane (40:60) to yield compound **5** (4.4 mg).

2.4. α-Glucosidase inhibitory activity assay

The α-glucosidase inhibitory activity was evaluated following previously reported procedures [11, 12]. The assay mixture, prepared freshly prior to use, contained 525 μL of the test sample in 0.01 M phosphate buffer (pH 7.0) and 50 μL of enzyme solution (α-glucosidase, 0.1 U/mL in the same buffer). After preincubation at 37 °C for 5 min, the enzymatic reaction was initiated by adding 50 μL of substrate solution (1.5 mM *p*-nitrophenyl-α-D-glucopyranoside in 0.01 M phosphate buffer, pH 7.0). The reaction mixture was then incubated at 37 °C for 30 min and subsequently terminated by the addition of 375 μL of 0.1 M Na_2CO_3 solution. Enzyme activity was determined by measuring the absorbance at 401 nm using a Shimadzu UV-1800 spectrophotometer. One unit of α-glucosidase activity was defined as the amount of enzyme required to release 1.0 μM of *p*-nitrophenol per minute. The percentage inhibition (I%) was calculated accordingly, and IC_{50} values were defined as the concentrations of inhibitors required to reduce enzyme activity by 50%. Acarbose was employed as a positive control.

3. RESULTS AND DISCUSSION

3.1. The α-glucosidase inhibitory activity of plant extracts

In this study, 23 extracts from the rhizomes of seven Zingiberaceae plants were tested for α-glucosidase inhibitory activity at five initial concentrations ranging from 10 – 250 μg/mL in the Table 1.

Table 1. α-Glucosidase inhibitory activity of extracts.

Plant name	Extracts	Percentage inhibition (%)				IC_{50} (μg/mL)
		250 μg/mL	100 μg/mL	10 μg/mL	1 μg/mL	
A. officinarum	Hexane	*	*	16.4 ± 1.6	–	17.1 ± 0.4
	EtOAc	*	*	*	48.24 ± 0.65	1.10 ± 0.05
	MeOH	*	89.77 ± 0.46	28.03 ± 0.61	–	27.5 ± 0.7
B. pandurata	$CHCl_3$	*	96.2 ± 1.9	27.5 ± 2.9	–	17.2 ± 0.4
	EtOAc	*	64.3 ± 1.8	–	–	60.9 ± 1.5
	MeOH	*	97.3 ±1.6	19.6 ± 2.9	–	30.4 ± 0.8
C. aromatica	Hexane	53.1 ± 2.9	27.9 ± 2.2	–	–	231.3 ± 3.5
	$CHCl_3$	*	*	65.93 ± 0.24	2.91 ± 0.50	7.2 ± 0.3
	EtOAc	*	*	60.19 ± 0.54	8.98 ± 0.77	8.1 ± 0.4

	H_2O	*	92.65 ± 0.81	36.8 ± 1.6	–	19.7 ± 0.5
C. zanthorrhiza	Hexane	*	*	31.5 ± 2.9	–	15.3 ± 0.4
	EtOAc	*	98.32 ± 0.48	10.6 ± 2.4	–	28.6 ± 0.7
	MeOH	*	*	51.84 ± 0.69	–	9.7 ± 0.4
C. zedoaria	Hexane	*	*	93.81 ± 0.74	–	5.8 ± 0.3
	EtOAc	*	89.89 ± 0.13	3.54 ± 0.45	–	40.9 ± 1.0
	MeOH	*	*	82.6 ± 2.9	–	6.9 ± 0.3
Z. montanum	Hexane	87.9 ± 1.8	49.1 ± 2.5	–	–	103.5 ± 1.6
	EtOAc	92.7 ± 2.8	58.1 ± 1.3	–	–	84.8 ± 2.1
	MeOH	46.1 ± 2.2	33.2 ± 2.1	10.2 ± 1.4	–	> 250
Z. zerumbert	Hexane	79.1 ± 2.8	18.6 ± 1.4	–	–	177.7 ± 2.7
	$CHCl_3$	94.8 ± 1.5	40.2 ± 2.9	–	–	126.9 ± 1.9
	EtOAc	72.1 ± 2.2	52.0 ± 3.2	–	–	95.7 ± 2.4
	MeOH	25.2 ± 2.0	7.6 ± 1.1	–	–	> 250
Acarbose[δ]						138.2 ± 2.1

*: I > 99 %; –: <1 %; [δ]: Positive control.

The results of screening showed that all the extracts illustrated *α*-glucosidase inhibitory activity both at concentrations of 250 μg/mL and 100 μg/mL, among which 21 samples displayed an inhibition rate greater than 50 % at 250 μg/mL and 17 samples exhibited an inhibition of more than 50 % at 100 μg/mL. Altogether, 15 extracts were found to be active at a concentration of 10 μg/mL, and 6 samples of them had an inhibition over 50 %. They continued to be tested for *α*-glucosidase inhibitory assay at four concentrations ranging from 1 – 10 μg/mL, and 3 samples possessed the inhibition at a concentration of 1 μg/mL. The detailed data of percentage inhibition of all concentrations were demonstrated in the table of supporting information; meanwhile, the Table 1 has been shortened to include only the IC_{50} values. In total, 21/23 samples showed IC_{50} values less than 250 μg/mL, 17 samples with IC_{50} values below 100 μg/mL, and 6 samples displayed IC_{50} values below 10 μg/mL. The IC_{50} value of acarbose, a positive control, was 138.2 μg/mL.

Among all of plants, the *A. officinarum* and *Z. zerumbert* are used more widespread than that of others [4] in daily life. Unlike *A. officinarum* has many diarylheptanoids and their derivatives, *Zingber zerumbert* has been previously reported containing a lot of flavonoids [7, 8]. The flavonoid is a special group of polyphenols ubiquitously distributed in plant kingdoms and important functional compositions of human diets. Some flavonoids have been previously reported to exhibit *α*-glucosidase inhibitory effects, showing both hypoglycemic and antioxidant effects in diabetic animals. Their roles could be directly associated with their specific structural features [9, 10, 13].

3.2. The structural elucidation of seven compounds from *Z. zerumbet*

The dried powdered rhizomes of *Z. zerumbet* were refluxed with MeOH to yield the MeOH-soluble extract. This extract was suspended in H_2O and then successively partitioned with *n*-hexane, $CHCl_3$, and EtOAc to yield the corresponding fractions. Present work targeted for phytochemical study of $CHCl_3$-soluble extract because the $CHCl_3$-soluble extract of the rhizomes of *Zingiber zerumbet* displayed positive inhibitory *α*-glucosidase activity with an IC_{50} value of 126.9 μg/mL compared to acarbose, a positive control, (IC_{50}, 138.2 μg/mL). Therefore,

the $CHCl_3$-soluble extract was subjected to a series of column chromatographic separation steps and preparative TLC to afford seven flavanones (Figure 1).

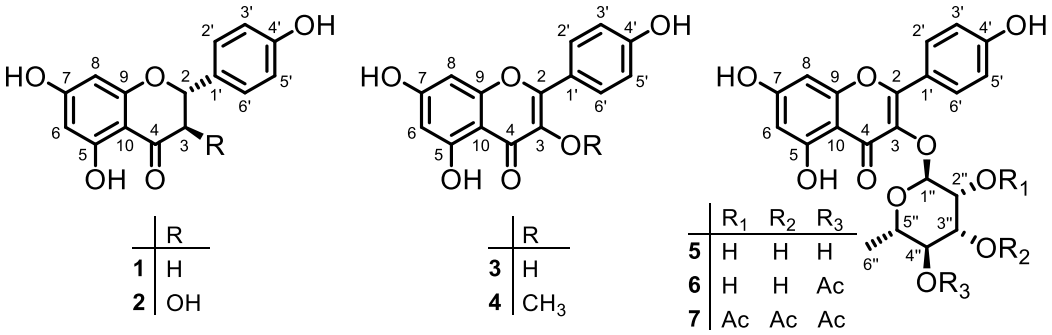

Figure 1. Chemical structures of isolated compounds.

Compound **1** was isolated as a white, amorphous solid. The compound showed a sodium adduct molecular ion at *m/z* 295.0577 $[M + Na]^+$ in HR-ESI-MS, corresponding to the empirical formula $C_{15}H_{12}O_5Na^+$ (calculated for 295.0577) thus confirming the molecular formula as $C_{15}H_{12}O_5$. The ^{1}H-NMR spectrum displayed signals corresponding to a set of two *meta*-coupled aromatic protons [δ_H 5.95 (1H; *d*; *J* = 2.1; H-6)] and [δ_H 5.96 (1H; *d*; *J* = 2.1; H-8)], symmetrical pairs of *orthro*-coupled aromatic protons [δ_H 7.39 (2H; *d*; *J* = 8.5; H-2', H-6')] and [δ_H 6.90 (2H; *d*; *J* = 8.5; H-3', H-5')], one oxymethine proton [δ_H 5.45 (1H; *dd*; *J* = 12.9, 3.0; H-2)], two methylene protons [δ_H 3.17 (1H; *dd*; *J* = 17.1, 12.9; H-3a)] and [δ_H 2.73 (1H; *dd*; *J* = 17.1, 3.0; H-3b)], together with a characteristic signal of a intramolecular hydrogen bonding hydroxyl group [δ_H 12.17 (1H; *s*; OH-5)] (Table 2). Additionally, the ^{13}C-NMR spectrum revealed 15 carbon signals including those for a ketone carbonyl carbon [δ_C 197.1 (C-4)]; four oxygenated aromatic carbons [δ_C 167.5 (C-5)], [δ_C 165.0 (C-7)], [δ_C 164.4 (C-9)], and [δ_C 158.7 (C-4')]; two quarternary aromatic carbons [δ_C 103.1, (C-10)] and [δ_C 130.8, (C-1')]; six aromatic methine carbons [δ_C 96.8 (C-6)], [δ_C 95.9 (C-8)], [δ_C 129.0 (C-2', C-6')], and [δ_C 116.2 (C-3', C-5')]; an oxymethine carbon [δ_C 79.9 (C-2)] and a methylene carbon [δ_C 43.6 (C-3)]. These data resembled analogous data for a flavanone having three hydroxyl groups. Therefore, the structure of compound **1** was concluded as naringenin by comparison of the spectroscopic data with literature values [14].

Table 2. NMR data of **1** and **2** in CD_3COCD_3.

Position	Compound **1**		Compound **2**	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2	5.45 (1H; *dd*; *J* = 12.9, 3.0)	79.9	5.08 (1H; *d*; *J* = 11.6)	84.4
3a	3.17 (1H; *dd*; *J* = 17.1, 12.9)	43.6	4.64 (1H; *d*; *J* = 11.6)	73.1
3b	2.73 (1H; *dd*; *J* = 17.1, 3.0)			
4		197.1		198.2
5		167.5		164.8
6	5.95 (1H; *d*; *J* = 2.1)	96.8	5.95 (1H; *d*; *J* = 2.1)	97.1
7		165.0		167.8
8	5.96 (1H; *d*; *J* = 2.1)	95.9	5.99 (1H; *d*; *J* = 2.1)	96.0
9		164.4		164.2
10		103.1		101.5
1'		130.8		129.2
2', 6'	7.39 (2H; *d*; *J* = 8.5)	129.0	7.42 (2H; *d*; *J* = 8.5)	130.3

3', 5'	6.90 (2H; *d*; *J* = 8.5)	116.2	6.89 (2H; *d*; *J* = 8.5)	116.0
4'		158.7		159.0
5-OH	12.17 (1H; *s*)		11.69 (1H; *s*)	

Compound **2** was obtained as a white, amorphous solid. The compound showed a *quasi*-molecular ion at *m/z* 289.0702 $[M + H]^+$ in HR-ESI-MS, corresponding to the empirical formula $C_{15}H_{13}O_6^+$ (calculated for 289.0707), thereby confirming the molecular formula as $C_{15}H_{12}O_6$. The ^{1}H- and ^{13}C-NMR spectra closely resembled those of **1**; however, the appearance of one more oxymethine group [δ_H 4.64 (1H; *dd*; *J* = 11.6; H-3) and δ_C 73.1 (C-3)] in **2** instead of the disappearance of the methylene group in **1**. The relative configuration at C-2 and C-3 was determined as a *trans*-form based on the large coupling constant (*J* = 11.6 Hz). Thus, the structure of compound **2** was assigned as aromadendrin by comparison with previous works [15].

Compound **3** was isolated as a yellowish, amorphous solid. The compound showed a *quasi*-molecular ion at *m/z* 287.0555 $[M + H]^+$ in HR-ESI-MS, corresponding to the empirical formula $C_{15}H_{11}O_6^+$ (calculated for 287.0555), thereby confirming the molecular formula as $C_{15}H_{10}O_6$. The ^{1}H-NMR spectrum showed signals due to a set of two *meta*-coupled aromatic protons [δ_H 6.27 (1H; *d*; *J* = 2.1; H-6)] and [δ_H 6.53 (1H; *d*; *J* = 2.1; H-8)] and symmetrical pairs of *orthro*-coupled aromatic protons [δ_H 8.15 (2H; *d*; *J* = 9.0; H-2', H-6')] and [δ_H 7.02 (2H; *d*; *J* = 9.0; H-3', H-5')], along with a characteristic signal of a intramolecular hydrogen bonding hydroxyl group [δ_H 12.17 (1H; *s*; OH-5)] (Table 3). In addition, the ^{13}C-NMR spectrum exhibited 15 carbon signals consisting of a ketone carbonyl carbon [δ_C 176.6 (C-4)]; four oxygenated aromatic carbons [δ_C 162.3 (C-5)], [δ_C 164.9 (C-7)], [δ_C 157.8 (C-9)], and [δ_C 160.2 (C-4')]; two substituted aromatic carbons [δ_C 104.2 (C-10)] and [δ_C 123.3 (C-1')]; six aromatic methine carbons [δ_C 99.2 (C-6)], [δ_C 94.5 (C-8)], [δ_C 130.5 (C-2', C-6')], and [δ_C 116.3 (C-3', C-5')], two oxygenated olefin carbons [δ_C 147.0 (C-2)], and [δ_C 136.6 (C-3)]. There are 12 aromatic carbons in both **3** and **2**, but the occurrence of two olefin carbons in **3** proves that **3** is a flavone with four hydroxyl groups. Hence, the structure of compound **3** was elucidated as kaempferol [16].

Table 3. NMR data of **3** and **4** in CD_3COCD_3.

Position	Compound **3**		Compound **4**	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2		147.0		157.4
3		136.6		139.7
4		176.6		180.0
5		162.3		163.7
6	6.27 (1H; *d*; *J* = 2.1)	99.2	6.25 (1H; *d*; *J* = 2.0)	99.9
7		164.9		165.3
8	6.53 (1H; *d*; *J* = 2.1)	94.5	6.48 (1H; *d*; *J* = 2.0)	94.9
9		157.8		158.4
10		104.2		106.4
1'		123.3		123.2
2', 6'	8.15 (2H; *d*; *J* = 9.0)	130.5	8.02 (2H; *d*; *J* = 9.0)	131.7
3', 5'	7.02 (2H; *d*; *J* = 9.0)	116.3	7.01 (2H; *d*; *J* = 9.0)	116.9
4'		160.2		161.4
3-OCH_3			3.86 (3H; *s*)	60.7
5-OH	12.17 (1H; *s*)		12.79 (1H; *brs*)	

Compound **4** was obtained as a yellowish, amorphous solid. The compound showed a sodium adduct molecular ion at *m/z* 323.0527 $[M + Na]^+$ in HR-ESI-MS, corresponding to the empirical formula $C_{16}H_{12}O_6Na^+$ (calculated for 323.0526), thereby confirming the molecular formula as $C_{16}H_{12}O_6$. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **3**, except for the presence of one methoxyl group [δ_H 3.86 (3H; *s*) and δ_C 60.7] in **4**. The location of the methoxyl group was affirmed at C-3 because of the HMBC correlation from the methoxyl proton to the oxygenated olefinic carbon. Consequently, the structure of compound **4** was determined as *iso*-kaempferide by comparison of the spectroscopic data with literature values [16].

Compound **5** was isolated as a yellowish, amorphous solid. The compound showed a sodium adduct molecular ion at *m/z* 455.0951 $[M + Na]^+$ in HR-ESI-MS, corresponding to the empirical formula $C_{21}H_{20}O_{10}Na^+$ (calculated for 455.0949), thereby confirming the molecular formula as $C_{21}H_{20}O_{10}$. The ^{1}H- and ^{13}C-NMR spectra showed that a part of these data closely resembled those for compound **3** and indicated the presence of a flavone skeleton. However, **5** displayed remaining signals due to a ketal group [δ_H 5.38 (1H; *d*; *J* = 1.7; H-1") and δ_C 103.5 (C-1″)]; four oxymethine groups [δ_H 4.22 (1H; *dd*; *J* = 3.4, 1.7; H-2") and δ_C 72.1 (C-2")], [δ_H 3.72 (1H; *m*; H-3") and δ_C 72.0 (C-3")], [δ_H 3.34 (2H; *m*; H-4") and δ_C 73.2 (C-4")], and [δ_H 3.34 (2H; *m*; H-5") and δ_C 71.9 (C-5")]; together with a methyl group [δ_H 0.92 (3H; *d*; *J* = 5.7; H-6") and δ_C 17.6 (C-6")] corresponding to the occurrence of a L-rhamnopyranosyl unit. In the HMBC spectrum of **5**, this is confirmed by the HMBC correlations in Figure 2. Moreover, the HMBC correlation from the oxymethine proton H-1" in the L-rhamnose unit to one of the oxygenated olefinic carbon C-3 in the aglycone moiety, suggesting the location of the L-rhamnose unit at C-3. Based on the good comparison of the NMR data of **5**, the structure of compound **5** was determined to be kaempferol-3-*O*-*α*-L-rhamnopyranoside or afzelin [17].

Table 4. ^{1}H-NMR data of **5** in CD_3OD and **6–7** in CD_3COCD_3.

Position	Compound **5**	Compound **6**	Compound **7**
6	6.20 (1H; *d*; *J = 2.1*)	6.27 (1H; *d*; *J* = 2.1)	6.28 (1H; *d*; *J* = 2.1)
8	6.38 (1H; *d*; *J* = 2.1)	6.47 (1H; *d*; *J* = 2.1)	6.49 (1H; *d*; *J* = 2.1)
2', 6'	7.76 (2H; *d*; *J* = 8.7)	7.84 (2H; *d*; *J* = 8.7)	7.88 (2H; *d*; *J* = 8.7)
3', 5'	6.94 (2H; *d*; *J* = 8.8)	7.04 (2H; *d*; *J* = 8.7)	7.06 (2H; *d*; *J* = 8.7)
1"	5.38 (1H; *d*; *J* = 1.7)	5.53 (1H; *d*; *J* = 1.2)	5.67 (1H; *d*; *J* = 1.5)
2"	4.22 (1H, *dd*, *J* = 3.4, 1.7)	4.24 (1H; *dd*; *J* = 3.2, 1.2)	5.63 (1H; *dd*; *J* = 3.5, 1.8)
3"	3.72 (1H; *m*)	3.86 (1H; *dd*; *J* = 9.7, 3.2)	5.27 (1H; *dd*; *J* = 10.2, 3.5)
4"	3.34 (1H; *m*)	4.83 (1H; *m*)	4.91 (1H; *m*)
5"	3.34 (1H; *m*)	3.41 (1H; *m*)	3.47 (1H; *m*)
6"	0.92 (3H; *d*; *J* = 5.7)	0.78 (3H; *d*; *J* = 6.2)	0.85 (3H; *d*; *J* = 6.2)
2"-CO*CH*$_3$			2.10 (3H; *s*)
3"-CO*CH*$_3$			1.96 (3H; *s*)
4"-CO*CH*$_3$		1.98 (3H; *s*)	1.97 (3H; *s*)
5-OH		12.67 (1H; *s*)	12.54 (1H; *s*)

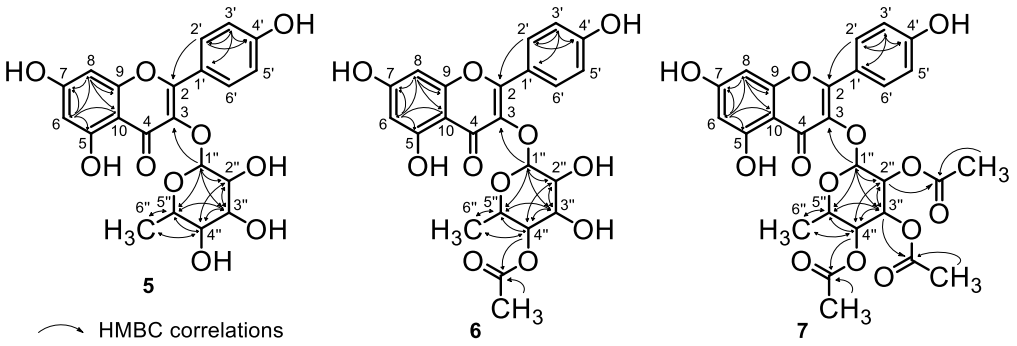

Figure 2. Key HMBC correlations for compounds **5**–**7**.

Compound **6** was obtained as a yellowish, amorphous solid. The ^{1}H- and ^{13}C-NMR spectra closely resembled those of **5**; nevertheless, signals due to another methyl group [δ_H 1.98 and δ_C 20.9] along with an ester carbonyl carbon [δ_C 170.1] suggested the appearance of one acetoxyl group in **6**. The location of the acetoxyl group was determined by the HMBC correlation from the oxymethine proton H-4" to the ester carbonyl carbon, thereby the acetoxyl group being attached to C-4". As a result, the structure of compound **6** was assigned as kaempferol-3-*O*-(4"-*O*-acetyl)-*α*-L-rhamnopyranoside by comparison with previous works [18].

Table 5. ^{13}C-NMR data of **5** in CD_3OD and **6** –**7** in CD_3COCD_3.

Position	**5**	**6**	**7**	Position	**5**	**6**	**7**
2	158.5	158.7	158.7	1"	103.5	102.3	99.0
3	136.2	135.6	134.6	2"	72.1	71.5	69.8
4	179.6	179.2	178.9	3"	72.0	69.8	70.8
5	163.2	163.3	163.2	4"	73.2	74.5	69.7
6	99.9	99.6	99.7	5"	71.9	69.0	69.2
7	165.9	165.1	165.4	6"	17.6	17.6	17.5
8	94.8	94.6	94.7	2"-*CO*CH_3			170.2
9	159.3	158.1	158.1	2"-CO*CH_3*			20.6$_0$
10	105.9	105.8	105.6	3"-*CO*CH_3			170.3
1'	122.6	122.5	122.0	3"-CO*CH_3*			20.6$_1$
2', 6'	131.9	131.7	131.6	4"-*CO*CH_3		170.1	170.4
3', 5'	116.5	116.3	116.5	4"-CO*CH_3*		20.9	20.6$_4$
4'	161.5	161.0	161.3				

Compound **7** was obtained as a yellowish, amorphous solid. The compound showed a sodium adduct molecular ion at *m/z* 581.1270 $[M + Na]^+$ in HR-ESI-MS, corresponding to the empirical formula $C_{27}H_{26}O_{13}Na^+$ (calculated for 581.1266), thereby confirming the molecular formula as $C_{27}H_{26}O_{13}$. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **6**; nonetheless, the appearance of additional two acetoxyl groups [δ_H 2.08 and 1.94; [δ_C 170.1, 170.0, 20.3$_8$, 20.3$_6$] in **7**. From the HMBC experiments, the oxymethine proton at δ_H 5.61 (H-2") the methyl proton at δ_H 2.08 with the ester carbonyl carbon at δ_C 170.0, and another oxymethine proton at δ_H 5.26 (H-3") and another methyl proton at δ_H 1.94 with another ester carbonyl carbon at δ_C 170.1 indicated C-2" and C-3" being carried the acetoxyl groups, respectively. As a consequence, the structure of

compound **7** was determined as kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl)-*α*-L-rhamnopyranoside by comparison of the spectroscopic data with literature values [18].

3.3. The α-glucosidase inhibitory activity of seven compounds from *Z. zerumbet*

The isolated compounds were tested for *α*-glucosidase inhibitory activity. The assay was carried out at several different concentrations ranging from 10 to 250 μM. All the results are illustrated in Table 6. The result showed that all isolated compounds possessed inhibitory activity with IC_{50} values less than 250 μM, ranging from 19.6 to 145.6 μM, and smaller than that of acarbose (IC_{50}, 185.2 μM), a positive control used clinically for the treatment of type 2 diabetes. Especially, **1** showed the most active compound with the IC_{50} value of 19.6 μM. Based on the results, there were significant differences in the relationship between biological activity and chemical structures. These observations displayed that the appearance of a methoxyl group or an acetoxyl group or an L-rhamnose moiety tends to reduce the activity (**1**, **2**, **3** > **4** > **5** > **6** > **7**).

Table 6. α-Glucosidase inhibitory activity of isolated compounds.

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
1	19.6 ± 0.5	**5**	98.6 ± 1.5
2	31.3 ± 0.8	**6**	129.4 ± 1.9
3	28.6 ± 0.7	**7**	145.6 ± 2.2
4	85.6 ± 1.1	Acarbose[§]	214.5 ± 3.2

[§] Positive control.

These findings suggest that the active compounds may serve as promising natural nutraceutical candidates for blood glucose regulation, owing to their potent *α*-glucosidase inhibitory activity. This is compared to reports to inhibit *α*-glucosidase of flavonoids as well as relationship between their structure and *α*-glucosidase inhibitory activity [9, 10, 13]. Additionally, in this study provide a series of potentially effective flavonoids as well as Vietnamese *Z. zerumbet* that could be considered as potential replacements for widely used α-glucosidase inhibitors in the treatment of type-2 diabetes mellitus.

4. CONCLUSION

In this paper, 23 extracts from the rhizomes of seven Zingiberaceae plants were tested for *α*-glucosidase inhibitory activity. The screening results showed that 21/23 samples showed IC_{50} values less than 250 μg/mL, 17 samples with IC_{50} values below 100 μg/mL, and 6 samples displayed IC_{50} values below 10 μg/mL. From the $CHCl_3$ extract of the rhizomes of *Z. zerumbet* Sm., seven flavonoids, comprising of naringenin (**1**), aromadendrin (**2**), kaempferol (**3**), *iso*-kaempferide (**4**), afzelin (**5**), kaempferol-3-*O*-(4''-*O*-acetyl)-*α*-L-rhamnopyranoside (**6**), and kaempferol-3-*O*-(2,3,4-tri-*O*-acetyl)-*α*-L-rhamnopyranoside (**7**), were isolated and identified by spectroscopic analysis and comparison of the spectral data with literature values. Among them, compounds **1**, **2**, **5**, and **7** have been found for the first time in this plant. All compounds possessed significant *α*-glucosidase inhibitory activity and showed more potent inhibitory activity than that of a positive control, acarbose (IC_{50}, 214.5 μM or 138.2 μg/mL). In particular, the compound **1** has been the most active compound.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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