The *in vitro* antioxidant and alphaglucosidase inhibitory activity of extracts from *Zingiber zerumbet* Linn rhizomes

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Abstract
The total methanol residue, *n*-hexane and chloroform extracts from *Zingiber zerumbet* Linn rhizomes have been evaluated for *α*-glucosidase inhibitory activity in an attempt to identify potential active ingredients in natural products. The results showed that the chloroform extract inhibited *α*-glucosidase more effectively than the positive control acarbose, with an IC_{50} value of 127.8 *μ*g/mL. This antioxidant activity of the crude extract; fractional extracts including: *n*-hexane extract, chloroform extract, and methanol extract of from *Zingiber zerumbet* has been investigated using DPPH assay. The results showed that the crude chloroform extract exhibited good antioxidant activities with IC_{50} value of 2.09 *μ*g/mL.

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Introduction

Zingiber zerumbet, commonly known as *Wild Ginger*, is also referred to as *Riềng gió* in Vietnamese, *Gingembre fou* in French, and *Fēng Jiāng* (风姜) in Chinese. The rhizome is tuberous and branched, with a pale white outer surface and a light yellow internal parenchyma. The leaves are arranged closely along the stem, nearly sessile, lanceolate in shape with an acuminate apex and tapering base. The adaxial (upper) surface is dark green, while the abaxial (lower) surface is paler and sparsely pubescent. The leaf sheath is glabrous except near the apex. The flowering stalk (inflorescence axis) measures approximately 30 to 60 cm in length and is enveloped by overlapping bracts with pubescent margins. The fruit is a capsule, ovoid in shape, containing black seeds [1].

Zingiber zerumbet is indigenous to India and is currently distributed across Polynesia, Hawaii, and various regions of Southeast Asia. In Vietnam, the species grows wild predominantly in the northern mountainous provinces. It typically thrives in moist, shaded habitats such as damp forested areas and along stream banks where the soil is rich and well-drained [2]. The rhizomatous tubers of Zingiber zerumbet are typically harvested in the autumn. After collection, they are thoroughly washed and may be used fresh—often macerated in alcohol for medicinal purposes—or sliced and subjected to drying, either by sun-drying or controlled dehydration.

Traditionally, the rhizomes are decocted and ground into a paste for the treatment of various ailments, including dental pain (odontalgia), cough, bronchial asthma, helminthiasis, leprosy, and other dermatological conditions. In Hawaiian ethnomedicine, the rhizome is crushed and topically applied to alleviate localized pain, bruises, and lacerations. Additionally, it is employed in the management of headaches, toothaches, ascariasis (roundworm infection), as well as musculoskeletal conditions such as arthralgia, sprains, and gastric discomfort [1].

The phytochemical constituents of *Zingiber zerumbet* include a variety of sesquiterpenes and essential oil components, such as citronellyl formate, curcuphenol, *α*-bisabolol, pinocarvone, elemol, germacrene D, bicyclogermacrene, α-copaene, bulnesol,

selin-11-en-4-α-ol, β-bisabolene và trans-α-bergamotene [3]. Additionally, the plant contains a range of aromatic compounds and flavonoids, including *p*-hydroxybenzaldehyde, vanillin, kaempferol-3-*O*-methylether, kaempferol-3,4'-*O*-dimethylether, kaempferol-3,4',7-*O*-trimethylether, 4"-*O*-acetylafzelin, 2",4"-*O*-diacetylafzelin, 3",4"-*O*-diacetylafzelin [4, 5].

Additionally, several novel compounds have been identified in the essential oil of *Zingiber zerumbet* rhizomes originating from Bangladesh. These include carveol, 4-terpineol, β-terpinyl acetate, trans-nerolidol, 4-methoxystilbene, norethynodrel, germacrene-4-ol, and 2-methylenecholestan-3-ol [6].

Several studies investigating the biological activities of Zingiber zerumbet have demonstrated that ethanol extracts, petroleum ether extracts, and chloroform extracts derived from fresh rhizomes exhibit significant antibacterial and antifungal properties. These extracts have shown inhibitory activity against five Gram-positive bacterial strains, eight Gram-negative bacterial strains, and three pathogenic fungal species [7]. The chloroform extract of *Zingiber zerumbet* has demonstrated anticarcinogenic activity in murine models, particularly through the action of zerumbone, a sesquiterpene compound, which has shown efficacy against colon and lung cancer [8]. Additionally, the rhizome of *Zingiber zerumbet* has exhibited a potent hypoglycemic effect in experimental rats, suggesting potential utility in the management of hyperglycemia and diabetes mellitus [9].

However, pharmacological studies on *Zingiber zerumbet* in Vietnam remain limited. This study aims to evaluate the *in vitro* antioxidant activity and α-glucosidase inhibitory potential of extracts from this species collected in Vietnam.

Materials and methods

Plant materials

Z. zerumbet rhizome samples were collected in Dak Lak province, Vietnam, in 2023. A voucher specimen has been deposited at the Faculty of Natural Science and Technology, Tay Nguyen University.

Extraction and isolation

The rhizomes were washed, cut into small pieces and dried in the oven at the temperature of 38 ºC in three days to obtain 5.3 kg dried sample. The dried sample was extracted with methanol (MeOH) (3.5 L × 3) at room temperature using conventional ultrasound-assisted technique. The extracting solution was then collected and evaporated under reduced pressure to yield 561.8 g methanol residue. The methanol residue was partitioned sequentially with solvents in the order of increasing polarity as following: *n*-hexane, chloroform, ethyl acetate (each solvent 3 L × 3). Concentration of the extracted solutions afforded *n*-hexane extract (140.0 g), chloroform (57.0 g), ethyl acetate extract (19.6 g), and methanol extract (304.0 g).

Inhibition of α-glucosidase assay

The *α*-glucosidase inhibitory protocol was carried out according to previously assay with slight modification [10]. Extracts and compounds were dissolved in DMSO and diluted with phosphate buffer (pH 6.8) to various concentrations of 250, 100, 50, 25 and 10 *μ*g/ml. In a 96-well plate, a reaction mixture containing 100 *μ*L of phosphate buffer (100 mM, pH 6.8), 40 *μ*L of extracts or compounds, 20 *μ*L *α*- glucosidase (0.4 U/mL, CAS No 9001-42-7, Sigma) were pre-incubated for 15 min at 37 °C. Then, 40 *μ*L substrate *p*-nitrophenyl-*α*-D-glucopyranoside (pNPG, 2.5 mM, CAS No 3767-28-0, Sigma) were added to the mixture. The reaction was stopped by 100 *μ*L of Na_2CO_3 (0.1 M) for each well after 20 min incubation. Without test compound was set up as a blank control and acarbose was used as a positive control. Experiments were done in triplicates. The absorbance of the released *p*-nitrophenol was measured at 405 nm with microplate reader (BioTek, USA). The results expressed as percentage inhibition, were calculated using the following equation:

Inhibition (%) = [(OD control – OD sample) / OD control] x 100

The IC_{50} value showed the concentration of compound inhibiting 50% of *α*-glucosidase activity. The IC_{50} value was calculated using TableCurve software.

Assessment of *in vitro* antioxidant activity

Analysis of the ability to scavenge free radicals generated by DPPH (1,1-diphenyl-2-picrylhydrazyl; Brand-Williams *et al*., 1995, Shela *et al*., 2003, Kumar *et al*., 2013) is a recognized method for quickly determining antioxidant activity. The test compound is dissolved in 100% dimethyl sulfoxide (DMSO) and DPPH is dissolved in 96% ethanol. The absorption of DPPH at λ = 515 nm is measured after adding DPPH to the sample solution in a 96-well microplate. Results of the experiments are expressed as the mean value of at least 3 repeated tests ± standard deviation ($p \leq 0.05$).

The samples were dissolved in 100% DMSO at a concentration of 4 mg/ml for crude extract and 1 mg/ml for purified sample. Flavonoid at 1 mM or ascorbic acid at 5 mM in 10% DMSO served as positive controls. Samples were applied onto a 96-well microplate with DPPH solution to achieve final concentrations ranging from 200 µg/mL to 12.5 µg/mL (for crude extract) and from 50 µg/mL to 3.1 µg/mL (for purified sample) in the reaction mixture.

Incubate at 37°C for 30 minutes and measure the optical density (OD) at a wavelength of λ = 515 nm using a spectrophotometer (Infinite F50, Tecan, Switzerland).

The scavenging capacity (SC%) of free radicals at various sample concentrations is calculated using Excel software according to the formula:

$$SC\% = [\frac{OD_{test} - OD_{DMSO}}{OD_{control(-)}} \times 100\%] \pm \sigma$$

The standard deviation σ is calculated using the formula by Duncan as follows:

$$\sigma = \sqrt{\left(\Sigma\left(xi - \bar{x}\right) \wedge 2\right) / \left(n - 1\right)}$$

Determining SC_{50}: The sample (test substance) is diluted into decreasing concentrations, repeated three times at each concentration. The scavenging efficiency against DPPH free radicals for each sample is calculated based on the percentage of free radical neutralization compared to blank and negative control samples. Samples showing antioxidant activity against the DPPH system undergo further steps to determine the IC_{50} value (µg/mL, µM/mL). The SC_{50} value is the concentration of the test substance at which 50% of free radicals are neutralized, determined using Table Curve AISN Software (Jandel Scientific, USA) based on SC% values and the range of corresponding test substance concentrations.

Results and Discussion

A-glucosidase inhibitory activity

The total methanol residue, *n*-hexane extract, chloroform extract and and two isolated compounds from the rhizomes of *Z. zerumbet* were evaluated for *α*-glucosidase inhibitory activity. As shown in Table 1, total methanol residue exhibited weak inhibition against *α*-glucosidase while n-hexane extracts did not exhibit inhibitory activity. Chloroform extract showed good inhibitory activity.

Table 1: *α*-glucosidase inhibitory activity of extracts from *Z. zerumbet* rhizomes

Extracts	Inhibition (I, %)					IC_{50} (*μ*g/mL)
	250 *μ*g/mL	100 *μ*g/mL	50 *μ*g/mL	25 *μ*g/mL	10 *μ*g/mL	
Total MeOH residue	87.5 ± 1.3	29.4 ± 2.5	2.2 ± 2.5	–	–	146.3
n-Hexane extract	68.2 ± 2.7	17.5 ± 1.2	–	–	–	181.6
Chloroform extract	97.4 ± 1.4	39.7 ± 2.6	6.5 ± 1.7	–	–	127.8
Acarbose						138.2

*: I > 100 %; –: I < 1 %

Results of the DPPH radical scavenging assay •

The DPPH radical scavenging activity assay was conducted on the crude extracts of *Zingiber zerumbet* rhizomes. The results are presented in Table 2.

Table 2: DPPH Radical Scavenging Activity of *Zingiber zerumbet* Rhizome Extracts

Extracts	Inhibition (I, %)				IC_{50} (μg/mL)
	100 μg/mL	50 μg/mL	25 μg/mL	10 μg/mL	
Total MeOH residue	23.10 ± 1.70	8.31 ± 0.67	3.56 ± 0.74	0.09 ± 0.86	>100
n-Hexane	9.13 ± 1.20	5.36 ± 0.84	–	–	>100
Chloroform	48.75 ± 0.96	31.59 ± 0.84	21.57 ± 1.78	2.09 ± 0.80	~100
Trolox*					2.73

* The positive control used in the experiment; (-): I % < 1 %

The results of the DPPH• radical scavenging activity assay on *Zingiber zerumbet* rhizome extracts showed that the *n*-hexane, chloroform, and methanol extracts exhibited inhibitory activity at a concentration of 100 μg/mL. Specifically, the n-hexane, chloroform, and methanol extracts displayed inhibition at a concentration of 50 μg/mL, while the chloroform, ethyl acetate, and methanol extracts exhibited activity at concentrations of 25 μg/mL and 10 μg/mL.

Among these, the chloroform extract demonstrated the strongest DPPH radical scavenging activity, with an IC50 value of 2.09 μg/mL, which was more potent than the positive control, trolox (IC_{50} = 2.73 μg/mL).

Conclusions

Three extracts and two compounds form *Z. zerumbet* rhizomes have been investigated for their inhibition activity against *α*-glucosidase. As the results, chloroform extract showed good activity with IC_{50} lower than IC_{50} of acarbose. The results of the DPPH radical scavenging activity assay indicated that all the extracts exhibited weak activity at the tested concentration of 100 μg/mL. Among them, the chloroform extract demonstrated stronger DPPH radical scavenging activity compared to the other extracts.

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