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Research Article

POTENTIAL ALPHA-GLUCOSIDASE INHIBITOR FROM SELECTED ZINGIBERACEAE FAMILY

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ABSTRACT

Objective: This study aimed to evaluate Zingiberaceae family as potential sources of alpha-glucosidase inhibitors (AGIs).

Methods: A 16 species of selected Zingiberaceae family were macerated by ethanol 96%. The extracts were filtered and concentrated by rotary evaporator. Phytochemical analysis carried out on the dry extract. AGI was assayed in 50 mM phosphate buffer at pH 6.8 with 5 mM p-nitrophenyl-α-D-glucopyranoside as the substrate. Alpha-glucosidase activity was determined by measuring the release of the yellow p-nitrophenol at 400 nm using a spectrophotometer UV-Vis, and the percentage of inhibition was calculated.

Results and Conclusion: The Zingiberaceae family showed IC_{50} against alpha-glucosidase varies range from 28.4 µg/ml to 269.2 µg/ml. *Curcuma longa* L., *Zingiber cassumunar* Roxb., *Curcuma heyneana* Val. Et van zijp, *Curcuma xanthorrhiza* Roxb., *Zingiber ottensii* Val., showed the highest inhibition against alpha-glucosidase, with IC_{50} 28.4, 49.0, 78.2, 78.9, and 79.0 µg/ml, respectively. The Zingiberaceae family is potential sources as an alternative medicine to treated diabetes mellitus.

Keywords: Zingiberaceae, Alpha-glucosidase inhibitor, Diabetes mellitus, Phytochemistry content.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder and is characterized by high blood glucose level which results from defects in both insulin secretion and/or insulin action [1]. DM is associated with reduced quality of life because of its complication and increased risk for mortality and morbidity. Long-term hyperglycemia is an important factor in the development and progression of micro- and macro-vascular complications, which include neuropathy, nephropathy, cardiovascular, and cerebrovascular diseases [2].

One therapeutic approach to treat diabetes is to delay the absorption of glucose via inhibition of enzymes, such as alpha-glucosidase, in the digestive organs. Alpha-glucosidase is a membrane-bound enzyme present in the epithelium of small intestine, which catalyzes the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides and facilitates glucose absorption by the small intestine. Inhibiting this enzyme retards the elevation of glucose following a carbohydrate meal [3].

Alpha-glucosidase inhibitors (AGIs) are recommended as first line therapy by American Association of Clinical Endocrinologists [4]. Their efficacy, safety, and cardiovascular benefits make them suitable for use in diabetes. AGIs can be used as monotherapy, combination therapy with other oral drugs and insulin, and as fixed dose combinations [5].

AGIs attenuate postprandial hyperglycemia (PPH) and reduce the risk of cardiovascular events in patients with impaired glucose tolerance or type 2 DM. PPH is one of the factors leading to endothelial dysfunction, which is an early event in the pathogenesis of atherosclerosis [6]. AGIs also have an indirect effect on fasting glycemia, and are able to reduce hemoglobin A1c levels effectively. So, AGIs preferred for diabetes control in carbohydrate consuming populations [7]. Moreover, AGIs showed various antidiabetic or anti-obesity effects in addition to the suppression of PPH. So, AGI could ameliorate body weight gain, insulin resistance, and inflammatory adipokine upregulation [8].

However, one of side effect of AGIs is indigestion because AGIs delays complex carbohydrate digestion. This mechanism of action leads to cause flatulence, diarrhea and abdominal pain, may lead to reducing patients' adherence. This is a challenge to discover the potential of natural medicine, especially from Zingiberaceae as AGIs and overcome the digestive disorder.

In recent years, many researches have been done to identify effective AGIs from natural sources as functional food or lead compounds for treating diabetes. Many AGIs that are phytoconstituents from plants, such as alkaloids, flavonoids, terpenoids, anthocyanins, glycosides, and phenolic compounds [9].

Zingiberaceae family is the most widely grown crop in the tropics, especially Southeast Asia. This plant is important for natural resources for humans as a source of food, spices, dyes, perfumes, food preservatives, food coloring, and a source of herbal medicine [10]. Zingiberaceae family have been known and used in traditional Indonesian medicine (jamu) to cope with various diseases such as antidiabetic, anti-inflammatory, hepatoprotector, blood cleanser, antioxidant, and antitumor [11].

Turmeric (*Curcuma longa* L.) is one of the most widely species of Zingiberaceae studied. Turmeric is known to have a wide range of pharmacological effects such as antidiabetic and antihyperlipidemic [12]. Turmeric can improve insulin secretion, inhibits the enzyme alpha-glucosidase through modulation of peroxisome proliferator-activated receptor (PPAR) alpha gene expression. Turmeric and its compounds are a promising alternative for patients who suffer from digestive disorders [13]. Currently, turmeric has become fitofarmaka drugs (standardized herbal medicine) in Indonesia.

In addition to, turmeric, *Curcuma xanthorrhiza* Roxb. is one of Zingiberaceae species that have been widely studied and used mainly in Indonesia. The main components of *C. xanthorrhiza* Roxb. and turmeric are curcuminoid. *C. xanthorrhiza* Roxb. is most widely used to increase appetite that mainly used in children.

Based on the description above, that turmeric and *C. xanthorrhiza* as species of family Zingiberaceae showed a wide range of pharmacological effects; we assumed that Zingiberaceae (species other than turmeric)

very potential to be developed as an antidiabetes from natural sources, especially as AGIs. This study aimed to screened potential sources of AGIs from 16 selected Zingiberaceae which commonly used especially in Indonesia.

METHODS

Plant materials

This study was conducted in the Pharmacology Laboratory, Bandung School of Pharmacy, Indonesia. The 16 species of selected Zingiberaceae were collected from Manoko, Lembang, Bandung, West Java, Indonesia and botanically identified at Herbarium Bandungense, ITB, Bandung. The following Zingiberaceae were used: *Zingiber officinale* var. Officinarum, *Z. officinale* var. Amarum, *Z. officinale* var. Rubrum, *Zingiber cassumunar* Roxb., *Zingiber ottensii* Val., *Zingiber zerumbet* (L.) J.E. Smith, *Zingiber aromaticum* Val., *Zingiber littorale* Nor., *C. longa* L., *C. xanthorrhiza* Roxb., *Curcuma mangga* Val., *Curcuma aeruginosa* Roxb., *Curcuma zedoaria* Rosc., *Curcuma heyneana* Val., *Curcuma rotunda* L., and *Curcuma petiolata* Roxb. The fresh Zingiberaceae were cut into small pieces, dried, and powdered. The powdered were macerated by ethanol 96% for 3 days. The extracts were filtered and concentrated by rotary evaporator. Phytochemical analysis carried out on the dry extract.

AGI activity

AGI was assayed in 50 mM phosphate buffer at pH 6.8 with 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) as the substrate [14]. A hundred units of alpha-glucosidase enzyme (purchased from Sigma) dissolved in 100 ml buffered phosphate pH 6.8 that contained 200 mg of bovine serum albumin. Extract of selected Zingiberaceae and acarbose (Dexa Medica) were dissolved in dimethyl sulfoxide in different concentration 1, 10, 50, 100, 200 µg/ml.

Briefly, A 50 µl aliquot of alpha-glucosidase enzyme (0.15 unit/ml) was mixed with 25 µl of extract. After incubation at 37°C for 5 minutes, 50 µl of PNPG was then added and incubated again at 37°C for 10 minutes. To stop the enzyme reaction, 0.9 ml of 0.1 M Na_2CO_3 was added. Alpha-glucosidase activity was determined by measuring the release of the yellow p-nitrophenol at 400 nm using a spectrophotometer UV-Vis, and the percentage of inhibition was calculated. Acarbose (Dexa Medica) was used as the positive control.

RESULTS AND DISCUSSION

Screening phytochemistry for 16 species of Zingiberaceae family showed a positive result for flavonoid except for *C. mangga* Val., *C. petiolata* Roxb., and *C. rotunda* L. (Table 1). Table 2 showed potential inhibition against alpha-glucosidase enzyme for 16 species of Zingiberaceae. The IC_{50} value for acarbose, a comercially AGI, was 123.4 µg/ml. The IC_{50} value for 16 species of Zingiberaceae ranging from 28.4 to 269.2 µg/ml (Table 2). 8 species of Zingiberaceae family showed the highest inhibition against alpha-glucosidase were *C. longa* L., *Z. cassumunar* Roxb., *C. heyneana* Val. Et van Zijp, *C. xanthorrhiza* Roxb., *Z. ottensii* Val., *Z. officinale* var. Rubrum, *Z. officinale* var. Amarum, and *C. mangga* Val.

Pharmacological activity of plants extract is mainly contributed by phytochemistry contained in them. Flavonoids are polyphenolic compounds that have beneficial effect on glucose and lipid homeostasis. Flavonoids showed different act and regulated different signaling pathways on various molecular targets in pancreatic β-cells, hepatocytes, adipocytes, and skeletal myofibers. Flavonoids have beneficial effects in diabetes by enhancing insulin secretion, improving hyperglycemia; reducing insulin resistance, and increasing glucose uptake in skeletal muscle and white adipose tissue [15]. Moreover, phenolic compounds are the contributors to the antioxidant activity in the plants [16]. Antioxidant could scavenge free radicals which contribute to degenerative diseases.

Recently, polyphenolic compounds are the most of the nutritional interest because its ability to bind and precipitate macromolecules, such as dietary protein, carbohydrate, and digestive enzymes, thereby reducing food digestibility. Many researches in phenolics have increased greatly because of the antioxidant and free radical-scavenging abilities associated with some phenolics and their potential benefits on human health [17].

Generally, 16 selected Zingiberaceae family showed potential inhibition against alpha-glucosidase enzyme. There are many constituent phytochemistry in Zingiberaceae may act as AGI activity. Our study found that *C. longa* L., *Z. cassumunar* Roxb., *C. heyneana* Val. Et van zijp, *C. xanthorrhiza* Roxb., *Z. ottensii* Val., showed the highest inhibition against alpha-glucosidase, with IC_{50} 28.4, 49.0, 78.2, 78.9, and 79.0 µg/ml, respectively (Table 2). Our result in accordance with other study that reported turmeric extract (*C. longa* L.) showed inhibition against alpha-glucosidase with IC_{50} 23.0 µM [9]. To our knowledge, there is no research that reported of *Z. cassumunar* Roxb., *C. heyneana* Val. Et van zijp, *C. xanthorrhiza* Roxb., *Z. ottensii* Val., as AGIs. Hence, our study as the first report for those species as AGIs.

Turmeric (*C. longa* L.), as well as, *C. xanthorrhiza* are the most common utilized species, possesses a wide range of pharmacology activity. Turmeric (*C. longa* L.) known as antihyperglycemia [18], antihyperlipidemia [19], etc. Turmeric contained curcuminoids and sesquiterpenoids that showed synergistic antihyperglicemia effects

Table 1: Phytochemistry compounds for 16 species of Zingiberaceae family

Zingiberaceae	Alkaloid	Flavonoid	Saponin	Steroid	Triterpenoid	Tanin	Polyphenol
Z. cassumunar Roxb.	−	+	−	−	+	+	−
Z. ottensii Val.	−	+	−	−	+	+	−
Z. zerumbet	−	+	+	+	−	−	−
Z. littorale Nor.	−	+	+	−	−	+	−
Z. aromaticum Val.	−	+	+	−	−	+	−
Z. officinale var. R.	+	+	−	−	−	+	−
Z. officinale var. O.	+	+	−	−	+	+	−
Z. officinale var. A.	−	+	−	+	−	−	+
C. longa L.	+	+	−	+	+	−	+
C. heyneana Val.	+	+	−	+	−	+	+
C. xanthorrhiza	+	+	+	+	+	+	+
C. mangga Val.	+	−	−	−	−	−	+
C. aeruginosa Roxb.	−	+	+	−	−	+	+
C. zedoaria	−	+	+	−	−	+	+
C. petiolata Roxb.	−	−	−	−	+	−	+
C. rotunda L.	+	−	−	−	−	+	+

C: Curcuma, Z: Zingiber, +: Present, −: Absent, *Z. zerumbet: Zingiber zerumbet* (L.) J.E. Smith, *Z. officinale* var. R: *Zingiber officinale* var. Rubrum, *Z. officinale* var. O: *Zingiber officinale* var. Officinarum, *Z. officinale* var. A: *Zingiber officinale* var. Amarum, *C. heyneana* Val.: *Curcuma heyneana* Val. Et van Zijp, *C. xanthorrhiza: Curcuma xanthorrhiza* Roxb., *C. zedoaria: Curcuma zedoaria* (Chrism.) Roscoe

Table 2: Potential inhibition against alpha-glucosidase enzyme for 16 species of Zingiberaceae

Zingiberaceae	Percentage of inhibition (%)					IC_{50} (µg/ml)
	1 (µg/ml)	10 (µg/ml)	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	
C. longa L.	41.20±0.15	46.09±0.25	55.40±0.01	57.70±0.06	59.50±0.10	28.4
Z. cassumunar Roxb.	43.97±0.14	44.69±0.05	48.78±0.10	53.25±0.21	68.85±0.08	49.0
C. heyneana Val.	39.00±0.14	42.80±0.12	48.50±0.13	53.00±0.16	59.70±0.13	78.2
C. xanthorrhiza Roxb.	39.80±0.09	43.90±0.27	46.00±0.02	53.40±0.26	65.60±0.10	78.5
Z. ottensii Val.	39.40±0.14	40.76±0.09	45.57±0.06	53.08±0.04	66.70±0.05	79.0
Z. officinale var. R.	35.52±0.05	36.33±0.14	42.61±0.10	47.14±0.21	64.27±0.08	106.6
Z. officinale var. A.	27.25±0.20	29.00±0.10	37.10±0.11	44.10±0.14	66.60±0.10	119.9
C. mangga Val.	15.77±0.75	18.10±0.13	21.10±0.43	42.40±0.05	53.50±0.02	121.4
C. aeruginosa Roxb.	14.90±1.14	27.90±0.16	36.70±0.49	44.70±0.30	53.70±0.17	126.6
Z. officinale var. O.	25.72±0.20	27.48±0.10	35.57±0.11	42.58±0.14	63.92±1.28	130.2
C. zedoaria	24.50±0.02	32.00±1.96	37.70±0.02	45.10±0.02	51.70±0.04	134.3
C. petiolata Roxb.	22.70±0.22	26.40±0.06	33.40±0.13	42.10±0.15	52.20±0.18	140.3
Z. aromaticum Val.	21.23±0.10	23.98±0.19	32.24±0.11	38.03±0.05	52.22±0.16	182.3
C. rotunda L.	9.79±0.02	12.20±1.77	21.30±0.08	29.00±0.01	35.60±0.01	204.8
Z. littorale Nor.	22.69±0.08	24.30±0.09	30.18±0.05	36.21±0.14	48.56±0.14	210.2
Z. zerumbet (L.)	19.60±0.11	22.61±0.10	27.57±0.04	31.70±0.06	42.37±0.17	269.2
Acarbose	33.02±3.02	37.72±7.54	40.61±3.87	48.37±3.03	58.89±3.03	123.4

C: Curcuma. Z: Zingiber. Values are expressed as mean±standard deviation (n=3). IC_{50} obtained from regression line of percentage inhibition. *Z. zerumbet: Zingiber zerumbet* (L.) J.E. Smith, *Z. officinale* var. R: *Zingiber officinale* var. Rubrum, *Z. officinale* var. O: *Zingiber officinale* var. Officinarum, *Z. officinale* var. A: *Zingiber officinale* var. Amarum, *C. heyneana* Val.: *Curcuma heyneana* Val. Et van Zijp, *C. xanthorrhiza: Curcuma xanthorrhiza* Roxb., *C. zedoaria: Curcuma zedoaria* (Chrism.) Roscoe

via PPAR-γ activation [20]. Yasni *et al.* and Afshari *et al.* found that extract of *C. xanthorrhiza* and *Z. cassumunar* showed antihyperglicemia in animal model diabetes induced by streptozotocin [21,22]. *Z. cassumunar* reported as an analgesic and anti-inflamatory [23] and *C. xanthorrhiza* attenuate the insulin resistance state of obesity-induced hyperglycemia [24]. We reported that one of antihyperglicemia effect of *C. xanthorrhiza* and *Z. cassumunar* came from inhibition of alpha-glucosidase enzyme (Table 2).

Lukiati *et al.* reported the action mechanism of *C. heyneana* Val. Et van zijp as antidiabetic was able to increase SOD activity and repair the pancreatic beta cells damage on DM rats induced by multiple low-dose streptozotocin [25]. Our result inline with their report, *C. heyneana* Val. reduced blood glucose levels by inhibition of alpha-glucosidase enzyme activity (Table 2).

It has been reported that the protein of *Z. ottensii* Val. showed activity as an inhibitor of alpha-glucosidase [26]. Our study showed that phytoconstituents in *Z. ottensii* Val. also inhibited alpha-glucosidase activity (Table 2). Based on these studies, that the protein and phytoconstituents in *Z. ottensii* Val. works synergistically as AGI.

Ginger (*Z. officinale)* modulated glucose metabolism in rats fed a high-calorie diet and suggest that ginger may be effective in preventing the development of metabolic syndrome and type 2 diabetes [27]. We reported that all variant of *Z. officinale* showed inhibition againt alpha-glucosidase enzyme (Table 2).

Our result showed that *Curcuma* species contained phenolic compounds (Table 1). Their medicinal properties correlated with the presence of phytochemicals such as phenolics in the rhizomes. Other study showed that phenolic compounds are contributors to the antioxidant activity in the plants. Antioxidant could scavenge free radicals which contribute to the pathogenesis of DM [28].

Scientific contributions of our results is Zingiberaceae family as inhibitors of alpha-glucosidase enzyme (especially those that have not been reported include *Z. cassumunar* Roxb., *C. heyneana* Val. Et van Zijp, *C. xanthorrhiza* Roxb., and *Z. ottensii* Val.) can be used as phytonutrients to prevent glucose intolerance and obesity causes insulin resistance, especially in populations with a large proportion of carbohydrates consumption. However, these studies require further research.

We confirmed that species in Zingiberaceae family (other than turmeric) such as *Z. cassumunar* Roxb., *C. heyneana* Val. Et van zijp, *C. xanthorrhiza* Roxb., and *Z. ottensii* Val. as AGIs and need more study to investigated its potiential role in ameliorated DM especially as AGIs.

CONCLUSION

The 16 species of Zingiberaceae showed potential inhibition against alpha-glucosidase enzyme. The highest inhibition against alpha-glucosidase were *C. longa* L., *Z. cassumunar* Roxb., *C. heyneana* Val. Et van zijp, *C. xanthorrhiza* Roxb., *Z. ottensii* Val., respectively. Pharmacology Activity of those Zingiberaceae as AGI may come from its various phytochemistry contents.

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