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Study on α-glucosidase inhibitory activity of 40 kinds of vegetables, tubers, and fruits in An Giang

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Abstract:

A study on the α-glucosidase inhibitory activity of 40 kinds of vegetables, tubers, and fruits found in the An Giang province was conducted. The results indicated that all 40 extracted samples displayed α-glucosidase inhibitory activity at a concentration of 250 μg ml^{-1}, 36 extracted samples showed an inhibition rate greater than 50% at 250 μg ml^{-1}, 25 extracted samples had over 50% at 100 μg ml^{-1}, 17 extracted samples possessed more than 50% at 50 μg ml^{-1}, 7 extracted samples sampled showed over 50% at 25 μg ml^{-1}, 5 extracted samples were greater than 50% at 10 μg ml^{-1}, and 1 extracted sample was greater than 50% at 1 μg ml^{-1}. Among the 40 samples, those taken from the seeds of *Areca catechu*, the fruits of *Cassia grandis*, the fruits of *Syzygium cumini*, the seeds of *Glycine max*, and the stems of *Enydra fluctuans* exhibited the strongest α-glucosidase inhibitory activity in methanol, with IC_{50} values of 0.15, 2.54, 4.05, 5.17 and 8.68 μg ml^{-1}, respectively, which were stronger than the positive control acarbose with an IC_{50} value of 214.5 μg ml^{-1}.

Keywords: **An Giang, α-glucosidase, *Areca catechu*, *Cassia grandis*, fruit, *Glycine max* and *Enydra fluctuans*, *Syzygium cumini,* tuber, vegetable.**

Classification number: **3.3**

Introduction

Diabetes is a disease known for symptoms of characteristically high blood sugar levels due to a lack of insulin with or without insulin resistance. Individuals with diabetes not only have high blood sugar but also have high sugar levels in their urine, so-called diabetes mellitus. Besides, diabetes also causes dangerous complications such as cardiovascular risks, stroke, and chronic kidney disease. Diabetes includes two types and diabetes type 2 accounts for about 90% of the total number of cases [1]. In the body, the small intestinal cell membrane secretes the α-glucosidase enzyme that plays a role in the hydrolysis of carbohydrates from food into oligosaccharides. Then, these hydrolysed oligosaccharides turn into glucose and enter the bloodstream via the small intestinal membrane to feed the body's cells. When the body has a metabolic disorder, carbohydrates and blood sugar levels will rise and lead to diabetes. By inhibiting the activity of the α-glucosidase enzyme, hydrolysis of carbohydrates slows down and blood sugar levels reduce [1].

Therefore, the search for vegetables, tubers, and fruits with the ability to inhibit the α-glucosidase enzyme may have significance in new diabetes treatments. Consequently, in this study, we conducted a screening of the α-glucosidase enzyme inhibitory activity of some vegetables, tubers, and fruits found in the An Giang province to further guide the research of diabetes treatments.

Materials and methods

Sample preparation

The 40 samples of vegetables, tubers, and fruits in this study were purchased in the An Giang province during the month of 1/2019 (Table 1) and were identified by Dr. Luong Minh Chau, Institute of Rice in the Mekong delta. These samples (of vegetables, tubers, and fruits) were selected according to three criteria: folk criteria (used to treat diabetes mellitus), references, and random selection. The dried vegetables, tubers, and fruits were minced, and then simmered under circulation 3 times with the solvent methanol for 3 h. The extracted samples were collected and concentrated at low pressure to obtain samples in a methanol solvent.

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Table 1. The list of 40 vegetables, tubers, fruits in An Giang.

Number	Science name	Familia	Parts used	Selection criteria
1	*Abelmoschus esculentus* L.	Malvaceae	Fruits	1
2	*Allium ramosum* L.	Alliaceae	Stems	3
3	*Amaranthus tricolor* L.	Amaranthaceae	Stems	3
4	*Asparagus officinalis* L.	Asparagaceae	Stems	3
5	*Azadirachta indica* A.	Meliaceae	Leaves	1
6	*Basella alba* L.	Basellaceae	Stems	1
7	*Benincasa hispida* Thunb	Cucurbitaceae	Fruits	3
8	*Brassica integrifolia (West.) O.E. Schulz*	Brassicaceae	Stems	3
9	*Brassica juncea* L. Czern	Brassicaceae	Stems	3
10	*Brassica rapa chinensis*	Brassicaceae	Stems	3
11	*Brassica rapa* subsp. *pekinensis* Lour.	Brassicaceae	Stems	3
12	*Cassia grandis* L.F.	Fabaceae	Fruits	3
13	*Centella asiatica* L.	Apiaceae	Stems	2
14	*Colocasia esculenta* L.	Araceae	Tubers	3
15	*Cucumis sativus* L.	Cucurbitaceae	Fruits	1
16	*Cucurbita pepo L.*	Cucurbitaceae	Fruits	2
17	*Eichhornia crassipes* M.	Pontederiaceae	Stems	3
18	*Enydra fluctuans* Lour.	Asteraceae	Stems	2
19	*Areca catechu* L.	Arecaceae	Seeds	3
20	*Glycine max* L. Merr.	Fabaceae	Seeds	3
21	*Ipomoea batatas* L.	Convolvulaceae	Tubers	1
22	*Ipomoea batatas* L.	Convolvulaceae	Stems	1
23	*Lagenaria siceraria* M.	Cucurbitaceae	Fruits	3
24	*Luffa cylindrica* L. M.	Cucurbitaceae	Fruits	1
25	*Momordica charantia* L.	Cucurbitaceae	Stems	2
26	*Momordica charantia* L.	Cucurbitaceae	Fruits	2
27	*Momordica charantia* L.	Cucurbitaceae	Seeds	2
28	*Morus alba* L.	Moraceae	Fruits	2
29	*Musa balbisiana* Colla	Musacea	Young fruits	1
30	*Peperomia pellucida* L.	Piperaceae	Stems	1
31	*Perilla frutescens* L.	Lamiaceae	Stems	3
32	*Phaseolus vulgaris* L.	Fabaceae	Fruits	3
33	*Piper sarmentosum* R.	Piperaceae	Stems	1
34	*Raphanus sativus* L.	Brassicaceae	Tubers	3
35	*Sauropus androgynus* L.	Phyllantheae	Stems	1
36	*Solanum lycopersicum* L.	Solanaceae	Fruits	3
37	*Solanum melongena* L.	Solanaceae	Fruits	3
38	*Spinacia oleracea* L.	Amaranthaceae	Stems	1
39	*Syzygium cumini* L.	**Myrtaceae**	Fruits	2
40	*Vigna unguiculata* sp.	Fabaceae	Fruits	3

1-folk criteria; 2-references; 3-random selection.

Process of inhibitory activity testing α-glucosidase enzyme

Methodological basis:

The α-glucosidase enzyme catalyses p-nitrophenyl-α-D-glucopyranoside into α-D-glucose and p-nitrophenol, which has a light yellow colour and maximum absorbance in the optical spectrum at 401 nm [2]. In the presence of enzyme inhibitors, the intensity of the absorption of the solution will decrease. Based on the absorption intensity of the solution with and without a sample, the percentage of inhibition of the α-glucosidase enzyme of the sample can be calculated. Construction of the line of representation between percent inhibitors and concentration of inhibitors determines the IC_{50} values, which is the concentration of the sample at which 50% of the enzymes are inhibited. Samples with higher activity have lower IC_{50} values.

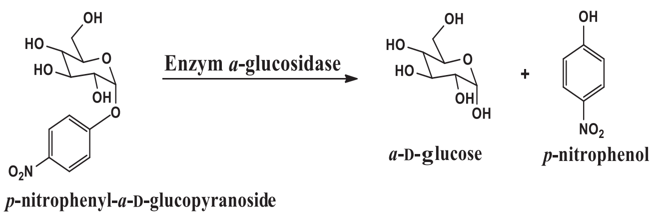

Diagram 1. Hydrolysis of the α-glucosidase enzyme with *p*-nitrophenyl-α-D-glucopyranoside substrate.

Chemicals:

- Phosphate buffer solution 0.01 M with pH=7.0
- α-glucosidase enzyme solution 0.1 U ml^{-1}
- p-nitrophenyl-α-D-glucopyranoside background solution 1.5 mM
- Na_2CO_3 solution 0.1 M

All the chemicals were supplied from Aldrich-Sigma.

Activation test procedure:

The sample was dissolved in a phosphate buffer, to which 50 µl of the enzyme was added and mixed well. After incubation at 37ºC for 5 min, 50 µl of background solution was added, mixed well, and incubated again at 37°C for 30 min. At the end of the second incubation period, 375 µl of Na_2CO_3 was added and the optical density of the solution was measured at a wavelength of 401 nm. Each sample was measured at 5 different concentrations (250, 100, 50, 25, 10 µg ml^{-1}), along with a blank sample that was similar to the test samples. Still, the enzyme solution was replaced with a phosphate buffer solution. The average of three optical density measurements at each concentration was calculated as the inhibitory percentage value (I%).

Results and discussion

The results of the enzyme α-glucosidase inhibitory activity of the 40 vegetables, tubers, and fruits from the An Giang province are presented in Table 2.

Table 2. The results of α-glucosidase enzyme inhibition activity of the 40 vegetables, tubers, fruits from the An Giang province.

Number	Samples	Inhibition (%)					IC$_{50}$ (µg ml^{1})
		250 (µg ml^{-1})	*100 (µg ml^{-1})*	*50 (µg ml^{-1})*	*25 (µg ml^{-1})*	*10 (µg ml^{-1})*	
1	*Abelmoschus esculentus*	*	96.89±0.71	78.71±1.45	49.74±0.98	24.67±0.99	25.22
2	*Allium ramosum*	*	87.51±0.81	60.96±1.19	40.43±1.13	31.06±1.51	36.65
3	*Amaranthus tricolor*	17.93±1.65	1.39±0.66	-	-	-	>250
4	*Asparagus officinalis*	*	96.01±0.85	62.72±0.46	22.01±0.97	1.6±0.58	42.19
5	*Azadirachta indica*	87.6±0.68	39.77±1.04	5.77±0.82	-	-	132.08
6	*Basella alba*	98.77±0.25	32.49±0.88	15.37±0.83	3.36±1.3	-	139.63
7	*Benincasa hispida* Thunb	*	54.47±0.73	22.87±0.8	7.1 ± 1.7	-	92.93
8	*Brassica integrifolia*	46.8±1.09	19.27±1.48	7.1±1.79	-	-	>250
9	*Brassica juncea*	*	51.52±1.01	23.43±0.53	6.46±0.48	-	97.29
10	*Brassica rapa chinensis*	93.02±0.63	40.5±0.86	18.19±0.91	7.95±0.85	-	127.13
11	*Brassicarapa* subsp. *pekinensis*	*	68.16±1.33	37.16±1.79	7.81±1.02	-	70.72
12	*Centella asiatica*	*	75.15±0.66	26.4±1.15	2.6±0.9	-	74.21
13	*Colocasia esculenta*	*	67.3±0.92	46.44±0.83	15.69±0.58	6.16±0.63	58.53
14	*Cucumis sativus*	23.78±0.98	3.57±1.63	-	-	-	>250
15	*Cucurbita pepo*	*	97.12±0.36	55.58±0.51	27.27±1.25	1.92±0.76	45.07
16	*Eichhornia crassipes*	*	73.44±1.34	28.04±0.77	11.39±0.93	-	74.18
17	*Ipomoea batatas*	81.57±0.15	33.5±0.91	27.28±0.95	10.8±0.52	3.54±1.41	151.50
18	*Ipomoea batatas*	*	86.61±0.86	76.09±1.01	58.47±0.78	49.22±0.73	11.26
19	*Lagenaria siceraria*	*	96.39±1.25	69.28±0.96	23.2±1.33	4.09±0.24	39.54
20	*Luffa cylindrica*	*	97.88±0.38	82.66±0.92	34.45±0.86	15.04±2.02	33.07
21	*Momordica charantia*	*	93.36±1.31	71.22±0.85	44.45±1.59	29.2±0.57	30.18
22	*Momordica charantia*	95.88±0.64	31.84±1.01	19.65±1.61	-	-	142.54
23	*Momordica charantia*	*	*	89.16±2.45	40.39±1.22	12.51±0.99	29.93
24	*Morus alba*	*	59.98±1.43	34.22±1.24	16.4±1.06	2.65±1.3	80.63
25	*Musa balbisiana*	*	*	97.7±0.15	68.06±0.97	26.65±0.74	18.46
26	*Peperomia pellucida*	96.09±0.38	39.65±1.55	21.13±1.39	12.28±0.91	-	126.76
27	*Perilla frutescens*	*	51.78±0.71	20.19±1.13	11.34±0.73	3.31±1.05	97.18
28	*Phaseolus vulgaris*	*	97.04±0.43	64.63±1.21	17.67±0.72	6.96±1.16	42.21
29	*Piper sarmentosum*	76.56±0.88	22.76±1	19.68±1.44	11.28±1.17	1.19±0.91	175.94
30	*Raphanus sativus*	98.68±0.27	48.71±0.97	5.62±1	-	-	103.86
31	*Sauropus androgynus*	96.78±0.51	26.72±1.17	17.41±1.42	2.25±1.21	-	149.84
32	*Solanum lycopersicum*	36.84±0.77	8.31±0.86	-	-	-	>250
33	*Solanum melongena*	98.34±0.44	41.77±1.43	15.84±1.15	-	-	121.82
34	*Spinacia oleracea*	45.78±1.64	13.38±1.07	3.08±1.08	-	-	>250
35	*Vigna unguiculata*	*	99.9±0.2	77.36±1.19	49.4±1.9	2.39±1.35	38.75
		-	10 (µg ml^{-1})	5 (µg ml^{-1})	2.5 (µg ml^{-1})	1.0 (µg ml^{-1})	
36	*Cassia grandis*	*	97.14±0.61	83.29±0.8	49.45±1.9	23.17±2.43	2.54
37	*Enydra fluctuans*	*	55.91±2.13	33.58±1.05	11.71±1.13	-	8.68
38	*Glycine max*	*	79.49±0.86	48.93±2.22	24.15±2.74	11.3±1.43	5.17
39	*Syzygium cumini*	*	98.59±0.73	64.6±1.27	26.34±1.12	9.79±1.53	4.05
		-	1.0 (µg ml^{-1})	0.5 (µg ml^{-1})	0.25 (µg ml^{-1})	0.1 (µg ml^{-1})	
40	*Areca catechu*	*	95.99±0.37	84.59±0.45	59.5±0.91	44.92±0.62	0.15
Acarbose							214.5

-: enzyme inhibitor concentration lower than 1% (I<1%); *: enzyme inhibitor concentration is higher than 100% (I>100%).

The results of this study showed that of the 40 samples of vegetables, tubers, and fruits, all 40 samples had inhibitory activity at a concentration of 250 µg ml^{-1}, while 36 samples had greater than 50% inhibition at a concentration of 250 µg ml^{-1}, 25 samples had greater than 50% inhibition at a concentration of 100 µg ml^{-1}, and 17 samples had greater than 50% inhibition at a concentration of 50 µg ml^{-1}. At lower concentrations, 7 samples had greater than 50% inhibition at a concentration of 25 µg ml^{-1}, 5 samples had greater than 50% inhibition at a concentration of 10 µg ml^{-1}, and 1 sample had greater than 50% inhibition at a concentration of 1 µg ml^{-1}. Of the 40 samples, the 5 with the most robust inhibition activity of the α-glucosidase enzyme should be further tested at lower concentrations, as the IC_{50} values of the 5 samples were 0.15, 2.54, 4.05, 5.17, and 8.68 µg ml^{-1}, respectively, which is much stronger than the positive control (IC_{50} value of acarbose was 214.5 µg ml^{-1}).

Areca catechu (areca nut, Fig. 1) is very popular in South Asia and the seed of these trees has been employed as traditional medicine. A lot of compounds have been identified from *Areca catechu* such as steroids, tannins, flavones, alkaloids, fatty acids, and triterpenes, which could be notable pharmacological activities [3].

Fig. 1. *Areca catechu*.

Cassia grandis (Fig. 2) has been found in the Mekong Delta for a long time. According to the dictionary of medicinal plants [4, 5] and accessible experience in Vietnam, *Cassia grandis* has a variety of medicinal uses. Its fresh leaves cure dermatophytosis, its green fruit cures dysentery, and its ripe fruit cures bone aches and pains. Meanwhile, chemical investigations of this plant are still limited. Besides traditional uses of the plant as in Vietnam, and particularly in South America where it is said to be the origin of *Cassia grandis*, its ripe fruits are a good remedy for people suffering from anaemia. Recent biological studies showed that this plant possesses several biological activities such as antimicrobial effects on saltwater shrimp, anti-diabetic, antioxidant, anti-inflammatory, analgesic, and hepatoprotective activity, etc. Preliminary chemical screening tests showed the presence of almost 9 large groups of natural compounds: flavonoid, anthracoid, alkaloid, steroid, terpenoid, glycoside, tannin, saponin, and coumarin [6].

Fig. 2. *Cassia grandis*.

Syzygium cumini (jambolan, Fig. 3) is one of the most commonly used anti-diabetic medicines. A lot of research shows that this plant contains anthocyanin, glucoside, ellagic acid, isoquercetin, kaemferol, and myricetin. Muniappan Ayyanar and Pandurangan Subash-Babu (2012) [7] found that alcaloid, jambosine, and glycoside jambolin or antimellin in *Syzygium cumini's* seed is a natural medicine.

Fig. 3. *Syzygium cumini*.

Glycine max (soybean, Fig. 4) is a globally grown legume, including Vietnam. Ghahari, et al. [8] studied the chemical composition of soybean oil and showed its antioxidant and antibacterial effects on various agriculturally harmful pathogens. By gas chromatography combined with mass spectrometry, the authors identified 40 components in soybean oil, accounting for 96.68% of the total amount of oil. In particular, the main components were carvacrol (13.44%), (E, E)-2.4-decadienal (9.15%), *p*-allylanisole (5.65%), *p*-cymene (4.87%), and limonene (4.75%). By disk diffusion and minimum inhibitory concentration techniques, the antibacterial activity of soybean oil was determined. In addition, the antioxidant activity of soybean oil was assessed by catalase, peroxidase, superoxide effuse and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The oil showed significant activity against *Pseudomonas syringae* subsp. syringae, *Rathayibacter toxicus* with MIC=25 µg ml^{-1}, and *Pyricularia oryzae* with MIC=12.5 µg ml^{-1}. In addition, the free radical scavenging capacity of the essential oil was determined with an IC_{50} value of 162.35 µg ml^{-1}. The results suggest that this plant may be a potential source of biocide, thus beneficial for economical and environmentally friendly disease control strategies. It may also be a good candidate for further biological and pharmacological investigations [8].

Fig. 4. *Glycine max*.

Enhydra fluctuans (Fig. 5), a natural vegetable, is used in Vietnamese meals. The *Enhydra fluctuans* belongs to the Asteraceae family and is gaining interest because of some of its amazing benefits to human health. According to folk medicine, its leaves have a slightly bitter taste, cure inflammation and skin diseases, can be used as a laxative, and to treat bronchitis, nervous affections, leucoderma, and biliousness. Some extracted compounds such as β-carotene, saponins, cholesterol, glucoside, and boostydrin are the main components in these plants. This vegetable has some great uses such as an antioxidant, for liver protection, central nervous system inhibition, and pain relief. The amazing pharmacological effects of this vegetable help scientists open up research pathways into herbs that can treat diseases without causing unwanted side effects that are caused by some synthetic drugs in use today [9].

Fig. 5. *Enhydra fluctuans*.

Conclusions

Results of the screening of the inhibitory activity of the α-glucosidase enzyme from the 40 samples of vegetables, tubers, and fruits showed that 1 sample had an IC_{50} value <1 μg ml^{-1}, 4 samples had IC_{50} values <10 μg ml^{-1}, 20 samples had IC_{50} values from 100 to 10 μg ml^{-1}, 10 samples had IC_{50} values from 250 to 100 μg ml^{-1}, and 5 samples had IC_{50} values >250 μg ml^{-1}. The positive control in this study was acarbose and 35 of the 40 samples had an α-glucosidase enzyme inhibitory activity stronger than the positive control, especially the seeds of *Areca catechu*, the fruits of *Cassia grandis*, the fruits of *Syzygium cumini*, the seeds of *Glycine max,* and the stems of *Enydra fluctuans*. These research results create a basis for the study of isolated substances originating from strong active samples and contribute to the investigation of diabetes treatments derived from herbs, which can have reduced side effects compared with synthesized active substances.

COMPETING INTERESTS

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

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