Research Article

Anti α-glucosidase, anti α-amylase, anti-oxidation, and anti-inflammation activities of *Etlingera elatior* rhizome

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ABSTRACT

The potentials of Etlingera elatior rhizome as an antidiabetic agent have been performed in the current study using anti α-glucosidase, anti α-amylase, anti-oxidation, and anti-inflammation activities. The macerated ethanolic crude extract (EE) and five fractions with different polarities, partitioned with n-C_6H_6 (F1), CH_2Cl_2 (F2), EtOAc (F3), n-BuOH (F4), and H_2O (F5), were prepared to be tested the biological activities. The results indicated that all crude extracts exhibited good inhibition for both α-glucosidase and α-amylase in the range of 28.36-99.79% and 35.91-58.13% at the concentration as 25 µg/mL, respectively. Most of them displayed equipotent to 9 fold higher than standard acabose, especially for anti α-glucosidase activity. Due to widely distribution of flavonoids in all partitioned extracts, flavonoids might play the important roles inα-glucosidase and α-amylase inhibition. EE andnon-polarfractions (F1 and F2), showed the potency in anti-inflammatory activity equal to standard indomethacin with IC_{50} 19.35, 17.79, 21.12and 19.81µg/mL, respectively. It is likely that the presence of terpenoid aglycones in F1 and F2fractions could possess anti-inflammatory activity of E. elatior rhizome. For antioxidation activities, the presence of tannin, phenolic and flavonoid was associated with good activity of EtOAc fraction (F3), when compared with standard ascorbic acid (IC_{50} 12.60 and4.22µg/mL, respectively) whereas F2, F3 and F4 revealed good anti-oxidation profile by FRAP assay. Our findings supported the ability of E. elatior rhizome in development as nutraceutical purpose for utilizing in diabetic care in near future.

Keywords: *Etlingera elatior*, anti*α*-glucosidase activity, anti *α*-amylase activity, diabetes mellitus, anti-inflammatory activity
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INTRODUCTION

Presently, diabetes mellitus (DM) has been remained to be the worldwide health problem and led to the decreasing in quality of life of the patients. It affected almost 366 million patients in 2011 and will be rising to 500 million in 2030 [1]. Type 2 diabetes mellitus (T2DM) or the most incidence among the other types of DM is often caused by the insulin resistance [2]and degenerative processes damaging pancreas, muscle, and fat tissues. This obnoxious processes are principally originated by the reactive species, such as oxygen free radical, peroxide, and superoxide species, which are subsequent to unwanted oxidation at the particular organ in the body. In addition, the inflammation process is another one reason that could defect the endocrine system by the releases of reactive intermediates, nitric oxide (NO), during the inflammation process.

α-Glucosidase and α-amylase are digestive enzymes which catalyzed the breaking down carbohydrates or polysaccharides to monosaccharides [3]. The blockage these enzymes can delay the carbohydrate digestion and provide lower absorbable sugars after meals and lead to lower glucose uptakes and blood sugars [4,5]. This becomes an attraction as the therapeutic option for DM combat.

Etlingera elatior is belonging to the family Zingiberaceae and widely grows in tropical area [6]. In tropical countries, *E. elatior* is often used as the food ingredients and as traditional medicines for DM, earache, cleaning wounds and post-partum women[6]. There were several reports determining the antidiabetic activity of *E. elatior* usually from leaves, however, of *E. elatior* rhizome have not been determined to date.

Due to the incurability of DM, the primary therapeutic successes in DM care involve the prevention in the occurrences of the vascular complications. Rather than the uses of potent antidiabetic drugs, the dietary is important as well in controlling the blood sugar level and reducing the complications. Therefore, in this study, we aim to investigate the anti α-glucosidase, anti α-amylase, anti-inflammation, antioxidant activities to evaluate the potentials of *E. elatior* rhizome to be developed in nutraceutical world.

EXPERIMENTAL SECTION

1. Chemicals
The solvents for extractions, hexane (n-C_6H_4), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), buthanol (n-BuOH) methanol (MeOH), chloroform ($CHCl_3$) and ethanol (EtOH) were purchased from Labscan Asia co., Thailand. Biological activities testing were performed using α-glucosidase and p-nitrophenyl-α-D-glucopyranoside from Sisco Research Laboratories Pvt. Ltd., India. Standard acarbose, α-amylase and starch azure were purchased from Sigma, Sigma-Aldrich, Germany. Lipopolysaccharide (LPS) from Escherichia coli, RPMI-1640 medium, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), standard indomethacin, phosphate buffer saline (PBS), dimethyl sulfoxide (DMSO), Tween 80, 2,2-diphenyl-1-picrylhydrazyl (DPPH), standard ascorbic acid, quercetin, potassium ferricyanide, trichloroacetic acid, ferric chloride, sodium hydroxide, concentrated sulphuric acid, Dragendroff reagent and hydrogen chloride were purchased from Sigma Aldrich (Sigma Aldrich, USA). Fetal calf serum (FCS) was from Gibco (Invitrogen, California, USA). Penicillin-streptomycin was from Invitrogen (Invitrogen, California, USA). 96-Well microplates were obtained from Nunc (Nunc, Birkrød, Denmark).

2. Plant materials
The rhizomes of *E. elatior* were collected from Songkhla province, Thailand in 2014 and were identified by the plant taxonomist, Dr. Oratai Neamsuvan. Voucher specimen has been deposited at the Faculty of Traditional Thai Medicine, Prince of Songkla University. The plant materials were cleaned with tap water to remove soil and other contaminants, sliced into smaller pieces, and then dried by hot-air oven for 48 hours at 50ºC. Dried plants were grinded to be fine powder and kept at 4ºC in the refrigerator until uses.

3. Crude extract preparation
2,600 g of rhizome powder were macerated using ethanolfor 48 hours. After collecting the filtrate, this ethanolic macerate was evaporated at 45ºC under reduced pressure to obtain ethanolic crude extract. Macerations were repeated in triplicates and all extracts were combined to give 127.33 g of ethanolic crude extract (EE). Aliquots of this crude extract (120 g) wereinitially dissolved with 10% aqueous MeOH and then proceed the sequential partition using several solvents including n-C_6H_6, CH_2Cl_2, EtOAc, n-BuOH, and H_2O to finally obtain five fractions (F1-F5), respectively. All of them were used to determine their biological activities and phytochemical screening.

4. Biological activities testing
4.1. α-amylase inhibitory activity
α-Amylase inhibitory activity was determined by the modified method of Gao and Kawabata (2005) and Hansawasdi et al. (2000)[7,8]. Two mg of starch azure, as enzyme substrate, were dissolved by boiling in 0.2 mL of 0.05 M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ at 100ºC for 10 min. The mixture was then cooled down to 50 ºC in 5 min and followed by pre-incubation at 37ºC for 5 min prior to further steps. The sample to be tested, approximately 2 mg, was prepared by dissolving in the mixture of 1 mL of DMSO and 0.1 mL of porcine pancreas α-amylase (1.6 unit/mL) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl solution. The reaction was initiated after mixing both substrate and enzyme containing extract solutions and it was allowed to incubate at 37ºC for 10 min. Finally, the chemical reaction was quenched by adding 0.5 mL of 50% acetic acid and the quenched reaction-mixture was centrifuged at 3000 rpm at 4ºC for 5 min. The absorbance of the resulting supernatant at 595 nm was measured. The % inhibition was calculated by following equation;

Inhibition (%) = [(A–B) / (A)] × 100;

Where *A* and *B* are the absorbance of negative control and sample, respectively.

The negative and positive controls were conducted using DMSO and acarbose, respectively.

4.2. α-glucosidase inhibitory activity
α-Glucosidase inhibitory activities were performed by the modified method of Kumar et al. (2013)[9]. 50 µL of sample solution in DMSO was mixed with 50 µL of α-glucosidase solution (0.57 unit/mL) in 50 mM phosphate buffer (pH 6.9). After pre-incubation at 37ºC for 10 min, this mixture was added by 50 µL of 5mM*p*-nitrophenyl-α-D-glucopyranoside in phosphate buffer (pH 6.9) and continuing the incubation at 37ºC for 20 min. The reaction was quenched by adding of 50 µL of 1 M Na_2CO_3 solution. The absorbance of the final solution was measured at 405 nm. The % inhibition was calculated based on the following equation (n = 4).

Inhibition (%) = [(*A*–*B*) / (*A*)] × 100;

Where *A* and*B* are the absorbance of negative control and sample, respectively.

The negative and positive controls were conducted using DMSO and acarbose, respectively.

4.3. DPPH radical scavenging assay
1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of extracts were determined by the modified methods of Brand-Williams et al. (1995)[10]. Briefly, 170 µL of 24% w/v DPPH solution was mixed with 30 µL of tested sample solution in DMSO. The mixture was allowed to stand at room temperature for 30 min with avoiding from light and was followed by the measurement of the absorbance at 515 nm (MeOH as blank and ascorbic acid as positive control). The % inhibition was calculated by following equation;

Inhibition (%) = [(*A*–*B*) / (*A*)] × 100;

Where *A* and*B* are the absorbance of negative control and sample, respectively.

IC_{50} were determined from the graph plotted between %inhibitions against sample concentrations (n = 4).

4.4. Ferric reducing antioxidant power (FRAP) assay
The reducing antioxidant power of samples were carried out by the modified method of Yildirim et al. (2001)[11]. Briefly, 200 µL of sample (250 µg/mL) were mixed with 500 µL of 0.2 M phosphate buffer (pH 6.6) and 500 µL of 1% w/v potassium ferric cyanide. The mixture was place in the incubator at 50ºC for 30 min and followed by the addition of 500 µL of 10% w/v trichloroacetic acid. After the centrifugation at 3000 rpm for 30 min, 600 µL of the supernatant were taken to mix with 600 µL of distilled water and 120 µL of 0.1% w/v of $FeCl_3$. The resulting mixture was measured at 700 nm. Reducing antioxidant power of tested samples were expressed as quercetin equivalents (mg quercetin/g sample).

4.5. Nitric oxide inhibitory activity
Inhibition the NO production in murine macrophage-like cell-line, RAW264.7 was evaluated by the method of Sudsai et al. (2014)[12].Cells were cultured in culture flask at 37°C with a humidified atmosphere containing 5% CO_2 using RPMI medium supplemented with 0.1% $NaHCO_3$, 2 mM glutamine, penicillin G (100 units/mL), streptomycin (100 µg/mL), and 10% FCS as culture medium. Cells were then harvested with trypsin-EDTA and suspended in a fresh medium. The cells were seeded in 96-well plates with 1×10^5 cells/well and allowed to adhere the bottom of well in the incubator. After 1 hour incubation, the medium was replaced by a fresh medium containing 25 µg/mL of LPS and the test samples or indomethacin (standard drug) at various concentrations (1-100 µg/mL).After 24 hours, the supernatant (100 µL) were collected and reacted with Griess reagent (100 µL) for measuring the accumulation of nitrite in the supernatant. The reaction was measured by spectrophotometer (Power Wave X from Bio-Tex Inc) at 570 nm. The % inhibition was calculated based on the following equation and IC_{50} were determined from the graph plotted between %inhibitions against sample concentrations (n = 4).

Inhibition (%) = [(*A* - *B*) / (*A* - *C*)] × 100

Where *A*, *B*, and *C* are the absorbance of LPS solution, sample with LPS, and sample without LPS, respectively.

Cytotoxicity was also determined using the MTT colorimetric method. After 24 hours incubation with the test samples, MTT solution (10 µL, 5 mg/mL in PBS) was added to the wells. After 2 hours incubation, the medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the

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cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm. The test samples were considered to be cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group.

5. Phytochemicals analysis

Ethanolic crude extract of *E. elatior* rhizome was phytochemically screened for the possible secondary metabolite including tannins, phenolic compounds, flavonoids, terpenoids, and alkaloids by the modified method of standard procedures[13].

5.1. Testing for tannins

25 mg of each sample were boiled in 5 mL of distilled water and then the suspension was filtered through filter paper (Whatman No.1). By the addition of a few drops of 0.1% ferric chloride, the sample filtrate became brownish green or blue black, if tannin is present.

5.2. Testing for phenolic compounds

25 mg of each sample in 2 mL of ethanol were mixed with a few drops of 5% ferric chloride solution. The presence of phenolic compounds could turn the solution to a bluish black color.

5.3. Testing for flavonoids

25 mg of each samplein 2 mL of ethanol were mixed with a few drops of 10% NaOH solution and led the solution turning to yellow. By adding 1 mL of conc. H_2SO_4, flavonoid-containing samples were de-colorized.

5.4. Testing for terpenoids

25 mg of each sample in 2 mL of chloroform were prepared. A few drop of conc. sulphuric acid was added carefully to from the layer. Formation of reddish brown color interface at the boundary indicated the presence of terpenoids.

5.5. Testing for alkaloids

25 mg of each sample were suspended in 5 mL of 10% HCl solution with boiling for 15 min. Two mL of diluted ammonia solution (pH > 8) was added after filtered the mixture solution through filter paper. The solution was then partitioned with 5 mL of chloroform. The part aqueous solution was collected and then mixed with 10 mL of acetic acid. The presence of alkaloids was determined by the reddish brown precipitate after a few drops of Dragendorff's reagent.

6. Statistical treatment

All results were expressed as mean±S.D. The IC_{50} values were calculated using the Microsoft Excel program. The comparison of the data was evaluated using one-way analysis of variance (ANOVA) using SPSS 16.0, followed by LSD.

RESULTS AND DISCUSSION

The powder of *E. elatior* rhizome was extracted by the maceration using ethanol to give 127.33 g of ethanolic crude extracts(EE; 4.76% dried weight). Of ethanolic crude extract, sequential partition was carried out to fractionate EE (120 g) into five fractions (F1-F5) to be different in the polarities. The weights, %yields, and physical appearances of each fraction were shown in Table 1.

The α-glucosidase and α-amylase inhibitory activities of all samples (EE and F1-F5) were demonstrated in Table 2. Interestingly, all tested samples at the concentration 25 µg/mL exhibited strong inhibition to α-glucosidase and more potent than acabose significantly. Their %inhibitions were in the range of 28-99% in which only F2 is lower than 80%. For α-amylase inhibitory activities, all tested sample, except F1displayed intermediate potency with by slightly higher than acabose significantly to blockα-amylase activity where F2, F4, and F5 showed the % inhibitions more than 50%.

In DPPH radical scavenging assay, the activities as $IC_{50}$of extracts were shown in Figure 1. F2-F4 could well scavenge the DPPH radical with IC_{50}12.60, 24.77 and 26.61 µg/mL, respectively as compare by ascorbic acid (IC_{50} 4.22µg/mL) while only F3exhibited most potent antioxidant capacity in FRAP assay with the highest quercetin equivalence (3.28 mg/g of quercetin) as shown in Figure 2.

Furthermore, the blockage of NO production of *E. elatior* rhizome extracts was also performed to determine the degree of anti-inflammation. Anti-inflammatory property of the extracts was compared with indomethacin as positive control. The results as % inhibition at any concentration and IC_{50} values were shown in Table 3. All crude

extracts could suppress NO production in a dose-dependent manner without cytotoxic effect at the concentration up to 100 µg/mL. The ethanolic crude extracts (EE) and the non-polar fractions (F1 and F2) illustrated satisfactory anti-inflammatory activity with the potency equal to standard indomethacin, while the moderate to high polar extracts (F3-F5) showed the moderate activity.

The phytochemical compositions of *E. elatior* rhizome were primarily screened. The general phytochemicals including tannins, phenolics, flavonoids, terpenoids and alkaloids in the crude extracts were evaluated in qualitative manner as showed in Table 4. Of ethanolic crude extracts prior to fractionation, it was comprised of tannins, phenolic compounds, flavonoids and terpenoids, but not alkaloids. When the fractionation was processed, flavonoids could distribute to all partitions. Tannin was absent in non-polar fraction (F1) and phenolic compound did notcontain in H_2Ofraction (F5). Surprisingly, terpenoids werespread into both non-polar (F1 and F2) and polar fractions (F4). It was probable that the terpenoids as glycoside were present in F4.

With the comparision of all biological activities, flavonoids played the important role for antidiabetic activities and the terpenoid aglycone modulated anti-inflammatory activity of *E. elatior* rhizome. Tannin, phenolic and flavonoid compounds would associate with the strong anti-oxidation activities in EtOAc fraction (F3).The results confirmed the potential of Zingiberaceous plants for anti-inflammation and antioxidation [12,14,15,16].

Table 1The amounts of each fraction and their physical appearances

Sample	Weight (g)	% Yield (*w/w*)	Physical appearance
F1	47.67	37.83 %	dark brown, semisolid
F2	6.60	5.24 %	yellow-brown, solid
F3	4.01	3.18 %	reddish-brown, solid
F4	18.03	14.30 %	light brown, solid
F5	27.01	21.43 %	brown, solid

Table 2α-Glucosidase and α-amylase inhibitory activities of crude extract and each fraction

Samples	% Inhibition[a]	
	α-glucosidase	α-amylase
EE	95.34±0.57[*]	45.39±3.34
F1	94.36±1.65[*]	35.91±2.52[*]
F2	28.36±8.95[*]	50.77±2.52[*]
F3	86.36±2.23[*]	43.56±3.46
F4	99.79±0.05[*]	55.31±0.96[*]
F5	92.10±0.80[*]	58.13±2.70[*]
Acarbose	11.12±5.93	43.39±1.19

[a]*the sample concentrations were at 25 µg/mL.*
[*]*p<0.05 as compared with standard acarbose.*

Table 3Anti-inflammatory activity of crude extract and each fraction

Sample	% inhibition at various concentrations (µg/mL)					IC$_{50}$ (µg/mL)
	1	3	10	30	100	
EE	7.12±3.01	14.43±3.12	33.46±3.29	56.93±0.41	90.87±0.60	19.36±3.08
F1	8.76±2.14	16.74±3.01	38.99±3.18	58.21±1.11	87.88±0.64	17.80±0.82
F2	4.92±3.58	16.54±2.85	29.93±0.89	55.06 ± 2.29	91.07±0.28	21.12±6.15
F3	2.21±2.17	5.24±3.53	13.34±2.39	31.77±2.50	68.43±1.09	52.13±2.73[#]
F4	-	7.14±0.54	13.98 0.82	30.15±3.62	70.86±1.13	51.00±4.10[#]
F5	-	5.43±0.25	13.60±1.06	23.50±1.00	69.72±1.45	58.89±1.35[#]
Indomethacin	23.32±1.28	34.08±1.35	44.94±0.70	52.73±0.53	85.90±0.44	19.82±0.96

[#] *significantly difference when compared with standard drug acabose,*
(-) = not determined.

Table 4 Phytochemical analysis of crude extract and each fraction

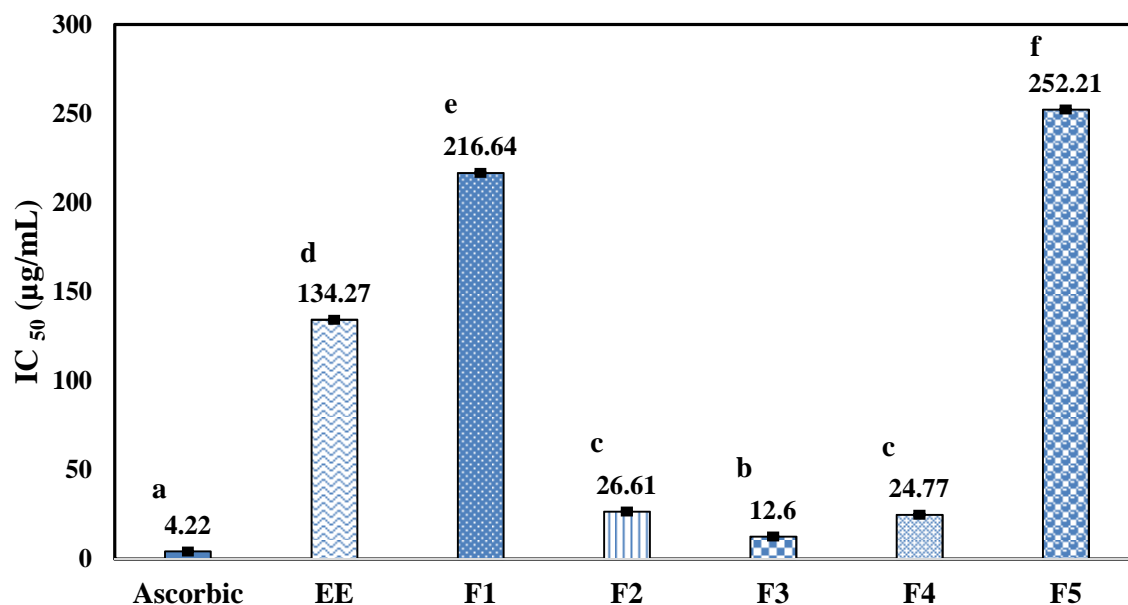| Sample	Tannins	Phenolics	Flavonoids	Terpenoids	Alkaloids
EE	+	+	+	+	−
F1	−	+	+	+	−
F2	+	+	+	+	−
F3	+	+	+	−	−
F4	+	+	+	+	−
F5	+	+	+	−	−

+to be present; - to be absent.

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Figure 1 IC$_{50}$ of crude extract and eachfractionin DPPH radical scavenging assay
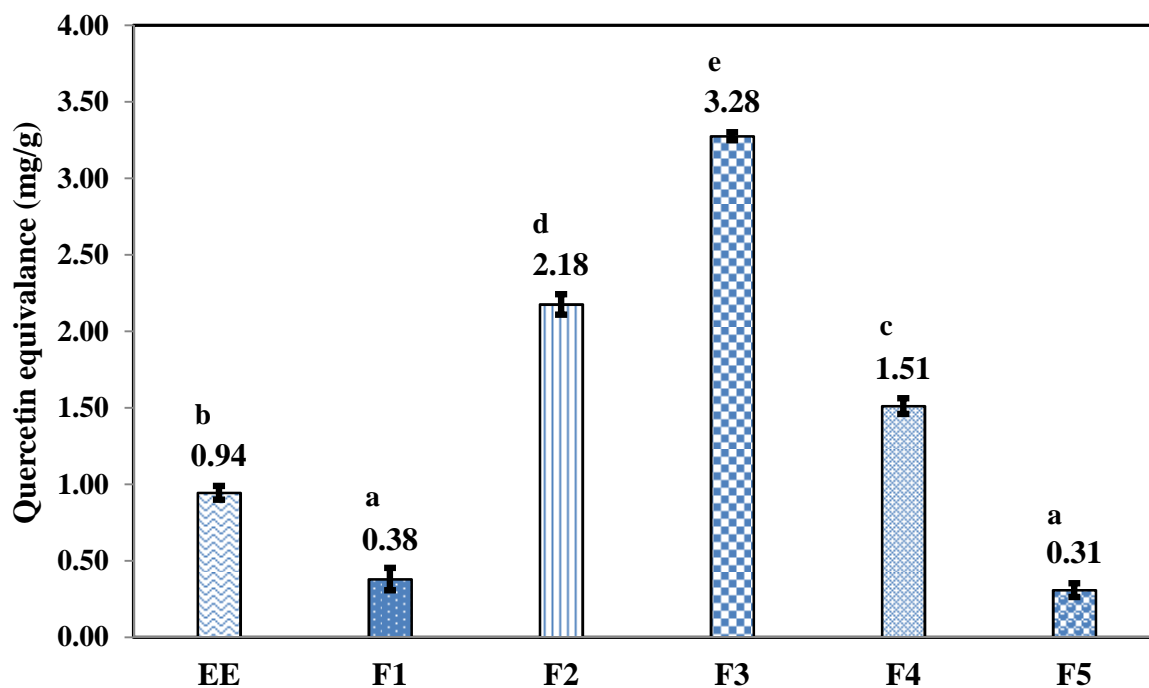*The letters indicate the statistically significant difference of the data.*

Figure 2 Quercetin equivalence of crude extract and eachfractionin FRAP assay
The letters indicate the statistically significant difference of the data.

CONCLUSION

E. elatior rhizomes satisfactorily exhibited anti α-glucosidase and anti α-amylase, antioxidantion, anti-inflammatory activities. It demonstrated the ability of *E. elatior* rhizomes as the potentials to use for nutraceutical purpose in diabetic care. The isolation of active components from *E. elatior* rhizome for antidiabetic activity is ongoing.

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