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Research Article

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A STUDY OF THE ABILITY TO INHIBIT α-AMYLASE AND α-GLUCOSIDASE ENZYMES FROM *GARCINIA MANGOSTANA* LINN'S PEEL EXTRACT

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ABSTRACT

The study examines the ability to inhibit α-amylase and α-glucosidase enzymes from *Garcinia mangostana* Linn's peel to exploit the potential of sources from the Mekong Delta. These are two important enzymes in the process of amylolysis into glucose, contributing to increase the blood sugar after meals. Inhibiting their activity helps control blood sugar levels. *Garcinia mangostana* Linn's peel is extracted with 70% ethanol, and the results show that the extraction efficiency reaches 15.59%. The ability to inhibit α-amylase is measured by spectrum at a wavelength of 660 nm, while the ability to inhibit α-glucosidase is measured through a reaction with para-nitrophenyl-α-D-glucopyranoside at a wavelength of 405 nm. The IC50 value shows that the inhibition levels of α-amylase, α-glucosidase and the ability to eliminate the free radical DPPHare 48 µg/mL, 56 µg/mL and 171 µg/mL respectively. The study also indentifies antioxidant compounds such as tannins, flavonoids, glycosides, alkaloids in the extract. The α-mangostin content in the dry peel is 21%, and the mechanism of enzyme inhibition is a mixed type.

Keywords: α-amylase, α-glucosidase, α-mangostin, DPPH, *Garcinia mangostana* Linn.

INTRODUCTION

In recent years, the number of people with diabetes has increased rapidly and become a major problem in public health. The number of people with diabetes is increasing, the total number of people with diabetes is expected to increase from 171 million in 2000 to 366 million in 2030, of which type 2 diabetes accounts for 90-95% (Wild *et al.,* 2004). Diabetes is one of the major threats to human health in the 21st century (Zimmet, 2000). This is the disease with the highest mortality rate among endocrine diseases due to many complications such as retinopathy, nephropathy, neuropathy and the high risk of cardiovascular diseases.Controlling blood sugar, especially blood sugar after meals is considered an important goal in the treatment of diabetes (Yao *et al.,* 2010). An approaching way to control the blood sugar after meals is to slow the glucose absorption through inhibition of carbohydrate digesting enzymes. Two enzymes that hydrolyze starch into glucose after meals are α-amylase and α-glucosidase. They play an important role in increasing the blood sugar after meals. Therefore, the inhibition of these two enzymes is one of the strategic solutions to control the blood glucose levels for the prevention and treatment of type 2 diabetes (Sim *et al.,* 2010; Hogan *et al,* 2010).

Diabetes control is a global problem and a complete cure for diabetes is still under the study (Mishra *et al.,* 2009). Current measures of diabetes control are to maintain the stability of blood glucose levels (Sulfonylurea or Biguanide), insulin secretagogues (Metformin), the inhibitors of digestion and starch absorption (Glucobay).. Although these therapies have certain effects, they are often accompanied by side effects. According to the World Health Organization (WHO, 2002), the research and development of hypoglycemic drugs which are originated from plants, especially medicinal plants that have been commonly used in traditional medicine aim at finding new drugs that are effective without side effects

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*Garcinia mangostana*Linnis a fruit tree which is grown in areas with tropical climates. *Garcinia mangostana*Linnis especially popular in India, Southeast Asia, and Sri Lanka. In Vietnam, *Garcinia mangostana*Linnis mainly grown in the Mekong Delta with the total area up to 4900 hectares with the yield of about 4500 tons. For ages, people have been using*Garcinia mangostana*Linnto treat diseases such as diarrhea, dysentery... and the main part that they use is the peel. *Garcinia mangostana* Linn's peel is a source of raw materials containing many biological activities such as antibacterial, antifungal, anti-inflammatory ones... Especially, it is the ability to lower blood sugar, so the ability to inhibit the α-amylase and α-glucosidase enzymes has a supportive effect in the treatment of diabetes.

Research objectives

A study of the ability to inhibit α-amylase and α-glucosidase enzymes from *Garcinia mangostana* Linn's peel extract is conducted to evaluate the ability to inhibit α-amylase and α-glucosidase enzymes of the extract, which is a basis for research on pharmaceutical production to support diabetes treatment.

SUBJECTS AND METHODS OF RESEARCH

Research subjects

Garcinia mangostana Linn's peel is used to extract.

Research scope

Evaluate the ability to inhibit α-amylase, α-glucosidase enzymes and the anti-oxidation of *Garcinia mangostana* Linn's peel extract.

Research content

Qualification of some natural compounds in ethanol extract of *Garcinia mangostana* Linn's peel according to the method of Yadav

et al., (2014). A study of the ability to inhibit the α-amylase enzyme of *Garcinia mangostana* Linn's peel ethanol extract according to Etoni *et al.,* (2010); Sudha *et al.,* (2011).

A study of the ability to inhibit the α-glucosidase enzyme of *Garcinia mangostana* Linn's peel ethanol extract according to Natasha et al (2012) with modifications.Determination of the inhibitory pattern of the α-amylase and α-glucosidase enzyme of *Garcinia mangostana* Linn's peel ethanol extract according to the methods of Cengis *et al.,* (2010); Gurudeeban *et al.,* (2012).

A study of the antioxidant ability of *Garcinia mangostana* Linn's peel ethanol extract by DPPH according to Ghasemzadeh *et al.,* (2012) with modifications. Determination of α-mangostin content in the extract according to the method of Pothitirat and Gritsanapan (2008)

Research means

Time and location of the research

- Time: The experiment was conducted from October 2023 to March 2024.
- Location: The experiment was conducted at the Biochemistry laboratory, Department of Molecular Biotechnology, Institute of Biotechnology Research and Development, Can Tho University.

Raw materials

*Garcinia mangostana*Linnwas collected in Xuan Hoa Commune, KeSach District, Soc Trang.

Main chemicals used in the experiment

- Extracted chemicals: C_2H_5OH 70%, CH_3OH (Xilong Chemical Co., Ltd; China).
- Qualitative chemicals: KI, I_2, $FeCl_3$ (Xilong Chemical Co., Ltd; China), H_3PO_4, $(CH_3)_2CO$ (VN- Chemsol Co., Ltd).
- Chemicals for testing the ability of enzyme inhibition: α-amylase enzyme is extracted from pig pancreas with an activity of 15 U, α-glucosidase enzyme is extracted from Saccharomyces serevisiae with an activity of 23 U, pNPG (Sigma, USA), DMSO, starch (Guangdong Guanghua Sci-Tech Co., Ltd, China), $Na_2HPO_4.12H_2O$, KH_2PO_4, I_2, HCl, Na_2CO_3 (Xilong Chemical Co., Ltd; China).
- Chemicals for α-mangostin quantification: standard a-mangostin (Chromadex, USA), CH3OH (Xilong Chemical Co., Ltd; China).
- Antioxidant chemicals: DPPH (Sigma, USA).

Main equipment and instruments used in the experiment

- Extraction equipment: Electronic balance (Sartorius, Germany); rotary evaporator (IKA, Germany); vacuum freeze-dryer (Virtis, UK).
- Experimental equipment: Analytical balance (Shimadzu, Japan); spectrophotometer (Thermo, USA); pH meter (Eutech, USA); micropipette (10-100 pL, 200 pL, 100-1000 pL); eppendorf (Bio-rad, USA).

Research method

The entire experiment is carried out according to the general diagram as shown in Figure 1

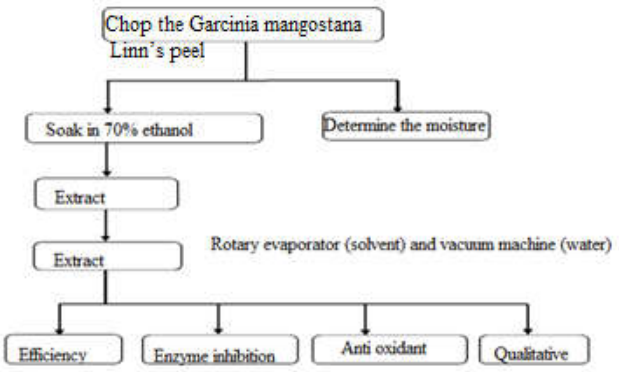

Figure 1. General diagram of the experiment

RESULTS AND DISCUSSIONS

Determination of α-mangostin content in ethanol extract of *Garcinia mangostana* Linn's peel

Garcinia mangostana Linn's peel contains many xanthone compounds, of which α-mangostin has the highest content. α-mangostin has hypoglycemic, anti-inflammatory, anti-cancer, anti-oxidant effects... The α-mangostin content is determined based on the α-mangostin standard line with methanol solvent measured at a wavelength of 320 nm (Pothitirat and Gritsanapan., 2008). Based on the method of Pothitirat and Gritsanapan. (2008), the α-mangostin standard line in methanol is constructed with concentrations from 5 - 20 µg/mL.

The α-mangostin content in the extract is calculated by the standard line equation y = 0.0884x + 0.178 with the correlation coefficient R2 = 0.9912 (Figure 4.2). It shows a linear correlation between the α-mangostin concentrations (µg/mL) and the absorbance at a wavelength of 320 nm.Of which, x is the α-mangostin concentration (µg/mL) and y is the absorbance.

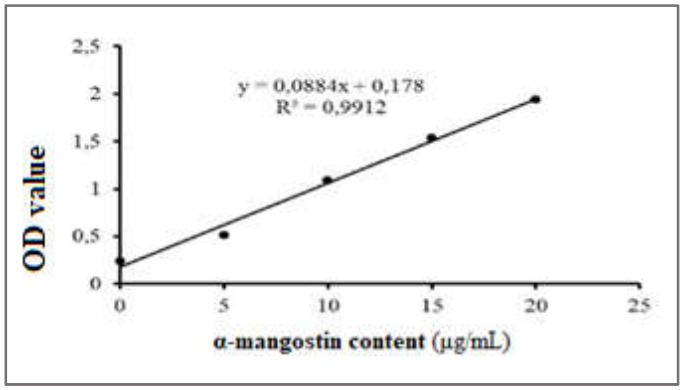

Figure 2. Graph with the relationship between a-mangostin and absorption

From the analysis results, the a-mangostin content is calculated and it accounts for 2.1% in 615 g of dry peel. Satong-aun (2011) extracts the *Garcinia mangostana* Linn's peel extract by three methods (shaking tank, Soxhlet, microwave extraction) for the α-mangostin content of 45.83 ± 0.02; 34.82 ± 0.17 and 49.79 ± 0.15% (w/w), respectively. Meanwhile, in this study, the influence of temperature for *Garcinia mangostana* Linn's peel drying at 55°C, 65°C and 75°C is investigated for the α-mangostin content in the extract of 35.98 ± 0.49; 40.32 ± 0.24 and 37.79 ± 0.34% (w/w).

According to WerayutPothitirat and WandeeGritsanapan (2008), the a-mangostin content in the extract and in the *Garcinia mangostana*

Linn's peel powder after drying in the East of Thailand reaches 9.55 ± 0.45 and 35.68 ± 3.79% (w/w). Meanwhile, the extract sample from the peel of *Garcinia mangostana*Linnin the South of Thailand gives a-mangostin content in the extract and the peel powder after drying of 10.39 ± 1.04 and 36.92 ± 5.55% (w/w), respectively.

Table 1. The result of α-mangostin content(%)

No.	Extract	Content (%)
1	In 95,85 g extract	13,21
2	In 615 g dried peel	2,1

This difference is due to different extraction methods for different α-mangostin contents. In addition, soil conditions in different areas and sampling time also affect the ripeness of the fruit peel, which causes differences in α-mangostin content in *Garcinia mangostana* Linn's peel. In addition, the experiment is to determine the α-mangostin content in the extract form, not pure a-mangostin, so the results may be affected by other substances in the extract, so the α-mangostin content results are lower than in other studies.

A study of the effect of the concentration of *Garcinia mangostana* Linn's peel ethanol extract on the ability to inhibit the enzyme α-amylase

The experiment which is implemented to study the ability to inhibit α-amylase enzymedepends on the initial amount of starch and the amount of starch remaining after the hydrolysis reaction to evaluate the hydrolysis level of the enzyme α-amylase. The larger the amount of starch remains after the reaction is, the stronger the ability to inhibit the α-amylase enzyme is (Le Ba Tuoc, 2013).

The ability to inhibit the α-amylase enzyme of the *Garcinia mangostana* Linn's peel ethanol extract is based on the efficiency of the starch hydrolysis reaction of the α-amylase enzyme. The percentage of inhibition of the α-amylase enzyme of the *Garcinia mangostana* Linn's peel extract is presented in Table 2.

Table 2. The ability to inhibit the α-amylase enzyme of the *Garcinia mangostana* Linn's peel ethanol extract

Independent variable	Extract concentration (g/mL)	Percentage of inhibited α-amylase enzyme (%)
Reference	0	$8,92^{g}$±0,37
1	5	$20,75^{f}$±0,43
2	10	$23,67^{e}$±0,33
3	20	$30,92^{d}$±0,34
4	40	$45,42^{c}$±0,25
5	80	$71,83^{b}$±0,21
6	160	$93,75^{a}$±0,42
7	200	$93,42^{a}$±0,31
8	240	$93,00^{a}$±0,65
9	320	$93,08^{a}$±0,15

The difference among following letters in the same column has no meaning in statistics at 5% level according to Tukey's test.

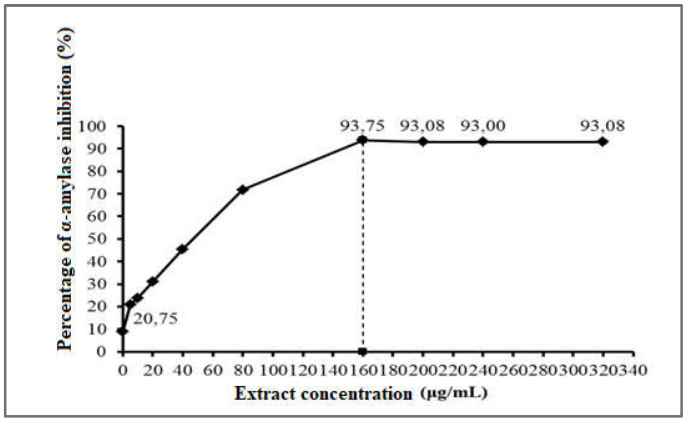

Figure 3. Graph shows the ability to inhibit the α-amylase enzyme of *Garcinia mangostana* Linn's peel ethanol extract

The inhibition of the α-amylase enzyme of *Garcinia mangostana* Linn's peel ethanol extract is proportional to the concentration of the extract. When the concentration of the extract increases, the ability to inhibit the α-amylase enzyme increases. Specifically, at a high concentration of 5 pg/mL, the inhibition percentage reaches $20,75^{f}$±0,43%. When the concentration is gradually increased to 10, 20, 40 pg/mL, the inhibition percentage also increases to $23,67^{e}$±0,33%, $30,92^{d}$±0,34%, $45,42^{c}$±0,25%, respectively. The results in Table 4.4 show that the ability to inhibit the α-amylase enzyme of the *Garcinia mangostana* Linn's peel ethanol extract is lowest at the concentration of 5 pg/mL ($20,75^{f}$±0,43%), highest at the concentration of 160 pg/mL ($93,75^{a}$±0,42%), and there is a statistically significant difference at the 5% level. However, when continuing to increase the concentration of the extract from 160 pg/mL to 200, 240, 320 pg/mL, the ability to inhibit the α-amylase enzyme of the extract does not change. Specifically, the percentage of inhibition among the concentrations of 160 pg/mL, 200 pg/mL, 240 pg/mL, 320 pg/mL is not significantly different with the meaning of statitics at 95%. This can be explained that the binding affinity of the inhibitor to its binding object, the enzyme, the substrate or the enzyme-substrate complex, is not absolutely tight and ideally reaches 100%. Therefore, at a certain time, the efficiency of the inhibition reaction will reach its maximum even if the inhibitor concentration continues to increase.

The ability to inhibit 50% of the α-amylase enzyme of the *Garcinia mangostana* Linn's peel ethanol extract is calculated based on the standard line equation $y = 0,7336^{x} + 14,631$ with a correlation coefficient R2 = 0.9805. At a concentration of 48 pg/mL, the extract has the ability to inhibit 50% of the α-amylase enzyme (Figure 4)

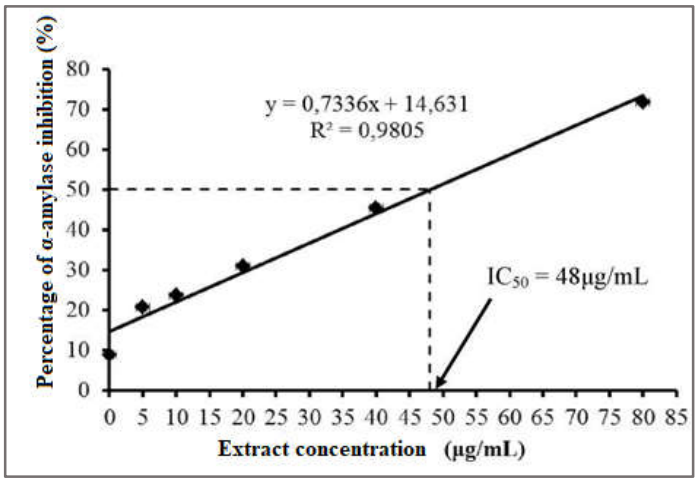

Figure 4. Standard lineshows the dependence of the percentage of α-amylase inhibition on extract concentration

To evaluate the strong or weak inhibitory ability of the experimental sample, people rely on the IC_{50} value for comparison. The lower the IC50 value of the sample is, the higher the inhibitory effect is. The 50% inhibitory activity of α-amylase enzyme of *Garcinia mangostana* Linn's peel ethanol extract has an IC_{50} value of 48 pg/mL, lower than the research results of Dejian Huang *et al.,* (2007) with an IC_{50} value of 5.4 pg/mL but higher than the research results of ThamilvaaniManaharan *et al.,* (2012) with an IC_{50} value of 41.7±3.2 mg/mL and of Adnyana *et al.,* (2016) with an IC_{50} value of 105.36±2.73pg/mL. The reason for this difference is due to the difference in extraction solvents as well as different extraction methods. The IC_{50} value of the positive control acarbose is IC_{50} = 90.7 pg/mL, 1.9 times higher than that of *Garcinia mangostana* Linn's peel ethanol extract, which proves that *Garcinia mangostana* Linn's peel ethanol extract has a higher ability to inhibit α-amylase than acarbose because acarbose selectively inhibits small intestinal enzymes such as glucoamylase, sucrose... The inhibitory effect of acarbose on the α-amylase enzyme also depends on the enzyme source and the origin of the drug, so the inhibitory effect of acarbose on the α-amylase enzyme is lower than that of the extract.

A study of the antioxidant capacity of *Garcinia mangostana* Linn's peel ethanol extract by DPPH method

Free radicals in the body are the cause of many degenerative diseases such as cancer, ulcerative colitis, Alzheimer's, aging, stroke... and these are also common complications in people with type 2 diabetes. Therefore, the elimination of free radicals is an effective measure to prevent degenerative diseases. *Garcinia mangostana* Linn's peel ethanol extract is a very potential source of raw materials in the production of antioxidant drugs. The ability to eliminate the free radical of *Garcinia mangostana* Linn's peel has been studied by different extraction methods and solvents, showing very promising antioxidant results.

The results of the evaluation of the ability to eliminate the free radical of *Garcinia mangostana* Linn's peel ethanol extract are presented in Table 4.12

Table 3. The ability to eliminate the DPPH free radical of *Garcinia mangostana* Linn's peel ethanol extract

Independent variable	Extract concentration (pg/mL)	The percentage of free radical elimination (%)
Reference	0	0^{k}
1	50	$18,36^{J}$±0,08
2	100	$32,42^{i}$±0,06
3	150	$46,89^{h}$±0,11
4	200	$56,45^{g}$±0,05
5	250	$70,44^{f}$±0,06
6	300	$71,85^{e}$±0,12
7	350	$75,23^{d}$±0,03
8	400	$77,63^{c}$±0,06
9	450	$79,09^{b}$±0,16
10	500	$82,79^{a}$±0,05
11	550	$82,55^{a}$±0,13
12	600	$82,55^{a}$±0,12
13	650	$82,53^{a}$±0,09
14	700	$82,60^{a}$±0,09
15	800	$82,59^{a}$±0,09

The difference among following letters in the same column has no meaning in statistics at 5% level according to Tukey's test.

The results from the experiment show that the efficiency of the free radical elimination of *Garcinia mangostana* Linn's peelethanol extract is proportional to the extract concentration. The higher the extract concentration is, the stronger the ability to eliminate the free radical is. The ability to eliminate the free radical of *Garcinia mangostana* Linn's peel ethanol extract is lowest at $18,36^{i}$±0,08% at a concentration of 50 pg/mL and highest at a concentration of 500 pg/mL with a free radical elimination efficiency of $82,79^{a}$±0,05%, this difference is statistically significant at the 5% level. However, when the concentration continues to increase to 550, 600, 650, 700, 800 pg/mL, the percentage of free radical elimination does not change. This is clearly shown through the result of statistical analysis at the 95% significance level, the values of corresponding inhibition percentage of concentrations from 500 to 800 pg/mL are not significantly different.

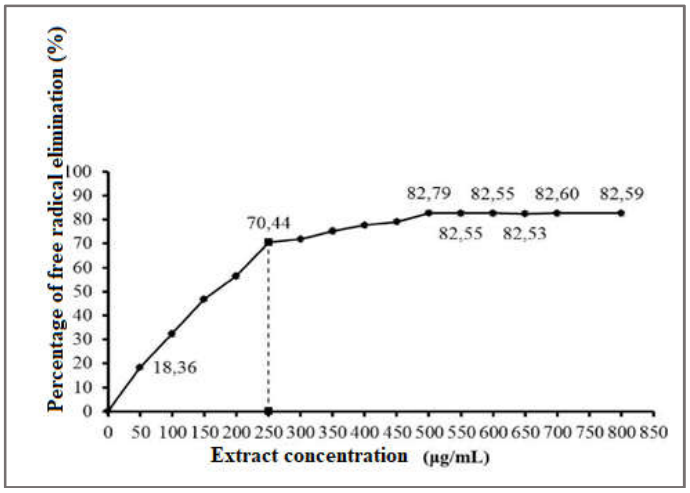

Figure 5. The ability to eliminate DPPH free radical of *Garcinia mangostana* Linn's peel ethanol extract

According to Figure 4.11, the percentage of DPPH free radical inhibition will increase from a concentration of 0 to 500 pg/mL. Conducting a standard line for the percentage of inhibition according to the concentration of the extract in the concentration range from 0 to 250 pg/mL will determine the IC_{50} value based on the standard line equation in the form y = ax + b (Figure 4.12)

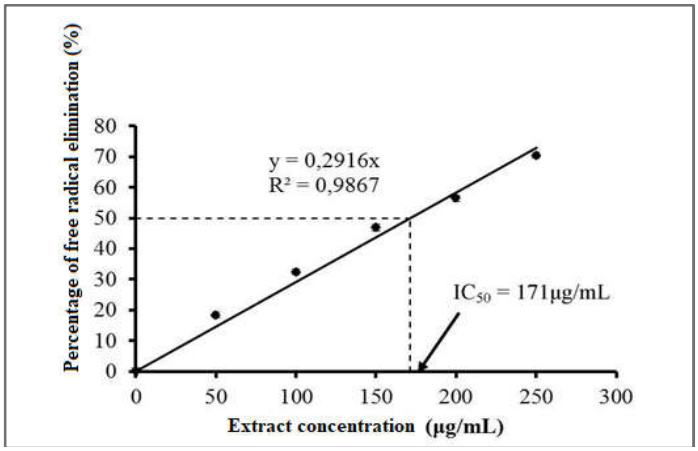

Figure 6: Standard lineshows the dependence of the percentage of DPPH free radical elimination on the concentration of*Garcinia mangostana* Linn's peel ethanol extract

The results show that the antioxidant activity by DPPH method of *Garcinia mangostana* Linn's peel ethanol extract at a concentration of 171 pg/mL is able to inhibit 50% of free radicals (IC50 = 171 pg/mL). The concentration of 50% free radical inhibition of *Garcinia mangostana* Linn's peel ethanol extract (IC50 = 171 pg/mL) is 4.4

times higher than that of the vitamin C control (IC50 = 39 pg/mL). This shows that *Garcinia mangostana* Linn's peelethanol extract has lower antioxidant activity than vitamin C. The reason for the difference is that *Garcinia mangostana* Linn's peel extract is extracted by soaking, filtering and evaporating. During the filtering process, all impurities may not have been removed, so the extract product is not purified. Vitamin C is a high antioxidant and a commercial product with higher purity, so it will have a higher free radical inhibition effect than *Garcinia mangostana* Linn's peel extract.

The ability to eliminate free radical of *Garcinia mangostana* Linn's peel ethanol extract is lower than the research results of Palakawong et al. (2010). According to the research of Palakawong *et al.,* (2010), 50% ethanol extract from the peel, leaves and stems of *Garcinia mangostana* Linnfruit gives free radical elimination effect by DPPH with IC50 values of 5.94 ± 0.14; 9.94 ± 0.39 and 6.46 ± 0.36 lig/ml, respectively. This result is also lower than the research results of ArasaliSulaimanZarena *et al.,* (2009), Weecharangsan *et al.,(*2006) and higher than the research results of ThamilvaaniManaharan *et al.,* (2012). In the research results of Weecharangsan *et al.,*(2006), the extract (water, 50% ethanol, 95% ethanol and ethyl acetate) from the pulp of *Garcinia mangostana*Linnfruit gives DPPH free radical elimination efficiency with IC50 values of 34.98 ± 0.24; 30.76 ± 1.66; 58.66 ± 0.98 and 77.84 ± 0.24 pg/ml, respectively. Meanwhile, the research results of ArasaliSulaimanZarena *et al.,* (2009), the extract (ethanol, methanol, acetone) gives DPPH free radical elimination efficiency with IC50 values of 69.43 pg/mL; 52.62 pg/mL; 33.32 pg/mL. The IC50 value in the study results of ThamilvaaniManaharan *et al.,(*2012) is 11.9±4.1 mg/mL. The reason for this difference is that the same extraction method can show different results because of differences in factors such as growth stage and experimental sampling time, and because of the use of different solvents during the extraction process. That is the reason why it gives different results of free radical elimination. In summary, the *Garcinia mangostana* Linn's peel ethanol extract has a 50% free radical elimination efficiency at a concentration of 171 pg/mL (IC50 = 171 pg/mL).

CONCLUSION

The experimental results show that the *Garcinia mangostana* Linn's peel has the moisture of 61.56% and the ethanol extraction efficiency of *Garcinia mangostana* Linn's peel was 15.59%. The a-mangostin content accounts for 2.1% in 615 g of dry peel.The results of qualitative analysis show that the ethanol extract of *Garcinia mangostana* Linn's peel contain polyphenol compounds such as tannin, flavonoid, glycoside, alkaloid, which have anti-diabetic effects, so the extract has the ability to inhibit the α-amylase and α-glucosidase enzymes, which are two enzymes that hydrolyze starch into sugar. The ability to inhibit the α-amylase and α-glucosidase enzymes of the ethanol extract of *Garcinia mangostana* Linn's peel with IC50 values is IC50 = 48 pg/mL and IC50 = 56 pg/mL, respectively.

In addition, these compounds also have the ability to eliminate free radicals - the cause of complications of type 2 diabetes. Therefore, the ability to reduce free radicals of *Garcinia mangostana* Linn's peel ethanol extract is IC50 = 171 pg/mL.

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