INVESTIGATION OF THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES IN VITRO OF THE ETHANOL EXTRACT FROM *COCCINIA GRANDIS*

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ABSTRACT

Coccinia grandis is a wild plant that is widely distributed in Vietnam, scattered throughout the provinces, especially the plains and high mountains. Not only is it a cooling and detoxifying food, it is also a traditional medicine with many uses such as anti-oxidation, anti-blood clotting, increasing insulin and preventing diabetes, anti-inflammatory, capable of protecting the liver and heart. This is a medicinal herb with a lot of potential, so the project "Investigating the antioxidant activity and in vitro sugar potential of ethanol extract from *Coccinia grandis* (L.) Voigt, Cucurbitaceae" was carried out. The results showed that the ethanol extract had a polyphenol content is 13.235 ± 0.32mg GAE/g extract and flavonoid is 11.125 ± 1.39mg QE/g extract. Antioxidant activity survey results show that ethanol extract has the strongest activity on $ABTS^{\bullet+}$ (EC_{50} = 361.26µg/mL), $NO^{\bullet}$ (EC_{50} = 394.82µg/mL), DPPH (EC_{50} = 482.42µg/mL), TAC (EC_{50} = 657.52µg/mL), RP (EC_{50} = 959.47µg/mL), and finally FRAP (EC_{50} = 1231.33µg/mL). *Coccinia grandis* grown in Vietnam showed that the EC_{50} value of *α*-amylase and *α*-glucosidase enzyme inhibitory *activity was 517.927µg/mL and 479.570µg/mL.*

Keyword: *Antioxidant, Coccinia grandis, flavonoid, hypoglycemia, polyphenol.*

1. INTRODUCTION

Currently, the rapidly increasing rate of diabetes indicates that our body's resistance is decreasing. Diabetes is now common among all social classes and age groups, leading to many health detriments, disabilities, and even death if not diagnosed and treated promptly. At present, Vietnam has more than 7 million people with diabetes, with reports showing that over 55% of patients experience complications, including 34% with cardiovascular complications, 24% with kidney complications, 39.5% with eye complications, and neurological complications. Diabetic patients with complications not only increase medical costs but also severely affect their quality of life.

The development of automation technology has led to a sedentary lifestyle. Moreover, people consume high-energy foods daily, resulting in an increasing obesity rate. Alarmingly, the age at which people are diagnosed with diabetes is becoming younger. To maintain stable blood glucose levels in the circulatory system, we need to be more active. After eating, the body releases insulin, a hormone that promotes cells to increase glucose absorption. Modern diets often include many types of high-calorie processed foods, which can weaken the body's self-regulation ability. Consequently, the body may become insulin resistant or produce insufficient insulin. *Coccinia*

grandis is a wild plant commonly found in Vietnam, scattered across various provinces, especially from the lowlands to the high mountains. Besides being a refreshing and detoxifying food, *Coccinia grandis* is also a traditional medicinal herb with various benefits, such as antioxidant, anticoagulant, and insulin-enhancing properties to prevent diabetes (Packirisamy et al., 2016), anti-inflammatory effects (Asmita et al., 2017), and liver and heart protection (Arnab et al., 2021). Additionally, in folk medicine, *Coccinia grandis* is researched for its potential to support healthy blood glucose levels naturally in adults. However, to date, specific studies on the biological effects of *Coccinia grandis* remain limited.

To contribute to building a scientific basis for the application of *Coccinia grandis* in supporting the treatment of diseases, the study titled 'Investigation of the Antioxidant and Antidiabetic Activities in Vitro of the ethanol extract from *Coccinia grandis*' is highly relevant and worthy of research.

2. MATERIALS AND METHODS

2.1. MATERIALS

The entire plant of Coccinia grandis was collected at coordinates 10.05792°N, 105.76861°E, alley 194, Cach Mang Thang 8 Street, Bui Huu Nghia Ward, Binh Thuy District, Can Tho City, Vietnam. The material was identified by observing the plant morphology, conducting microscopic surveys, and comparing it with botanical classification documents. The material was dried in the shade until dry and ground into powder for research purposes.

Solvents - chemicals

Trolox (Sigma-Aldrich), $K_2S_2O_8$ (Merck), Na_2CO_3 (Merck), gallic acid (Merck), Folin-Ciocalteu's phenol reagent (Merck), $K_3Fe(CN)_6$ (Merck), Cl_3CCOOH (Merck), $FeCl_3$ (Sigma-Aldrich), $NaNO_2$ (Xilong), 2, 2-Diphenyl-1-Picrylhydrazyl (Sigma-Aldrich), 2,2- azino-bis (3-ethylbanzthiazoline-6-sulphonic acid (Germany), 2, 4, 6-tripyridyl-s-triazine (Sigma-Aldrich), $NH_4Mo_7O_{24}.4H_2O$ (Xilong), Dimethyl sulfoxide (Merck), enzyme α-amylase (Sigma-Aldrich), enzym α-glucosidase (Sigma-Aldrich), acarbose (Sigma-Aldrich), thibarbituric acid (Merck) ….

Equipment

Moisture meter, electronic scale, electric stove, refrigerated centrifuge (Mikro 12-24, Hettich, Germany), analytical balance (AB104-S, Mettler Toledo, Switzerland), drying oven (BE 200, Memmert, Germany), incubator (Memmert, Germany), vortex mixer (ZX3, Velp, Italy), micropipettes 100 µL, 500 µL, 1000 µL (ThermoLabsystems), spectrophotometer (Thermo Scientific Multiskan GO, Finland).

2.2. METHODS

Preparation of extract

Coccinia grandis has been used in research as the entire plant above ground. After harvesting, it was thoroughly washed, dried at 50°C, and ground into powder. The moisture content of the herbal powder was determined by using heat to evaporate all the water vapor in the material. The *Coccinia grandis* powder was soaked in 96% ethanol at a ratio of 1 g of herbal material to 10 ml of 96% ethanol, at room temperature for 24 hours. The *Coccinia grandis* powder was soaked three times, and the extracts from each soaking were combined. The solvent was removed using a

vacuum rotary evaporator under reduced pressure at 50°C to obtain a concentrated extract. The *Coccinia grandis* extract was stored in glass bottles, labeled, and kept in the refrigerator compartment for preservation.

Method for quantifying total polyphenols

The polyphenol content was determined using the Folin-Ciocalteu assay as described by Rebaya et al., 2014 with modifications. To 250 µL of the extract, 250 µL of water and 250 µL of the Folin-Ciocalteu reagent were added, and the mixture was shaken well. Then, 250 µL of 10% Na_2CO_3 solution was added, followed by incubation for 30 minutes at 40°C in a water bath. The absorbance was measured at a wavelength of 765 nm using a spectrophotometer. The polyphenol content in the extracts was determined based on the standard curve of gallic acid.

Method for quantifying total flavonoids

The determination was conducted using the colorimetric method with $AlCl_3$ according to Bag et al., (2015) with adjustments. To 1 mL of the extract was mixed with 1 mL of water and shaken well. Then, 200 µL of 5% $NaNO_2$ was added and allowed to stand for 5 minutes. Subsequently, 200 µL of 10% $AlCl_3$ was added, mixed well, and left to stand for 6 minutes. Afterward, 2 mL of 1 M NaOH was added. Finally, water was added to make up the volume to 5 mL, and the absorbance was measured at a wavelength of 510 nm using a spectrophotometer. The total flavonoid content in the extracts was determined based on the standard curve of quercetin.

Investigation of in vitro antioxidant activity

The free radical scavenging activity was determined using the $ABTS^{\bullet+}$ assay (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid))

The determination was conducted using the $ABTS^{\bullet+}$ colorimetric reduction method as described by Nenadis et al., (2004). $ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate. The mixture was incubated in the dark at room temperature for 12-16 hours before use. Subsequently, the mixture was diluted, and the absorbance was measured at a wavelength of 734 nm. The assay was performed by adding 10 µL of the extract to 990 µL of $ABTS^{\bullet+}$ at room temperature for 6 minutes. The absorbance was then measured at a wavelength of 734 nm.

The free radical scavenging activity was determined using the DPPH assay (2, 2-Diphenyl-1-Picrylhydrazyl)

The antioxidant activity of the extracts was determined using the DPPH free radical scavenging method described by Sharma & Bhat (2009) with modifications. 40 µL of DPPH solution (1000 µg/mL) was mixed with 960 µL of the extract. The mixture was incubated in the dark at 30°C for 30 minutes. The absorbance was then measured at a wavelength of 517 nm.

The potential iron-reducing activity was determined using the FRAP assay (Ferric Reducing Antioxidant Power)

The potential antioxidant activity of the extracts was determined using the Ferric Reducing Antioxidant Power (FRAP) assay, as described by Benzie IF and Strain JJ in 1996. This method is based on the reduction of the ferric-tripyridyltriazine complex. Different concentrations of the

extract (10 µL) were reacted with the FRAP solution (990 µL) for 30 minutes under dark conditions. The absorbance was then measured at a wavelength of 593 nm.

Iron-reducing activity RP (Reducing power)

The iron reduction capability was performed according to the modified method of Ferreira (2007). Add 500µL of the extract, 500µL of phosphate buffer (0.2M, pH=6.6), and 500µL of 1% $K_3Fe(CN)_6$. Incubate at 50°C for 20 minutes, then add 500µL of 10% CCl_3COOH and centrifuge at 3000 rpm for 10 minutes. After centrifugation, withdraw 500µL and add it to 500µL of water and 100µL of 0.1% $FeCl_3$, mix well. Measure the absorbance at 700nm.

The activity of inhibiting the formation of $NO^{\bullet}$ (nitric oxide) free radicals

The investigation was carried out according to the modified method of Alisi & Onyeze (2008). Add 200µL of the extract and 400µL of 5mM sodium nitroprusside. Incubate for 60 minutes at 25°C, then centrifuge at 11000 rpm for 15 minutes. Add 600µL of Griess reagent to the supernatant. Incubate for another 5 minutes and measure the absorbance at 546nm.

The total antioxidant capacity (TAC)

The evaluation was carried out using the phosphomolybdenum method as described by Prieto et al., 1999 with modifications. Extracts at various concentrations with a volume of 300µL were combined with 900µL of the reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). Incubate at 95°C for 90 minutes. Measure the absorbance at 695nm.

The investigation of *in vitro* antidiabetic activity

Survey of the α-amylase inhibitory activity of the extracts.

Follow the modified method of Xiao-Ping et al. (2010). Mix 50µL of phosphate buffer solution (pH=7) with 50µL of the extract and 50µL of α-amylase enzyme (3U/mL), and incubate at 37°C for 5 minutes. Add 50µL of starch solution (2mg/mL) and continue incubating at 37°C for 15 minutes. Add 200µL of concentrated HCl solution to stop the reaction. Finally, add 300µL of iodine reagent solution to detect the remaining starch based on the characteristic blue color reaction. Measure the absorbance at a wavelength of 660nm.

Survey of the α-glucosidase inhibitory activity of the extracts.

Follow the modified method of Shai et al. (2011). Mix 100µL of phosphate buffer solution (100mM, pH=6.8), 20µL of α-glucosidase enzyme (1U/mL), and 40µL of the extract. Incubate at 37°C for 15 minutes. Add 40µL of p-nitrophenyl-α-D-glucopyranoside (5mM) and incubate at 37°C for 20 minutes. Stop the reaction by adding 100µL of Na_2CO_3 (0.1M). Measure the absorbance of the released p-nitrophenol at a wavelength of 405nm.

2.3. DATA PROCESSING AND ANALYSIS

In vitro antioxidant activity survey

Antioxidant activity assays were conducted using Trolox as a positive control. The antioxidant capacity of the extracts was compared to Trolox by determining the concentration (µg/mL) at which the standard or extract reduces or neutralizes 50% of the free radicals (EC_{50} - effective

concentration of 50%). The EC_{50} values for the extracts and Trolox were determined as described by Piaru et al., (2012).

Investigation of *in vitro* antidiabetic activity

The inhibitory activity of α-amylase enzyme by various plant extracts was investigated.

Acarbose is used as a positive control. The results are expressed as percentage inhibition, calculated using the formula:

$$\text{Percentage inhibition (\%)} = 100 - ((A_o - A_1) / Ao \times 100).$$

Where: A_o is the absorbance of the control solution. A_1 is the absorbance of the solution after the reaction.

The *α*-amylase inhibitory activity of Coccinia grandis extracts is determined based on the concentration at which the extract or standard inhibits 50% of enzyme activity (IC_{50} - inhibitory concentration of 50%).

The inhibitory activity of α-glucosidase enzyme by various extracts was investigated.

Acarbose was used as the standard. The results are expressed as percentage inhibition, calculated using the formula:

$$\text{Inhibition activity (\%)} = (1 - A_o/A_1) \times 100.$$

Where: A_o is the absorbance of the control solution. A_1 is the absorbance of the solution after the reaction.

The *α*-glucosidase inhibitory activity of Coccinia grandis extracts is determined based on the concentration at which the extract or standard inhibits 50% of enzyme activity (IC_{50} - inhibitory concentration of 50%).

3. RESULTS AND DISCUSSION

The total polyphenol and flavonoid content

Measure the absorbance of standard gallic acid at concentrations of 10, 20, 30, 40, 50, 60 µg/mL; standard quercetin at concentrations of 10, 20, 40, 60, 80, 100 µg/mL. From the absorbance and initial standard concentration, linear equations can be plotted (Figure 1, Figure 2).

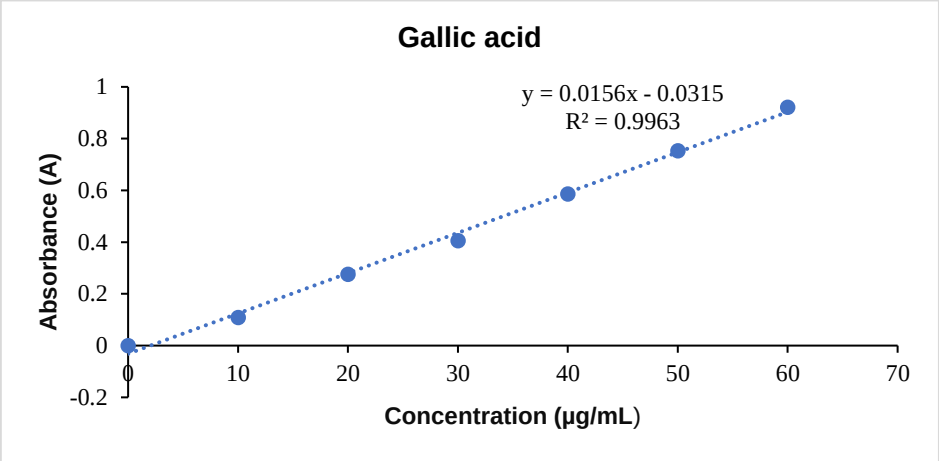

Figure 1. The correlation graph between the absorbance and the concentration of gallic acid

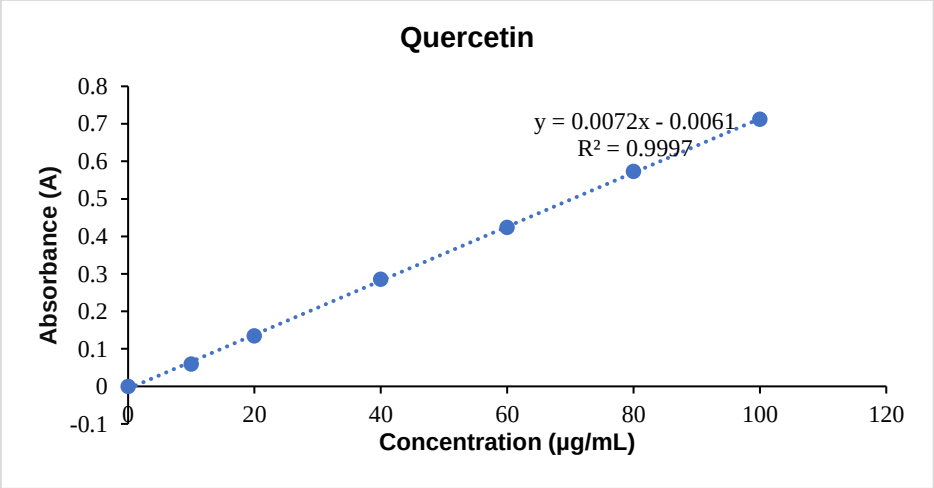

Figure 2. The correlation graph between the absorbance and the concentration of quercetin.

The total polyphenol and flavonoid content in the extracts were determined to be equivalent to the content of gallic acid and quercetin using the standard equations for gallic acid (y = 0.0156x – 0.0315; R^2 = 0.9936) and quercetin (y = 0.0072x – 0.0061; R^2 = 0.9997). Substituting the absorbance values into the y-value of the equations, the results obtained were a polyphenol content of 13.235 ± 0.32 mg GAE/g extract and a flavonoid content of 11.125 ± 1.39 mg QE/g extract for *Coccinia grandis* extract (Table 1).

Table 1. The content of polyphenols and flavonoids in *Coccinia grandis* extract.

Sample	Polyphenol content (mg GAE/g extract)	Flavonoid content (mg QE/g extract)
Coccinia grandis extract	13.235 ± 0.32	11.125 ± 1.39

The results of antioxidant and anti-diabetic activity *in vitro*

The results of antioxidant activity in vitro

The *Coccinia grandis* extract contains numerous secondary metabolite groups, indicating antioxidant potential. The results were evaluated through the determination of the ability to neutralize or scavenge free radicals, demonstrated through 6 assay methods: $ABTS^{\bullet+}$, DPPH, $NO^{\bullet}$, FRAP, RP and TAC.

The scavenging efficiency of $ABTS^{\bullet+}$, DPPH, $NO^{\bullet}$ free radicals expressed as the equivalent antioxidant concentration in µg/mL of trolox based on the respective standard curves were y= 0.1362x + 0.7876 (R^2 = 0.9939); y= 0.1132x – 4.5592 (R^2 = 0.9858); y= 0.4165x – 1.2572 (R^2 = 0.9998). As the concentration of the extract increased from 100 µg/mL to 600 µg/mL, the corresponding antioxidant content for $ABTS^{\bullet+}$, DPPH, $NO^{\bullet}$ increased from 15.351 ± 0.553% to 83.183 ± 0.08%; 6.109 ± 0.649% to 65.988 ± 2.336%; and 8.376 ± 1.356% to 84.97 ± 1.420% (Figure 3). These results indicate a proportional relationship between antioxidant activity and extract concentration. Additionally, the EC_{50} values of $ABTS^{\bullet+}$, DPPH, $NO^{\bullet}$ for Coccinia grandis extract were 361.26 ± 4.05 µg/mL; 482.42 ± 19.77 µg/mL; and 394.82 ± 2.89 µg/mL, respectively, indicating lower free radical scavenging activity compared to the positive control compound Trolox (Table 2).

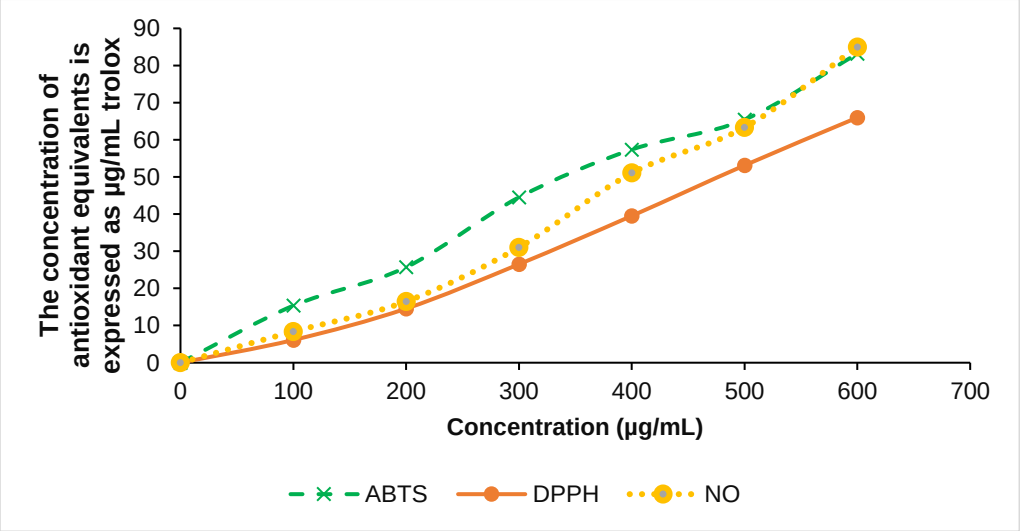

Figure 3. The antioxidant activity *in vitro* is assessed using the method $ABTS^{\bullet+}$, DPPH, $NO^{\bullet}$

The antioxidant efficiency in terms of FRAP, RP, and total antioxidant capacity (TAC) with the concentration of antioxidant equivalents expressed as µg/mL trolox was determined based on the following standard curves: y = 0.0013x + 0.0225 (R^2 = 0.9906); y = 0.0005x – 0.0203 (R^2 = 0.9936); y = 0.0007x + 0.0397 (R^2 = 0.9766), respectively. The concentration of the high extract increased from 100µg/mL to 1000µg/mL, corresponding to FRAP, RP, and TAC antioxidant equivalents from 0.108 ± 0.012% to 1.29 ± 0.038%; 0.076 ± 0.005% to 0.473 ± 0.011%; and 0.086 ± 0.002% to 0.701 ± 0.011%, respectively (Figure 4). The results indicated that antioxidant activity is directly proportional to the concentration of the high extract. Additionally, the EC_{50} values of FRAP, RP, and TAC in the *Coccinia grandis* extract were determined to be 1231.33 ± 28.51µg/mL, 959.47 ± 14.96µg/mL, and 657.52 ± 3.08µg/mL, respectively, indicating lower FRAP radical neutralization activity compared to the positive control Trolox (Table 2).

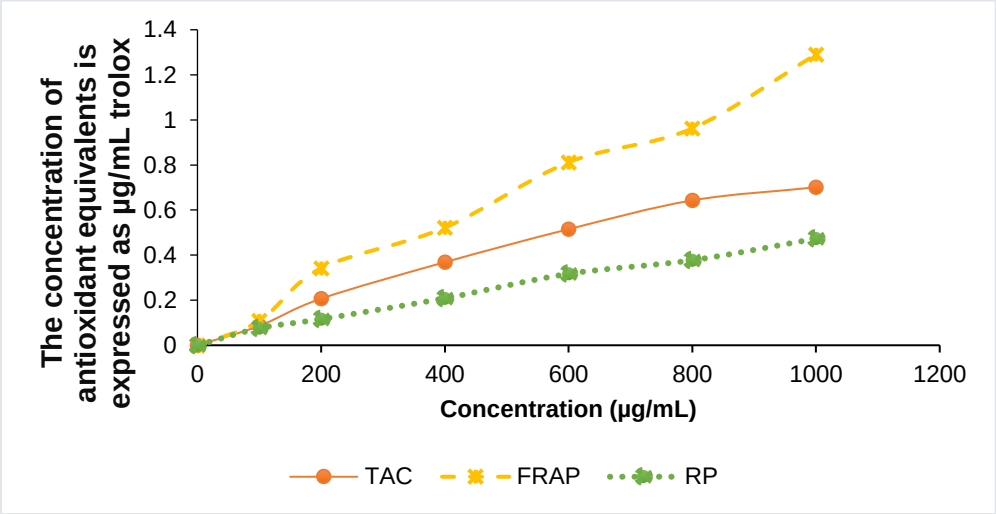

Figure 4. The antioxidant activity *in vitro* is assessed using the method FRAP, RP, TAC

Table 2. EC_{50} values of antioxidant methods

Sample	Values EC_{50} (µg/mL)					
	$ABTS^{\cdot+}$	**DPPH**	**$NO^{\cdot}$**	**FRAP**	**RP**	**TAC**
Coccinia grandis extract	361.26^{a}±4.05	482.42^{a}±19.77	394.82^{a}±2.89	1231.33^{a}±28.51	959.47^{a}±14.96	657.52^{a}±3.08
Trolox	6.81^{b} ± 0.11	6.4^{b} ± 0.35	63.88^{b} ± 4.83	4.04^{b} ± 0.04	5.14^{b} ± 0.07	13.79^{b} ± 0.17

The antioxidant capacity of *Coccinia grandis* extract was compared to trolox by using the concentration (µg/mL) at which the extract or trolox scavenged or neutralized 50% of free radicals (EC_{50}) (Table 2). Extract from Coccinia grandis exhibited antioxidant activity with EC_{50} values ranging from 361.26 ± 4.05 to 1231.33 ± 28.51 µg/mL. Among these, the extract showed the strongest antioxidant activity with the lowest EC_{50} value in the $ABTS^{\cdot+}$ method, while it exhibited the weakest antioxidant activity in the FRAP method.

The results of anti-diabetic activity in vitro

Plant-derived compounds that inhibit the enzymes *α*-amylase and α-glucosidase, involved in carbohydrate metabolism, hinder the rapid hydrolysis of carbohydrates into monosaccharides. This slowdown in glucose absorption helps regulate blood glucose levels, making glucose control crucial.

This study investigated the inhibitory efficiency of *α*-amylase at concentrations of 15.625 µg/mL, 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, and 500 µg/mL, corresponding to 6.130%, 8.453%, 11.648%, 16.043%, 30.099%, and 46.279%, respectively. Additionally, the inhibitory efficiency of *α*-glucosidase ranged from 8.018 ± 1.408% to 66.827 ± 1.435%, corresponding to concentrations from 100 µg/mL to 600 µg/mL, indicating that the inhibitory efficiency of *α*-amylase

and *α*-glucosidase increased gradually with concentration (Figure 5). Furthermore, the EC_{50} values for *α*-amylase and *α*-glucosidase were 517.927 ± 11.608 µg/mL and 479.570 ± 5.663 µg/mL, respectively, compared to the standard drug Acarbose, which had corresponding values of 5.504 ± 0.344 µg/mL and 5.042 ± 1.03 µg/mL (Figure 5).

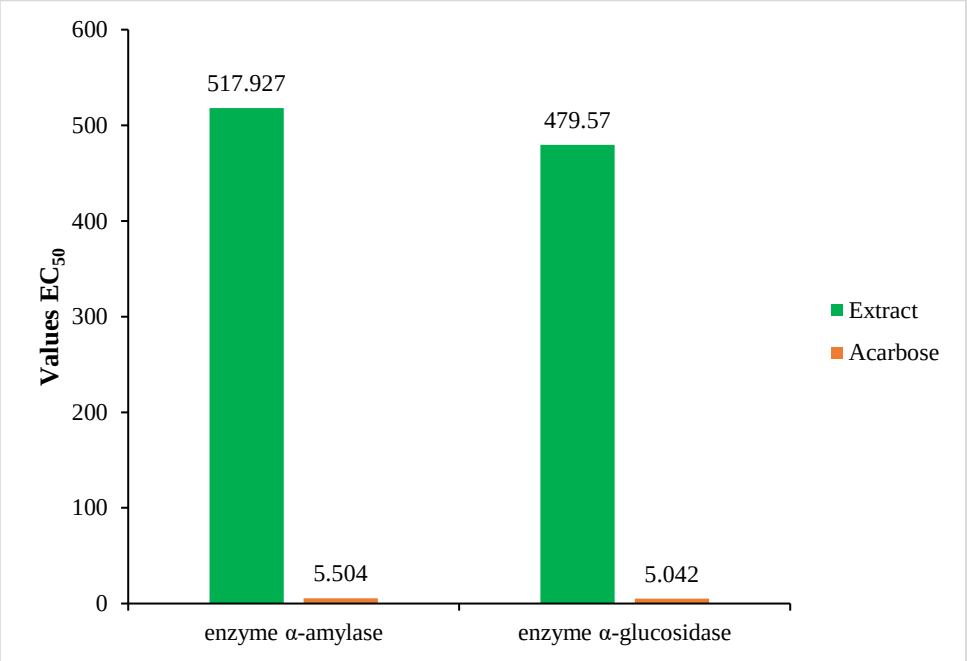

Figure 5. Concentration of 50% inhibition of *α*-amylase and *α*-glucosidase enzyme activity

The survey indicates that the ability to inhibit *α*-glucosidase enzyme by *Coccinia grandis* extract is stronger than *α*-amylase, Ratananikom et al., (2024) studied the inhibition ability of *α*-amylase enzyme in the ethanol leaf extract of dried *Coccinia grandis* leaves. According to the survey results, the inhibition activity of *α*-amylase enzyme of the leaf extract showed an efficiency of 35.12%, which is higher than the efficiency of 96% ethanol extract (30.099%) at a concentration of 250 µg/mL but lower than the efficiency of 96% ethanol extract (46.279%) at a concentration of 500 µg/mL.

4. CONCLUSION

The results from the *in vitro* antioxidant and anti-diabetic activity assessment of ethanol extract from *Coccinia grandis* revealed that the ethanol extract contains 13.235 ± 0.32 mg GAE/g of polyphenols and 11.125 ± 1.39 mg QE/g of flavonoids. The antioxidant activity survey showed that *Coccinia grandis* ethanol extract exhibited the strongest activity on $ABTS^{\bullet+}$ (EC_{50} = 361.26 µg/mL), $NO^{\bullet}$ (EC_{50} = 394.82 µg/mL), DPPH (EC_{50} = 482.42 µg/mL), TAC (EC_{50} = 657.52 µg/mL), RP (EC_{50} = 959.47 µg/mL), and finally FRAP (EC_{50} = 1231.33 µg/mL). *Coccinia grandis* extract demonstrated EC_{50} values for the inhibition of *α*-amylase and *α*-glucosidase activities at 517.927 µg/mL and 479.570 µg/mL, respectively. These findings lay the groundwork for further studies on the biological activities as well as chemical composition of *Coccinia grandis* in Vietnam.

ACKNOWLEDGMENT

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REFERENCES

1. Alisi C.S., Onyeze G.O.C. (2008). Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata* (Linn.). *African Journal of Biochemistry Research*, 2(7): 145-150.
2. Arnab B., Sandip M., Bithin K. (2021*).* Efficacy of *Coccinia grandis* against monosodium glutamate induced hepato-cardiac anomalies by inhibiting NF-kB and caspase 3 mediated signalling in rat model. *Hum Exp Toxicol,* 40(11): 1825 - 1851.
3. Asmita P., Dibyendu P.K. N., Tapati C. (2017*). Coccinia grandis (L.)* Voigt leaf extract exhibits antileishmanial effect through pro-inflammatory response: An In Vitro study. *Curr Microbiol*, 74: 59 - 67.
4. Bag G. C., Devi P. G., & Bhaigyabati T. H. (2015). Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three Hedychium species of Manipur valley. International Journal of Pharmaceutical Sciences Review and Research, 30(1), 154-159.
5. Benzie I.F, Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1): 70-76.
6. Ferreira I.C.F.R., Baptista P., Vilas-Boas M. and Barros L. (2007). Free-Radical scavenging capacity and reducing power of wild edible mushrooms from northeast portugal: individual cap and stipe activity. *Food Chemistry*, 100 (4): 1511-1516.
7. Nenadis N., Wang L.F., Tsimidou M., Zhang H.Y. (2004). Estimation of scavenging activity of phenolic compounds using the $ABTS^{\bullet+}$ assay. *Journal of Agricultural and Food Chemistry*, 52: 4669-4674.
8. Packirisamy M., Ayyakkanuu P., Sivaprakasam M. (2016). Antioxidant, antiglycation and insulinotropic properties of *Coccinia grandis* (L.) in vitro: Possible role in prevention of diabetic complications. *Journal of Traditional and Complementary Medicine*, 7(1): 54 - 64.
9. Piaru S.P., Mahmud R., Abdul Majid A.M.S., Mahmoud Nassar Z.D. (2012). Antioxidant and antiangiogenic activities of the essential oils of Myristica fragrans and Morinda citrifolia. *Asian Pacific Journal of Tropical Medicine*, 5(4): 294-298.
10. Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, *269*(2): 337-341.
11. Ratananikom K., Nitisuk P., Wongpreedee P., & Kubola J. (2024). Inhibitory Activities of Thai Culinary Vegetables against Key Enzymes Relevant to Diabetes Mellitus and the Kinetics of Enzyme Inhibitions. *Journal of Food Quality*.
12. Rebaya, A., Belghith S.I., Baghdikian B., Leddet V.M., Mabrouki F., Olivier E., Cherif J. K., Ayadi M.T. (2014). Total phenolic, total flavonoid, tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae). *Journal of Applied Pharmaceutical Science*, 5(1): 52-57.
13. Shai L.J., Magano S.R., Lebelo S.L., Mogale A.M. (2011). Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. *Journal of Medicinal Plants Research*, 5(13): 2863-2867.
14. Sharma O.P., Bhat T.K. (2009). DPPH antioxidant assay revisited. *Food chemistry*, 113(4): 1202-1205.

15. Xiao-Ping Y., Chun-Qing S., Ping Y., RenGang M. (2010). *α*-Glucosidase and *α*-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. *Chinese Journal of Natural Medicines*, 8(5): 349-352.