Pharmacogn J. 2022; 14(5): 511-520
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Antioxidant Activity of DPPH, CUPRAC, and FRAP Methods, as well as Activity of Alpha-Glucosidase Inhibiting Enzymes from *Tinospora crispa* (L.) Stem Ultrasonic Extract

Candra Irawan[1], Imalia Dwi Putri[1], Maman Sukiman[2], Andita Utami[3], Ismail[3,*], Ratna Komala Putri[3], Anisa Lisandi[3], Andrean Nur Pratama[4]

ABSTRACT

Aims: The goal of this work was to get the stem extract of *Tinospora crispa (L.)* using ultrasound-assisted extraction (UAE) with duration and amplitude changes to achieve optimal extraction conditions. The antioxidant potential of the extract as measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), CUPRAC (Cupric Ion Reducing Antioxidant Capacity), FRAP (Ferric Reducing Ability of Plasma), and its antidiabetic potential. **Results:** The third test of antioxidant methods showed that the measurement of antioxidant activity with, DPPH, CUPRAC, and FRAP gave significantly different results, but CUPRAC and FRAP gave the same response to the ethanol extract of *Tinospora crispa* stems, it can be concluded that the extract of *Tinospora crispa* stems in all treatments had strong antioxidant activity using either the CUPRAC or methods FRAP. In addition, it is very active as an antidiabetic by inhibiting alpha glucosidase. The optimum extract selected was extract B (extraction time was 35 minutes and amplitude 65%) with IC_{50} values for antioxidant activity in the CUPRAC method of 72.53 ± 0.18 mg/L and the FRAP method of 152.29 ± 2.16 mg/L, while the inhibition of alpha glucosidase was 0.30 ± 0.006 mg/L.

Key words: Antidiabetic, Antioxidant, *Tinospora crispa (L.)*, Ultrasound-assisted extraction.

Candra Irawan[1], Imalia Dwi Putri[1], Maman Sukiman[2], Andita Utami[3], Ismail[3,*], Ratna Komala Putri[3], Anisa Lisandi[3], Andrean Nur Pratama[4]

[1]*Department of Food Nanotechnology, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.*

[2]*Department of Industrial Waste Treatment, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.*

[3]*Department of Chemical Analysis, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.*

[4]*Research and Development Planning Agency, Tanggamus District, Lampung, INDONESIA.*

Correspondence

Ismail

Department of Chemical Analysis, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.

E-mail: ismail.abusyafiq@gmail.com

History

- Submission Date: 18-06-2022;
- Review completed: 21-07-2022;
- Accepted Date: 15-08-2022.

DOI : 10.5530/pj.2022.14.128

Article Available online

http://www.phcogj.com/v14/i5

INTRODUCTION

Diabetes mellitus is a major public health issue that affects people all over the world. In newly industrialized and developing countries, such as Indonesia, the prevalence and incidence of this disease have risen dramatically.[1] Indonesia is one of the countries with the most diabetes sufferers aged 20-79 years, which is ranked 7th in the world.[2] Diabetes mellitus is a metabolic disease characterized by hyperglycemia (high blood glucose levels) due to insulin deficiency.[3] This enzyme has the ability to raise blood sugar levels. As a result, an inhibitor of the glucosidase enzyme is required to prevent blood sugar from rising. Standard diabetes medications have more side effects than antihyperglycemic treatments.[4] Therefore, it is very important to find an effective and safe glucosidase enzyme inhibitor for pharmaceutical applications.

Medicinal plants are sources of active compounds that are very important in drug development because of their diverse and unique chemical structures.[5] Brotowali (*Tinospora crispa (L.)*) is an Indonesian natural plant that can be used as medicine. The Brotowali plant has been found to have antimalarial, antidiabetic, antipyretic, and antihyperglycemic properties.[6] Alkaloids, flavonoids, glycosides, and terpenes are found in Brotowali stems.[7] It is necessary to extract the antioxidant and anti-diabetic chemicals found in the brotowali stem first. The maceration procedure is the most commonly utilized extraction method. Plants are macerated by immersing them in water for 18 hours.[8] The use of conventional extraction methods such as maceration has several disadvantages including low efficiency and potential for environmental pollution due to the large volume of organic solvents used, as well as long extraction times. For that we need an alternative extraction method that is very good when compared to conventional extraction methods, mainly due to the lack of need for organic solvents and the relative extraction time including Microwave Assist Extraction (MAE), and Ultrasonic Assist Extraction (UAE).[9]

Ultrasonic-assisted extraction (UAE) is one of the ultrasonic-assisted extraction methods. Ultrasonic waves are sound waves that have a frequency above human hearing (≥ 20 kHz). This extraction method is used to obtain a higher antioxidant content in a relatively short time. Ultrasonic is non-destructive and non-invasive so it can be easily adapted to various applications.[10] With the help of ultrasonics, the process of extracting organic compounds in plants and grains using organic solvents can take place more quickly. The cell wall of the material is broken down by ultrasonic vibrations so that the contents in it can be easily removed.[11] Variations in time and amplitude conditions were carried out in the extraction to obtain optimum conditions to obtain the yield with the highest antioxidant and antidiabetic activity.

METHODS

Simplicia setup

The correctness of the identity of the plants utilized was previously determined in the Herbarium Bogoriense, Botany Field of the National Research and Innovation Agency, Cibinong, Bogor Regency, West Java. Farmers in Bogor Regency, West Java, provided *Tinospora crispa* stem samples (Figure 1).

Cite this article: Irawan C, Putri ID, Sukiman M, Utami A, Ismail, Putri RK, et al. Antioxidant Activity of DPPH, CUPRAC, and FRAP Methods, as well as Activity of Alpha-Glucosidase Inhibiting Enzymes from *Tinospora crispa* (L.) Stem Ultrasonic Extract. Pharmacogn J. 2022;12(5): 511-520.

Figure 1: The stem samples of *Tinospora crispa*.

Simplicia was crushed in a blender, then stored in a dry container, labeled, and kept out of direct sunlight.

Simplicia extraction

This procedure refers to the research conducted by Irawan using the UAE method.[12] The dried powder was weighed approximately 104.5 grams and put into a 250 mL glass beaker for 4 repetitions. Then 70% technical ethanol was added, the solvent was added until the dried fruit peel powder was submerged and stirred until everything was mixed. Stem simplicia was extracted with UAE using time variation parameters (minutes) and amplitude (%) at 30 minutes-60%; 35 minutes-65%; 45 minutes-60%; and 45 minutes-65%. The extraction result is filtered to separate the liquid extract from the dregs and put into a beaker that has been weighed. Each beaker is 250 mL. To achieve a higher yield, the ethanol solvent was removed from the filtrate in the 250 mL beaker by evaporation using an oven set at 40 °C and allowed to evaporate until all of the ethanol had evaporated. The percent yield value was derived by weighing the extract without the solvent.

Antioxidant activity test using 1,1-diphenyl-2-pikrilhidrazil (DPPH) method

Test for antioxidants: the DPPH method refers to the method used by Irawan.[13] An amount of 5 mg of stem bark extract was dissolved with methanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1,000 mg/L. DPPH 39 mg/L solution was added to five 5 mL measuring flasks, then each was put into five 5 mL measuring flasks, then each was added 2 mL of DPPH 39 mg/L solution, then measured with methanol pa, and homogenized (sample concentrations of 40, 80, 160,320 and 640 mg/L). The solution was incubated for 30 minutes at room temperature (25°C), then the absorbance of the solution was measured using a visible light spectrophotometer at a wavelength of 515 nm. The work is carried out in five repetitions. The same work was carried out to make a comparison solution of BHT with concentrations of 2, 4, and 8 mg/L.

Antioxidant activity is expressed as percent inhibition (% inhibition) with the following equation:

$$\%\ Inhibition = \frac{(A_{blank} - A_{sample})}{A_{blank}} x100\%$$

Details:

A_{blank} = Absorbance without sample

A_{sample} = Absorbance of the sample

The % inhibition value associated with the concentration in ppm (mg/L) will produce a linear equation ($Y=b_X + a$). The IC_{50} value was obtained from the calculation when the % inhibition was 50%.

Antioxidant activity test using Cupric Ion Reducing Antioxidant Capacity (CUPRAC) method

Antioxidant testing of the CUPRAC method using the procedure that has been used by Irawan *et al.*[14] The work was done on the crude ethanol extracts of treatments A, B, C, and D that were obtained during the extraction process with the UAE. sample concentrations range of 40 to 160 mg/L was prepared from a mother liquor of 1000 mg/L stem ethanol extract. Each solution was placed in five 5 mL volumetric flasks, followed by 1 mL of CUPRAC solution. After that, each solution was homogenized with ethanol pa. The solution was incubated for 30 minutes at 37°C, then the absorbance of the solution was measured using a visible spectrophotometer at a wavelength of 459 nm. The same steps were conducted on a standard solution of butylated hydroxytoluene (BHT) with a concentration of 1, 2, and 3 mg / L. Reducing activity can be calculated with the following equation:

$$\%.Reduction\ Power = \frac{(A_{sample} - A_{blank})}{A_{sample}} x100\%$$

Details :

A_{blank} = Absorbance without sample

A_{sampel} = Absorbance of sample

The calculated values were tranformed into a linear equation (Y = bX + a) with the ppm concentration (mg / L) as the abscissa (X-axis) and the % value of the reduction as the ordinate (Y-axis). The IC_{50} value was obtained from the calculation when the % reduction was 50%.

$$IC50 = \frac{50 - a}{b}$$

Antioxidant activity test using Ferric Reducing Antioxidant Power (FRAP) method

Antioxidant testing of the FRAP method using the procedure that has been used by Irawan *et al.*[15] The work was done on the crude ethanol extracts of treatments A, B, C, and D that were obtained during the extraction process with the UAE.A solution of stem ethanol extract with a concentration range of 40 to 160 mg/L was prepared from a mother liquor of 1000 mg/L stem ethanol extract. Each solution was placed in five 5 mL volumetric flasks, then 0.4 mL of 0.001 M citric acid was added; 0.2 mL of 0.002 M Fe^{3+} solution; and 0.4 mL of 0.2% o-phenanthroline, then filtered with distilled water and homogenized. The solution was incubated for 35 minutes at 37°C, then the absorbance of the solution was measured using a visible spectrophotometer at a wavelength of 510 nm. The same steps were conducted on a standard solution of gallic acid with a concentration of 0.25, 0.5, and 0.75 mg/L. The IC_{50} value can be calculated based on the equation as in the determination of the CUPRAC method.

Alpha-Glucosidase inhibitor activity test

Testing of alpha-glucosidase inhibitory activity refers to the procedure used by Budiarso.[12] The work was done on the crude ethanol extracts of treatments A, B, C, and D that were obtained during the extraction process with the UAE. Acarbose as a standard and stem ethanol extract samples were weighed and dissolved in phosphate buffer pH 6.8. Then, standard and sample solution were diluted into some concentrations. Thirty microliter standard and sample solution were added to 17 µL ofpara-Nitrophenyl-α-D-glucopyranosidesubstrate 4mM. The solutions were incubated at 37°C for 5 minutes, then 17 µL alpha-glucosidase solution was added. The solution was incubated again at 37°C for 15 minutes. After that, 100 µL sodium carbonate 200 mM was added, then the absorption of the solution was measured using a microplate reader at λ 405 nm. The same operation was carried out for sample control and blank control, but the addition of sodium carbonate was carried out before the addition of alpha glucosidase.

RESULT AND DISCUSSION

Antioxidant activity of the DPPH method

Antioxidants are substances that can slow down or prevent the oxidation process. This substance is significantly able to slow down or inhibit the oxidation of substances that are easily oxidized even in low concentrations. DPPH is a free radical which is stable at room temperature and in methanol and produces a purple solution. When free radicals react with antioxidants, their free radical properties are lost because the chains are broken and the color changes from purple to light yellow.[16] DPPH is determined by calculating the IC50 value through linear regression calculation, namely the concentration of the test compound that can reduce 50% of DPPH radicals. The concentration of the evaluated *Tinospora crispa* stem extract ranged from 40 to 640 mg/L.

The results of the antioxidant activity test with DPPH were expressed as % inhibition (Table 1), which was then linked to a series of samples or standard concentrations to produce a curve, as shown in Figure 2.

The regression equation for BHT and *Tinospora crispa* stem ethanol extract with treatments A, B, C, and D obtained y = 6.024x + 24.12; y = 0.077x + 1.007; y = 0.074x + 3.550; y = 0.069x + 9.769; and y = 0.075x + 2.627, respectively. From this equation, the IC50 values for BHT, treatment extracts A, B, C, and D were respectively 4.30 ± 0.01, 631.34 ± 0.85, 623.48 ± 0.74, 581.36 ± 0.91, and 626.62 ± 0.73 mg/L. In general, BHT had better DPPH inhibitory activity than the ethanol extract of *Tinospora crispa* stem. Based on the standard level of antioxidant activity proposed.[17] That the smaller the IC50 value indicates the higher the antioxidant activity, where the antioxidant capacity is very strong, namely 50 ppm, strong 50-100 ppm, moderate 101-150 ppm and weak >150 ppm. Based on this category, the antioxidant activity of *Tinospora crispa* stem extract with treatments A, B, C and D was included in the weak category because the IC_{50} value was in the range of 581-631 mg/L.

Antioxidant activity test CUPRAC method

The CUPRAC (cupric reducing antioxidant capacity) method has the advantage that this method is simple, selective, stable, and sensitive for thiol antioxidants, and can measure the ability of phenol compounds in the sample. From the research conducted for the Cuprac method, the IC_{50} results of the Ethanol extract of T. *crispa* stems with treatments A, B, C, and D were expressed as % inhibition (Table 2). which is then

Table 1: Antioxidant activity test results of the DPPH method.

Sample	Concentration (mg/L)	Mean % Inibition ± SD	IC_{50} ± SD
	2	34.1577 ± 0.05	
BHT	4	51.2365 ± 0.01	4.30 ± 0.01
	8	71.3122 ± 0.02	
	40	3.8930 ± 0.02	
	80	6.6604 ± 0.02	
A	160	13.6022 ± 0.03	631.34 ± 0.85
	320	26.9324 ± 0.06	
	640	50.1407 ± 0.07	
	40	6.4493 ± 0.05	
	80	8.1378 ± 0.06	
B	160	15.6191 ± 0.05	623.48 ± 0.74
	320	29.6904 ± 0.07	
	640	50.1876 ± 0.07	
	40	10.1078 ± 0.04	
	80	16.6979 ± 0.03	
C	160	23.2176 ± 0.04	581.36 ± 0.91
	320	30.4878 ± 0.06	
	640	54.1744 ± 0.04	
	40	5.7223 ± 0.03	
	80	7.3405 ± 0.08	
D	160	15.4315 ± 0.07	626.62 ± 0.73
	320	27.9549 ± 0.06	
	640	50.4221 ± 0.06	

Table 2: Antioxidant activity test results cuprac method.

Sample	Concentration (mg/L)	Mean % Inibition ± SD	IC_{50} ± SD
	1	27.4168 ± 0.29	
BHT	2	54.3370 ± 0.17	2.01 ± 0.01
	3	67.2272 ± 0.13	
	40	27.0866 ± 0.06	
A	80	48.0650 ± 0.06	106.76 ± 0.01
	160	63.3267 ± 0.03	
	40	31.0498 ± 0.18	
B	60	44.5840 ± 0.13	72.53 ± 0.18
	80	53.3736 ± 0.10	
	40	29.0924 ± 0.38	
C	80	46.9036 ± 0.10	104.80 ± 0.57
	160	64.2884 ± 0.07	
	40	30.9985 ± 0.41	
D	80	43.0504 ± 0.12	112.60 ± 0.61
	160	61.4808 ± 0.07	

Table 3: Antioxidant activity test results frap method.

Sample	Concentration (mg/L)	Mean % Inibition ± SD	IC_{50} ± SD
	0.25	29.2134 ± 0.34	
Gallic Acid	0.50	46.9845 ± 0.14	0.60 ± 0.01
	0.75	56.8985 ± 0.74	
	40	8.2872 ± 0.72	
A	80	14.8717 ± 0.94	159.07 ± 2.25
	160	41.3427 ± 0.47	
	40	20.4761 ± 0.62	
B	80	29.5358 ± 0.61	152.29 ± 2.16
	160	52.4216 ± 0.65	
	40	32.9317 ± 0.62	
C	80	39.7112 ± 0.59	126.58 ± 1.52
	160	57.0694 ± 0.34	
	40	29.2372 ± 0.66	
D	80	48.7730 ± 0.46	98.63 ± 1.44
	160	67.6356 ± 0.26	

Table 4: The results of the alpha-glucosidase activity inhibition test.

Sample	Concentration (mg/L)	% Reduction Power	IC_{50} (mg/L)
	10	27.01± 0.2	
Acarbose	50	47.68± 0.07	55.84 ± 0.2
	100	71.59± 0.4	
	4	37.58 ± 0.82	
A	8	53.65 ± 0.10	7.28 ± 0.09
	12	66.50 ± 0.03	
	0.25	47.27 ± 0.17	
B	0.5	60.97 ± 0.04	0.30 ± 0.006
	1	88.54 ± 0.09	
C	0.25	7 ± 0.32	
	0.50	48.08 ± 0.17	0.53 ± 0.001
	0.75	81.31 ± 0.08	
D	0.75	38.57 ± 0.12	
	1	48.34 ± 0.18	1.11 ± 0.01
	2	73.99 ± 0.08	

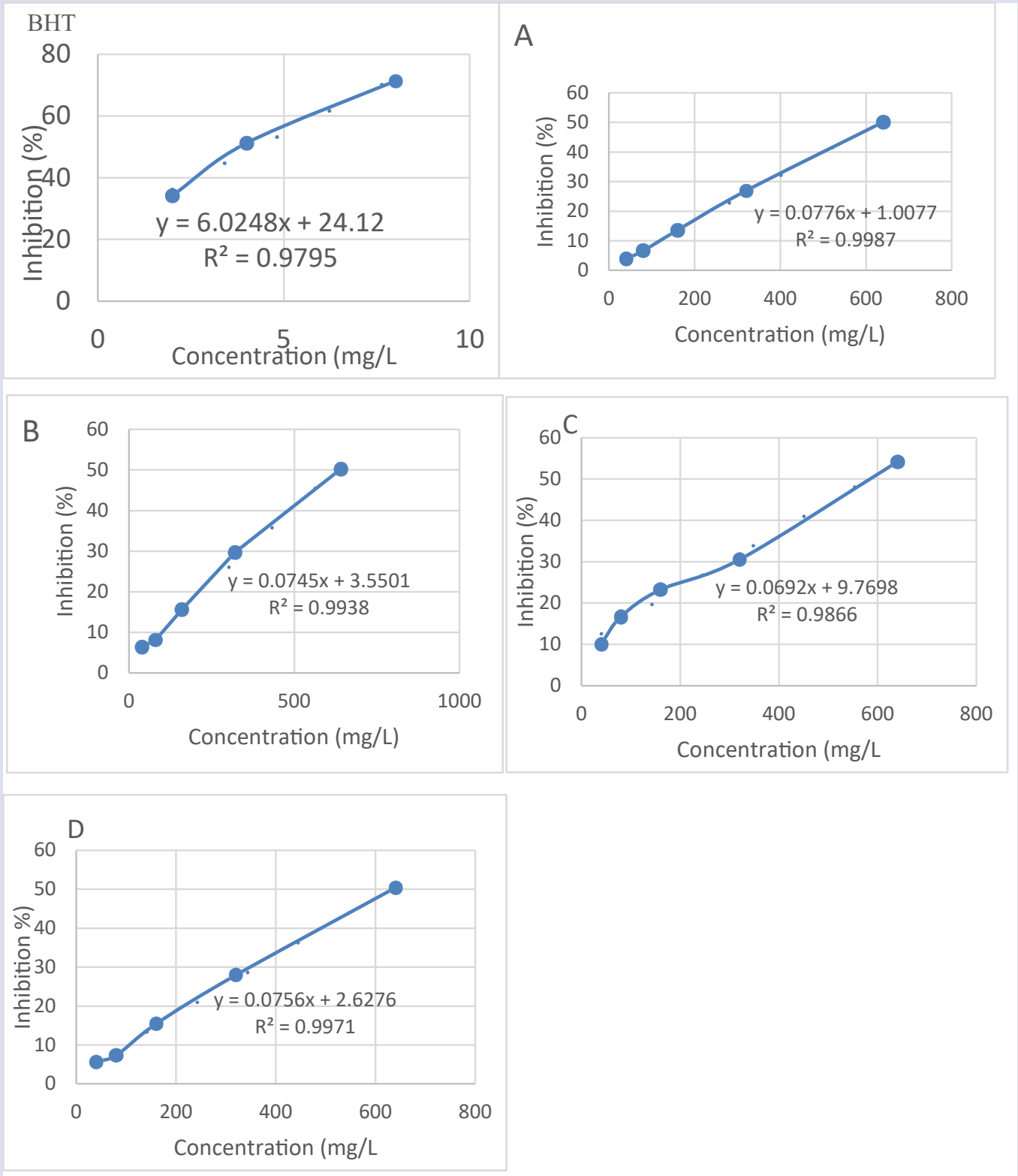


Figure 2: Antioxidant activity curve of DPPH method (a) BHT, (b) *Tinospora crispa* stem ethanol extract in extraction time of 30 minutes-60% amplitude, (b) with extraction time of 35 minutes-65% amplitude, (c) with extraction time 45 min-60% amplitude, and (d) with extraction time 45 min-65% amplitude.

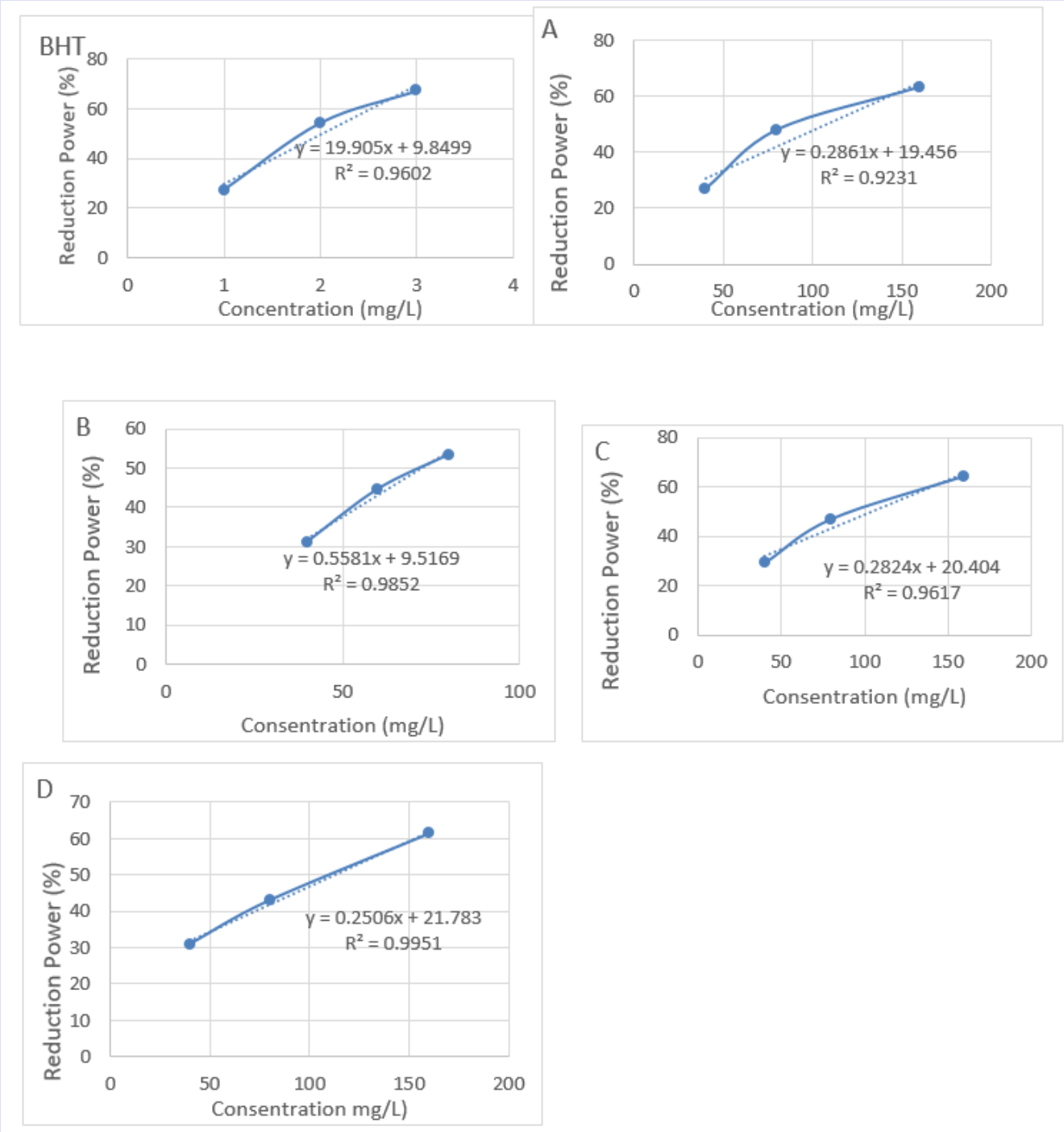


Figure 3: Antioxidant activity curve of CUPRAC method (a) BHT, (b) *Tinospora crispa* stem ethanol extract in extraction time of 30 minutes-60% amplitude, (b) with extraction time of 35 minutes-65% amplitude, (c) with extraction time 45 min-60% amplitude, and (d) with extraction time 45 min-65% amplitude.

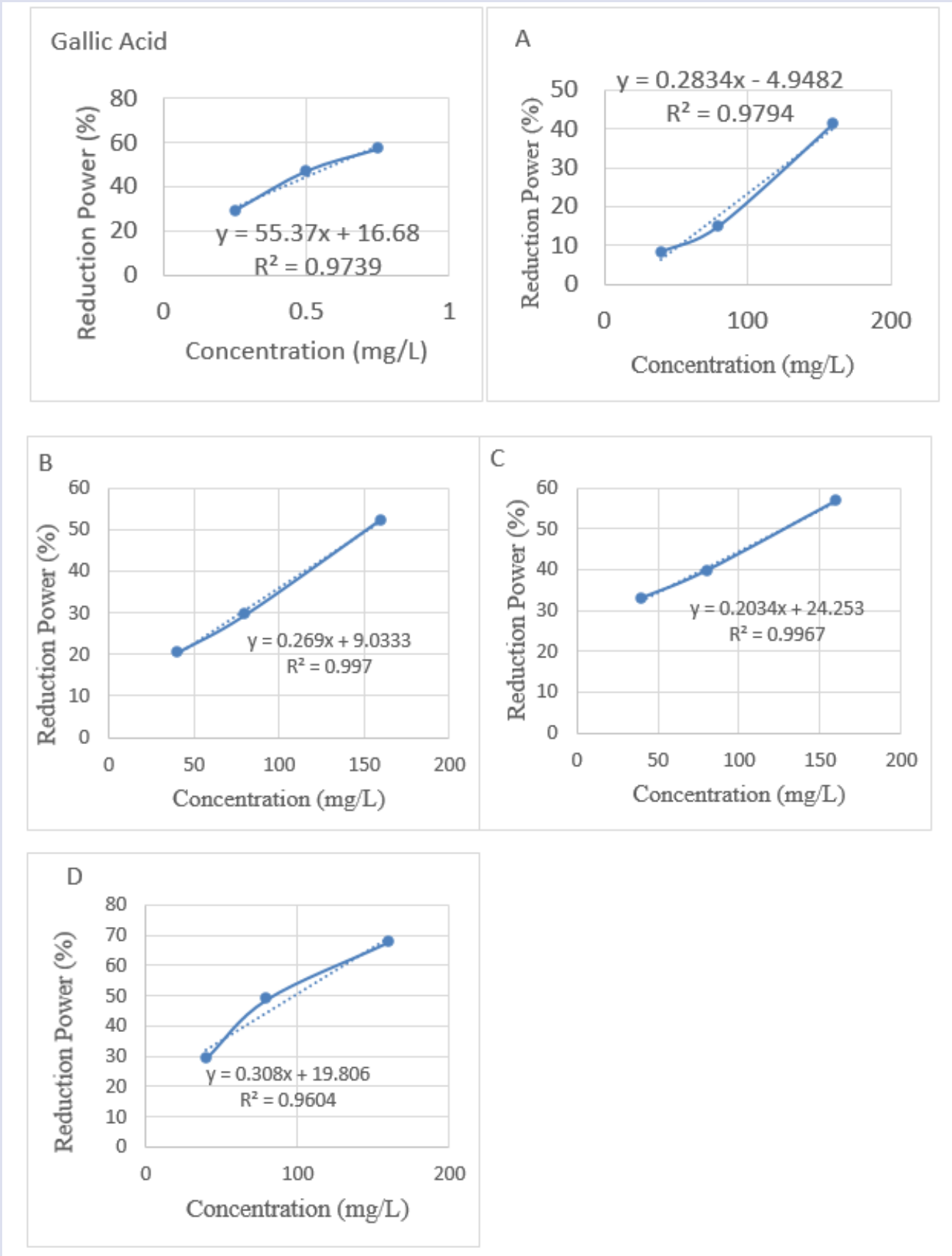


Figure 4: Antioxidant activity curve of FRAP method (a) Gallic Acid, (b) *Tinospora crispa* stem ethanol extract in extraction time of 30 minutes-60% amplitude, (b) with extraction time of 35 minutes-65% amplitude, (c) with extraction time 45 min-60% amplitude, and (d) with extraction time 45 min-65% amplitude.

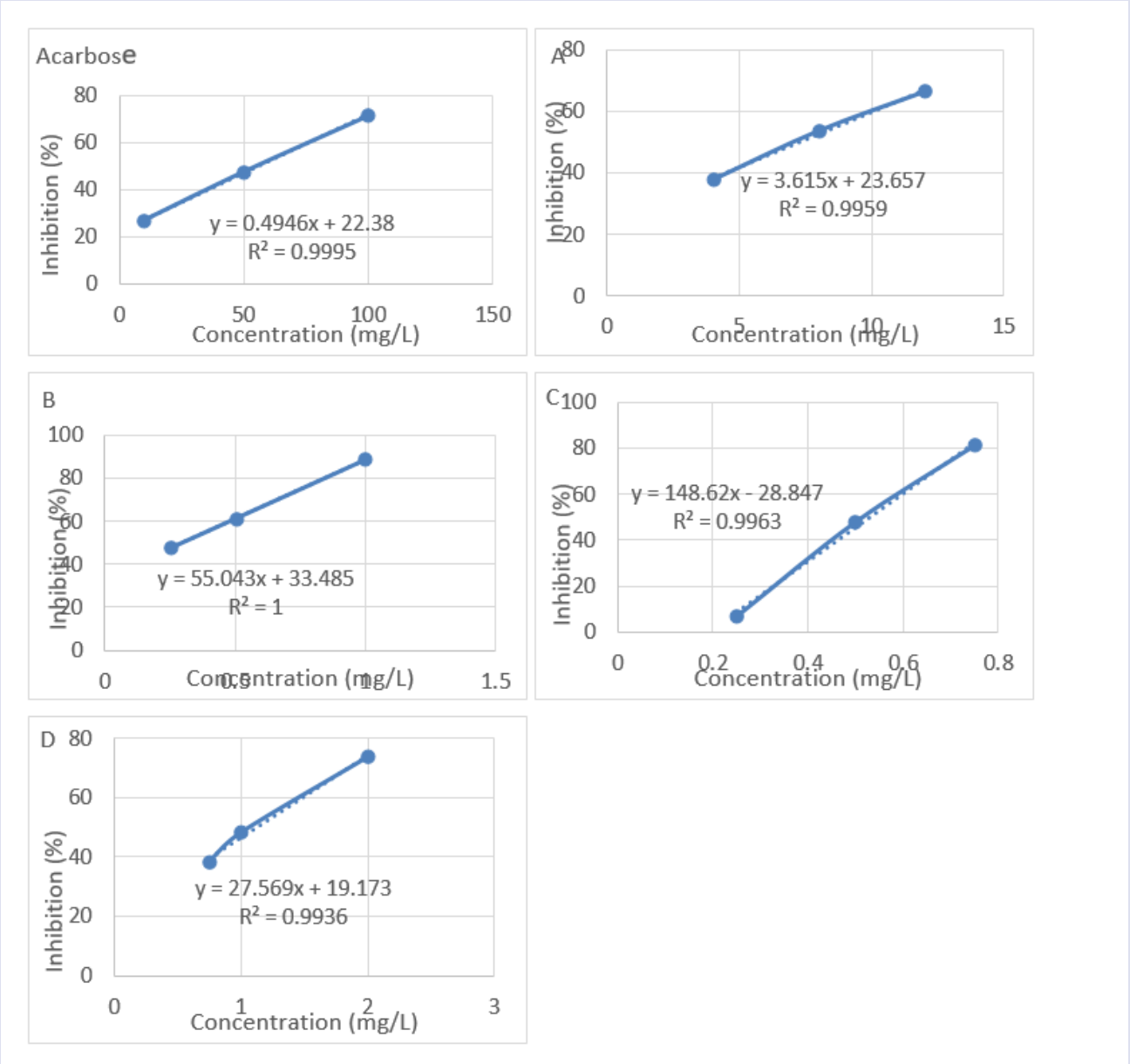


Figure 5: Graph of the relationship between concentration and % inhibition for IC_{50} determination acarbose, (A) ethanolic extracts of *Tinospora crispa* stem in 30 minutes extraction time-60% of amplitude, (B) with 35 minutes extraction time-65% of amplitude, (C) with 45 minutes extraction time-60% of amplitude, and (D) with 45 minutes extraction time-65% of amplitude.

linked to a series of samples or standard concentrations to produce a curve, as shown in Figure 3.

Table 2. Shows that the IC50 on the stems of T. crispa for treatments A, C, and D is relatively the same, while for treatment B it is lower. The smaller the IC_{50} value, the higher the antioxidant activity.

The regression equation for BHT and T. crispa stem ethanol extract with treatments A, B, C, and D obtained y = 19,90x + 9,849; y = 0.286x + 19.45; y = 0.558x + 9.516; y = 0.282x + 20.40, and y = 0.250x + 21.78; consecutive. From this equation, the IC_{50} values for BHT, treatment extracts A, B, C, and D were 2.01 ± 0.01, 106.76 ± 0.01, 72.53 ± 0.18, 104.80 ± 0.57, and 112.60 ± 0.61 mg/L. Based on the category, the antioxidant activity of *Tinospora crispa* stem extract with treatments A, B, C and D was included in the strong to moderate category because the IC_{50} value was in the range of 72-112 mg/L.

Antioxidant activity test FRAP method

The FRAP (ferric reducing ability of plasma) method has the advantage that it gives quick and easy results, and shows antioxidants in matrix complexes. From the research conducted for the FRAP method, the IC_{50} of *Tinospora crispa* stem ethanol extract with treatments A, B, C, and D was expressed as % inhibition (Table 3). which is then linked to a series of samples or standard concentrations to produce a curve, as shown in Figure 3. The regression equation for Gallic Acid and *Tinospora crispa* stem ethanol extract with treatments A, B, C, and D obtained y = 55.37x + 16.68; y = 0.283x - 4.948; y = 0.269x + 9.033; y = 0.203x + 24.25; and y = 0.308x + 19.80, respectively. From this equation, the IC_{50} values for BHT, treatment extracts A, B, C, and D were 0.60 ± 0.01, 244.36 ± 2.25, 152.29 ± 2.16, 126.58 ± 1.52, and 98.63 ± 1.44 mg/L. Based on the category, the antioxidant activity of *Tinospora crispa* stem extract with

treatments A, B, C and D was included in the strong to weak category because the IC_{50} value was in the range of 98-159 mg/L.

From the results of the antioxidant activity tests of the three methods, the DPPH method gave a different response (IC50 value) where the results showed a small antioxidant activity compared to the CUPRAC and FRAP methods. In this study, the CUPRAC and FRAP methods have the strongest antioxidant activity. Measurement of antioxidant activity is assumed that the extract obtained has antioxidant compounds that can reduce Fe(III) TPTZ under thermodynamic reaction conditions and have a fairly fast reaction rate. In addition, the oxidized antioxidant and all secondary reaction products must have a maximum absorption at the absorption of Fe(III) TPTZ.[18] The antioxidant capacity of plant origin is often associated with the content of phenolic compounds and flavonoids. Phenolic compounds have been reported to have antioxidant activity due to their oxidation-reducing properties. Phenolic compounds act as reducing agents, hydrogen donors, singlet oxygen absorbers and as potential chelators. Flavonoid compounds can react as antioxidants based on their ability to ward off free radicals by giving hydrogen atoms to these free radicals.

The third test of antioxidant methods showed that the measurement of antioxidant activity with DPPH, CUPRAC, and FRAP gave significantly different results, but CUPRAC and FRAP gave the same response to the ethanol extract of *Tinospora crispa* stems. Phenol is not correlated with flavonoid content but highly correlated with its antioxidant activity. Flavonoids have no correlation with antioxidant activity. The number of active compounds to be targeted and the matrix components present in the extract are important factors in measuring antioxidant capacity. In addition, the suitability of pH between the method and the sample to be analyzed can provide an initial guess on the choice of method.

Potential inhibition of alpha-glucosidase activity of leaves ethanol extract

Based on the antidiabetic activity test, acarbose was used as standard at concentrations of 10, 50 and 100 ug/mL and had 27.01%, 47.68%, and 71.59% inhibition, respectively. The ethanol extract of *Tinospora crispa* stem extract with treatments A, B, C, and D was expressed as % inhibition (Table 4). which is then linked to a series of samples or standard concentrations to produce a curve, as shown in Figure 4. The regression equation for Acarbose and *Tinospora crispa* stem ethanol extract with treatments A, B, C, and D obtained y = 0.4949x + 22.38; y = 3.615x – 23.65; y = 55.05x + 33.48; y = 148.6x + 28.84; and y = 27.56x + 19.17, respectively. From this equation, the IC_{50} values for Acarbose, treatment extracts A, B, C, and D were respectively 55.84 ± 0.2; 7.28 ± 0.09; 0.30 ± 0.006; 0.53 ± 0.001, and 1.11 ± 0.01 mg/L.

Based on the results of the antidiabetic activity test in Table 4, it can be seen that the antidiabetic activity of the *Tinospora crispa* stem ethanol extract in each treatment was higher than the acarbose standard used, namely IC_{50} of 55.84 mg/L. This could be due to the ethanol extract of *Tinospora crispa* stem which is suspected as an antidiabetic agent. Flavonoids are naturally occurring organic compounds found in plants in general. Many natural flavonoids play an important role in the prevention of diabetes and its complications.[19] *Tinospora crispa* stem ethanol extract in treatment C had high antidiabetic activity compared to other extracts with IC_{50} levels of 0.30 mg/L. Therefore, the optimum condition chosen for this extraction was an extraction time of 35 minutes with an amplitude of 65% (B).

The percentage of inhibition indicates the percentage of the enzyme that is inhibited by the concentration of the sample, so the greater the percentage value indicates the greater the inhibition of the enzyme, and vice versa.[20] IC_{50} indicates the ability of the sample to inhibit enzyme activity by 50 percent, so the smaller the IC_{50} value, the higher the inhibitory activity, and vice versa. IC_{50} < 50 ug/mL is very strong if it is 50-100 ug/mL, while if IC_{50} is 100-150 ug/mL, weak if IC_{50} is 150-200 ug/mL, and very weak. if IC_{50} value > 200 ug/mL.[20] Based on the results of the antidiabetic activity test of *Tinospora crispa* stem ethanol extract, the IC50 value for treatment A was 7.28 ± 0.09 mg/L, treatment B was 0.30 ± 0.006, treatment C was 0.53 ± 0.001 and treatment D was 1.11 ± 0.01 mg/L. It showed that the ethanol extract of *Tinospora crispa* stems had very strong inhibitory values. Judging from the IC_{50} value in each treatment which was in the range of values <50 mg/L.

CONCLUSION

Based on the results of the study, it can be concluded that the stem extract of *Tinospora crispa* has antioxidant activity and is very active as an antidiabetic by inhibiting the alpha glucosidase enzyme. The optimum extract selected was extract B (extraction time 35 minutes and amplitude 65%). *Tinospora crispa* stem ethanol extract has the potential as a source of antioxidants and antidiabetic.

ACKNOWLEDGMENT

This work was supported by the Industrial Resources Development Agency of the Ministry of Industry, Indonesia.

CONFLICTS OF INTEREST

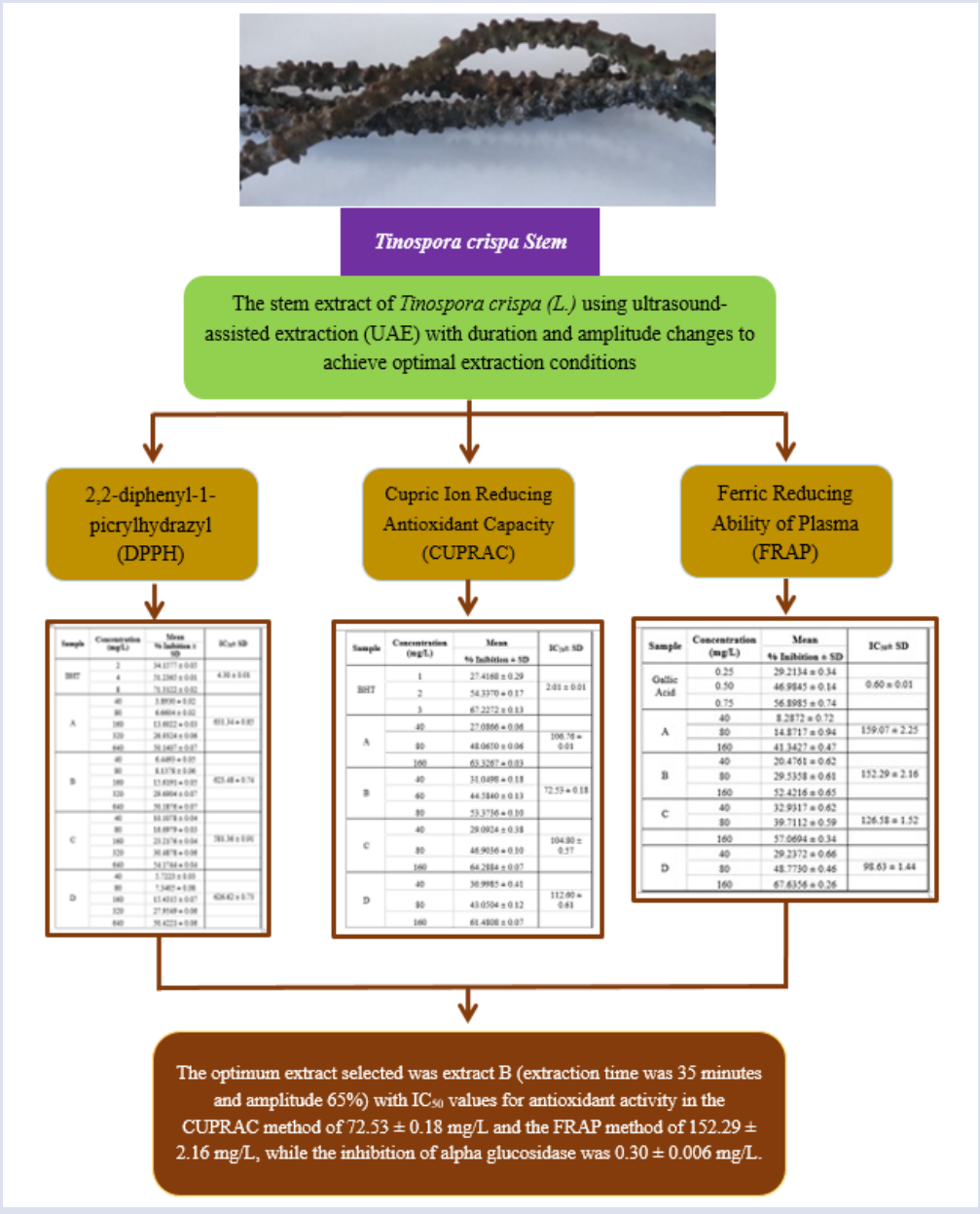
The authors declare that they have no conflicts of interest.

REFERENCES

1. Krisnatuti D, Yenrina R, Rasjmida D. Healthy diet for people with diabetes mellitus. East Jakarta: Self-help spreader. 2014.
2. International Diabetes Federation. Nam Han Cho, David Whiting, Leonor Guariguata, Pablo Aschner Mantoya, Wolfgang Rathmann, Gojka Roglic, Jonathan Shaw, Martin Silink, DRR. Williams, Ping Zhang. 2013. IDF DIABETES ATLAS, 9th Edition.
3. Afifah UN. Activity Test Antidiabetic Ethanol Extract 96% Bitter gourd (*Momordica charantia.) L.*) against Strain Male Rats Alloxan Induced Wistar. Thesis Pharmacy Study Program. 2017.
4. Niwa A, Tajiri T, Higashino H. Ipomoea batatas and Agarics blazei Ameliorate Diabetic Disorders with Therapeutic Antioxidant Potential in Streptozotocin Induced Diabetic Rats. J Clin Biochem Nutr. 2011;48(3):194-202.
5. Handayani S, Hermawab A, Meiyanto E, Udin Z. Induction of Apoptosis on MCF-7 Cells by *Selaginella* Fractions. J Appl Pharm Sci. 2013;3(4):31-4.
6. Noor H, Aschroft SJ. Pharmacological Characterization of the Antihyperglycaemic Properties of *Tinospora crispa* Extract. J Ethnopharmacol. 1998;62(1):7-13.
7. Apriliani ND, Saputri FA, Pharmacy F, Padjadjaran U. Review: Potential for Inhibition of Alpha-Glucosidase Enzymes in Indonesian Traditional Medicinal Plants. Pharmacy. 2018;16(1):169-77.
8. Rosandi MJ, Oktavia AI. The Effect of Solvent Type Variations on the Quality of Tempuyung (*Sonchus Arvensis L.*) Leaf Extract with Maceration Method. Indonesian Male Pharmacy Academy Malang. 2018;1-10.
9. Novi FU, Sely MN, Sutanto, Usep S. Effect of Various Extraction Methods on Determination of Flavonoid Levels of Leaf Ethanol Extract (*Plectranthus scutellarioides*). J Pharmacy. 2020;10(1):76-83.
10. McClements DJ. Advances in the application of ultrasonic in food analysis and processing. Trends Food Sci Techn. 1995;6(9):293-9.
11. Mason TJ, Lorimer JP. Applied Sonochemistry: The uses of power ultrasonic in chemistry and processing. Weinheim (DE): Wiley- VCH Verlag GmBH and Co. 2003;314.

12. Irawan C, Elya B, Hanafi M, Saputri FC. Application of Ultrasound-Assisted Extraction on the Stem Bark of Rhinachantus *Nasutus (L.) Kurz*, Total Phenolic, and Its Potential as Antioxidant and Inhibitor of Alpha-Glucosidase Enzyme Activity. Pharmacogn J. 2021;13(5):1-7.
13. Irawan C, Utami A, Styani E, Putri ID, Putri RK, Dewanta A, *et al*. Potential of Ethanolic Extract from Ripe *Musa balbisiana Colla* Fruit Using Ultrasound-Assisted Extraction as an Antioxidant and Anti-Gout. Pharmacogn J. 2021;13(6):1332-40.
14. Azmir J, Zainudin ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F. Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. J Food Eng. 2013;117:426-36.
15. Rostagno MA, Palma M, Barroso CG. Ultrasound Assisted Extraction of Soy Isoflavones. J Chromatogr A. 2003;1012(2):119-28.
16. Badarinath AV, Rao K, Chetty CMS, Ramkanth S, Rajan TVS, Granaprakash K. A Review on In-vitro Antioxidant Methods: Comparisions, Correlations and Considerations. Int J Pharmtech Res. 2010;2(2):1276-85.
17. Blois MS. Antioxidant Determination by the Use of a Stable Free Radical. J Nature. 1958;181(4617):1199-200.
18. Apak R, Guclu, K, Demirata B, Ozyurek M, Celik SE, Betasoglu B, *et al*. Comparative evaluation of various total antioxidant capacity assay applied to phenolic compounds with the CUPRAC assay. Molecules. 2007;12(7):1496-547.
19. Jack. Synthesis of Antidiabetic Flavonoids and Their Derivative. Medical Research page 180. 2012.
20. Hardoko, Febrianti A, Siratantri T. In Vitro Antidiabetic Activity of Agar, Agarose, and Agropectin from *Gracilaria gigas Seaweed*. Indones Fish Res J. 2015;18(2):128-39.
21. Mardawati E, Filianty F, Marta H. Study of Antioxidant Activity of Mangosteen Peel Extract (*Garcinia mangostana*) in the Context of Utilizing Mangosteen Peel Waste in Puspahiang District, Tasikmalaya Regency. Final Report of Young Researcher Research, Padjadjaran University. 2008.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Candra Irawan is a Lecturer at the Department of Food Nanotechnology, Politeknik AKA Bogor, Indonesia. He has research experience in the field of Phytochemistry and Natural Product.

Imalia Dwi Putri is a lecturer at D-IV Department of Food Nanotechnology, Politeknik AKA Bogor, Indonesia. Research focus in functional foods, antioxidants from various plant extracts, and halal food.

Maman Sukiman is a Lecturer at the Department of Industrial Waste Treatment, Politeknik AKA Bogor, Indonesia. He has research experience in the field of Natural Product and Chemical Environment.

Andita Utami is a Lecturer at the Department of Chemical Analysis, Politeknik AKA Bogor, Indonesia. She has research experience in the field of Natural Product and Antioxidants.

Ismail is a Lecturer at Chemical Analysis Department, Politeknik AKA Bogor and Doctoral Natural Resources and Environmental Management Student at IPB University, Bogor, West Java 16143, Indonesia. He has research experience in field of Natural Resources and Modelling.

Ratna Komala Putri is a laboratory staff at Politeknik AKA Bogor. Ratna continuing her Postgraduate study at IPB University, Indonesia, majoring Food Science. She is focusing on research in the field of Functional Foods, Microbiology and Food Technology.

Anisa Lisandi is a College student at Politeknik AKA Bogor. She participates in research on natural products.

Andrean Nur Pratama is a Researcher at the Research and Development Planning Agency, Tanggamus District, Lampung, Indonesia. He has research experience in statistics.

Cite this article: Irawan C, Putri ID, Sukiman M, Utami A, Ismail, Putri RK, et al. Antioxidant Activity of DPPH, CUPRAC, and FRAP Methods, as well as Activity of Alpha-Glucosidase Inhibiting Enzymes from *Tinospora crispa* (L.) Stem Ultrasonic Extract. Pharmacogn J. 2022;12(5): 511-520.