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Antioxidant activity and inhibition of α-glucosidase from extract and fraction of leaves and stems of *Vernonia amygdalina*

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ABSTRACT

Previous studies have shown that bitter leaf (*Vernonia amygdalina* Del.) has the ability as an antioxidant and an α-glucosidase inhibitor. Still, the difference in antioxidant activity and α-glucosidase inhibition based on the leaves and stems parts have not yet been determined. The effect of flavonoid-enriched extract on antioxidant activity and inhibition of α-glucosidase has not yet been determined. This research aimed to assess the impact of flavonoid-enriched extract from the leaves and stems part of the bitter leaf. The leaves and stems part of the bitter leaf were extracted using Soxhlet apparatus with 80% methanol and then underwent successive fractionation with petroleum ether, chloroform, and ethyl acetate. The crude extract and the fraction were concentrated and followed by the determination of total flavonoid, total phenolic, antioxidant activity, α-glucosidase inhibition activity, and calculated the IC_{50} of α-glucosidase inhibition. This research showed that chloroform-ethyl acetate leaf fraction was the best fraction with the higher total flavonoid (24.091±0.972 mg QE/g DW), total phenolic (84.299±4.589 mg GAE/g DW), diphenylpicrylhydrazyl (DPPH) antioxidant activity (33.881 µM TE/g DW), ferric reducing antioxidant power (FRAP) antioxidant activity (312.022±1.745 µM TE/g DW) and α-glucosidase inhibition activity with an IC_{50} value 1.23 mg/mL.

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1. INTRODUCTION

Indonesia is enriched with varieties of herbal plants widely used in the pharmaceutical and food industry. Herbal plants have pharmacological properties due to their bioactive compounds [1]. Around 80% of the world's population uses herbal products as food supplements, nutraceutical products, and alternative medicine. Bitter leaf (*Vernonia amygdalina*) is a promising medicinal plant in tropical Asia and Africa [2]. Previous studies reported that *Vernonia amygdalina* has several secondary metabolites, which include alkaloids, phenols, anthraquinones, cardiac glycosides, tannins, terpenoids, saponins, and terpenoids [3]–[5]. Previous studies have also reported that *Vernonia amygdalina* has several biological activities such as anti-hyperuricemia [6], antibacterial and antimicrobial [4], antioxidant [7], and inhibition of α-glucosidase enzyme [8].

Previous studies have shown that *Vernonia amygdalina* has antioxidants and inhibition of α-glucosidase activity. Thanh and Tran [7] reported that IC_{50} of antioxidant activity of *Vernonia amygdalina* from the maceration of ethyl acetate, 80% methanol, and 70% ethanol was 424.16 µg/mL, 290.93 µg/mL,

and 259.03 µg/mL. Djeujo *et al*. [8] reported that IC_{50} of α-glucosidase inhibition of *Vernonia amygdalina* from maceration extraction of aqueous and 70 % ethanol were 0.44 mg/mL and 0.36 mg/mL. Igbindu and Nimenibo [9] have also reported that *Vernonia amygdalina* has α-glucosidase inhibition with IC_{50} of methanolic extract, aqueous fraction, n-hexane fraction, and dichloromethane fraction were 12.17 µg/mL, 12.93 µg/mL, 13.59 µg/mL, dan 59.83 µg/mL. Ajebli and Eddouks [10] reported that flavonoid-enriched extract (FEE) from Warionia saharae can increase diphenylpicrylhydrazyl (DPPH) antioxidant activity with an IC_{50} 111.74±1.02 µg/mL.

According to the international diabetes federation (2019), around 463 million people worldwide are affected by diabetes mellitus, which is expected to increase to 700 million people by 2045. In this context, polyphenols can be useful nutraceuticals and supplementary treatments for various aspects of diabetes mellitus. Based on several in vitro and animal models and clinical studies, polyphenols may play a role in many metabolic processes. They can regulate carbohydrate and lipid metabolism, insulin resistance, and dyslipidemia, reduce hyperglycemia, improve adipose tissue metabolism, and alleviate oxidative stress [10]. The beneficial role of flavonoids in treating diabetes mellitus is evident due to their ability to reduce side reactions and complications. Numerous in vitro, in vivo, and epidemiological studies confirm flavonoids' hypoglycemic and hypolipidemic action [11], [12].

Previous research has shown that bitter leaf (*Vernonia amygdalina* Del.) has the ability as an antioxidant and an α-glucosidase inhibitor. It can be used as a candidate for herbal medicine for diabetes mellitus patients. Still, the difference in antioxidant activity and α-glucosidase inhibition based on the leaves and stems parts have not yet been determined. The effect of flavonoid-enriched extract on antioxidant activity and inhibition of α-glucosidase has not yet been determined. This study aims to determine the impact of flavonoid-enriched extract from the leaves and stem parts of *Vernonia amygdalina*.

2. RESEARCH METHODS

2.1. Study site and sample preparation

This research was conducted at the Laboratorium of the Department of Biochemistry, IPB University. The leaves and stems parts of *Vernonia amygdalina* were obtained from Tropical Biopharmaca Research Center, IPB University, Bogor, Jawa Barat, Indonesia (6°32′25.47″ LU, 106°42′53.22″ BT, 142.60 meters above sea level). The material was dried using the oven at 45 °C for 24 h. The dried material was ground to yield an 80-mesh powder.

2.2. Flavonoid-enriched extraction

Extraction of plant material modified from Ajebli and Eddouks [10], 100 g of the powdered leaves and stem of vernonia amygdalina were extracted in a soxhlet apparatus for 9 hours, using 1 L of 80% methanol as an organic solvent. Th *Vernonia amygdalina* e resulting methanolic extract was then filtered to remove particulate particles using a millipore filter (0.22 µm, MF-millipore). using a separatory funnel, the filtrate obtained underwent successive fractionations, and this extract was concentrated using rotary vacuum evaporator apparatus (labtech) at 45 °C. The residue was dissolved in distilled water, filtered, and refrigerated overnight. This residue was defatted with petroleum ether (1:1, v/v) five times to remove certain organic waste (lipids and chlorophyll). The organic phase was discarded, and the aqueous phase was fractionated five times using chloroform (1:1, v/v) to extract aglycone flavonoids. The organic phase from the previous fractionation was combined in an Erlenmeyer flask. The aqueous fraction was subjected to a third fractionation using ethyl acetate, which was repeated five times to extract flavonoid glycosides. The two fractions (organic phase of chloroform and ethyl acetate) were mixed, then concentrated using a rotary vacuum evaporator (labtech) and stored at 4 °C until further analysis.

2.3. Stock solution of solutions

To prepare the stock solution extracts for further analysis, a precise procedure was followed. Initially, 100 milligrams of crude and fraction extracts were meticulously weighed and transferred into 100 mL volumetric flasks. Methanol was added to each flask until the desired volume was reached, yielding the stock solution extracts ready for subsequent analysis.

2.4. Quantification of total phenolic content (TPC)

Quantifying TPC based on Nurcholis *et al*. [13] with modification, using folin-ciocalteau reagent and gallic acid (0-105 mg/L) as standard. The crude extract and fraction (20 µL) were added to 120 µL of folin-ciocalteau reagent in a microplate 96 well and then incubated in a dark room for 5 min. The mixture was added to 80 µL of 10% Na_2CO_3 and then incubated for two h at room temperature. Using a nano-spectrophotometer (SPECTROstarNano BMG labtech, Offenburg, Germany), the absorbance was measured

at 760 nm. The standard calibration resulted in a line equation of y = 0.0092 + 0.0337, with an R^2 of 0.9902, and the total phenolic was expressed in mg of gallic acid equivalent per g of DW (mg GAE/g DW).

2.5. Quantification of total flavonoid content (TFC)

Quantifying total TFC based on Nurcholis *et al.* [13] method with modification, using $AlCl_3$ and quercetin (0-225 mg/L) as standard. The crude extract and fraction (10 µL) were added to 120 µL distilled water, 10 µL of 10% $AlCl_3$, 10 µL CH_3COOK, and 50 µL of methanol in a microplate 96 well. The solution was incubated for 30 min, and the absorbance was recorded at 434 nm using a nano-spectrophotometer (SPECTROstarNano BMG labtech, Offenburg, Germany). The standard calibration resulted in a line equation of y =0.0035 + 0.0058, with an R^2 of 0.9984, and the total flavonoid were expressed in mg of quercetin equivalent per g of DW (mg QE/g DW).

2.6. Diphenylpicrylhydrazyl (DPPH) antioxidant assay

DPPH antioxidant assay based on Nurcholis *et al*. [13] with modification, using trolox (0-35 µM). The crude extract and fraction (50 µL) were added to 150 µL DPPH reagent in microplate 96 well. The solution was incubated for 20 min in a dark room, and then the absorbance was measured at 517 nm using a nano-spectrophotometer (SPECTROstarNano BMG labtech, Offenburg, Germany). A change in solution color from deep purple to pale yellow indicates antioxidant activity. The standard calibration resulted in a line equation of y = 2.4305 + 5.8409, with an R^2 of 0.981, and the antioxidant activity was expressed in trolox equivalent antioxidant capacity or µmol TE/ g DW.

2.7. Ferric reducing antioxidant power (FRAP) antioxidant assay

FRAP antioxidant assay based on Nurcholis *et al*. [13] with modification, using trolox (0-700 µM). FRAP solution was prepared by mixing acetate buffer pH 3.6, 10 mM tripyridyltriazine (TPTZ) dissolved in 40 mM HCl, and 20 mM $FeCl_3$ solution (1:1:10). The crude extract and fraction were added to 290 µL of FRAP solution and then incubated for 30 min in a dark room at 37 °C. The absorbance of the solution was recorded at 593 nm using a nano-spectrophotometer (SPECTROstarNano BMG labtech, Offenburg, Germany). Antioxidant activity is indicated by a change of color of the solution from colorless to intense blue. The standard calibration resulted in a line equation of y = 0.0013–0.0024, with an R^2 of 0.9928, and the antioxidant activity was expressed in trolox equivalent antioxidant capacity or µmol TE/ g DW.

2.8. α-glucosidase inhibition test

The Procedure was modified from Sancheti *et al*. [14], using acarbose as a control positive. A total of 50 µL 0.1 M phosphate buffer pH 7.0, 25 µL p-nitrophenyl-α-d-glucopyranoside (p-NPG) 50 mM (dissolved in 0.1 M buffer phosphate pH 7.0), and 10 µL of crude extract or fraction and then incubated for 5 min at 37 °C. After incubation, 25 µL of α-glucosidase 0.1 Unit/mL in 0.1 M phosphate buffer pH 7.0 was added and then incubated at 37 °C for 30 min. The reaction was stopped by adding 100 µL of 0.2 M Na_2CO_3 and then measured at 405 nm using a nano-spectrophotometer (SPECTROstarNano BMG labtech, Offenburg, Germany). The α-glucosidase inhibitory was expressed as percent inhibition and IC_{50}. Percent inhibition was calculated as (1).

$$\text{Percent inhibition} = \left(1 - \left(\frac{B-C}{A}\right)\right) x\ 100 \quad (1)$$

Where A is absorbance with α-glucosidase without a sample, B is absorbance with α-glucosidase and sample, and C is absorbance with a sample without α-glucosidase. The composition of each solution is presented in Table 1.

Table 1. Composition of the solution test of α-glucosidase inhibition test

Reagent	A (µL)	B (µL)	C (µL)
Phosphate buffer 0.1 M pH 7.0	50	50	75
p-NPG	25	25	25
Sample	10 (Aquadest)	10	10
Incubated at 37 °C for 5 min			
Enzyme	25	25	-
Incubated at 37 °C for 30 min			
Na_2CO_3 0.2 M	100 µL	100 µL	100 µL
Total	210 µL	210 µL	210 µL
Measured at 405 nm			

2.9. Data analysis

The data of TPC, TFC, antioxidant activity, and IC_{50} of α-glucosidase were expressed as means ± standard deviations (SDs). Statistical differences among the means were measured using one-way analysis of variance (ANOVA) followed by Tukey honest significant difference (HSD) post hoc test. Differences in means were significant when $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Yield of extraction

The total yield of crude extract and the fraction of *Vernonia amygdalina* is presented in Figure 1. Compared to previous studies, the yield obtained from the leaf crude extract (LCE) and stem crude extract (SCE) were higher at 19.765% and 15.243% (Figure 1). Adefisayo *et al.* [15] obtained a yield of 8.18% by macerating vernonia amygdalina leaves in 70% methanol for 72 hours, while Ogbuagu *et al.* [16] obtained 8.96% using soxhlet apparatus with 25 g of *Vernonia amygdalina* leaves in 250 mL of 80% methanol for 18 hours. Similarly, Gasaliyu *et al.* [17] yielded 8% from 3.5 kg of *Vernonia amygdalina* leaves macerated with 10.5 L of methanol for 48 hours. Additionally, the yield of chloroform-ethyl acetate leaf fraction (CELF) and chloroform-ethyl acetate stem fraction (CESF) were 11.982% and 9.407% (Figure 1), which was higher than Adefisayo *et al.* [15] study that obtained yields of 0.70%, 2.10%, 3%, and 3.28% for n-hexane, ethyl acetate, n-butanol, and aqueous fractions, respectively. The difference in yield results suggests that the amount of yield obtained from the extraction process is determined by the extraction method, type of solvent, extraction time, temperature, and biomass-to-solvent ratio [18].

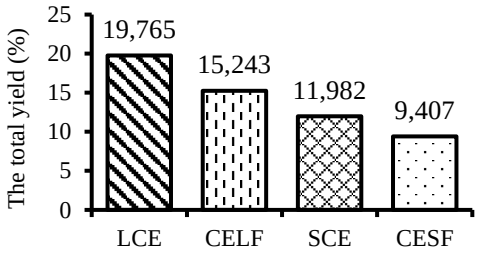

Figure 1. Total phenolic contents of LCE, CELF, SCE, and CESF. Different letters notation indicated that there was a significant difference between the groups analyzed by ANOVA with Tukey's test at a 95% confidence level

3.2. Total flavonoid and phenolic contents

Phenolics and flavonoids are the plant secondary metabolites with several pharmacological activities, including antimicrobial, anti-inflammatory, anticancer, antioxidant, and other biological activities antioxidant, anti-inflammatory, antimicrobial, anticancer, and other biological activities [19]. Phenolics and flavonoids serve as antioxidants and donate electrons or hydrogen atoms to free radicals, forming stable intermediates due to the electron delocalization of the aromatic ring when it reacts with free radicals, which leads to aromatic ring resonance to prevent chain reactions [20], [21].

The determination of TPCs was carried out by colorimetry assay using the folin-ciocalteau reagent [13]. The principle of this measurement is based on the reduction-oxidation reaction between the hydroxyl groups of phenolic compounds and the folin-ciocalteau reagent. This reaction will form a blue-colored molybdenum-tungsten complex that can be quantified at 760 nm. The intensity of the blue color formed is directly proportional to the total phenolic content [22]. Gallic acid is used as a standard because it is one of the phenolic compound derivatives of hydroxybenzoic acid that is pure and stable, making it easy to react with the folin-ciocalteau reagent [23].

The total phenolic content of each sample is presented in Figure 2. The highest total phenolic content was found in the chloroform-ethyl acetate leaf fraction (CELF), which was 84.299±4.589 mg GAE/g DW, while the lowest was found in the stem crude extract (SCE), which was 15.960±0.426 mg GAE/g DW. These values are higher compared to previous studies. The total phenolic content of the ethanol, n-hexane, ethyl acetate, n-butanol, and water were 2.51±0.07 mg GAE/g DW, 0.05±0.04 mg GAE/g DW, 3.61±0.03 mg GAE/g DW, and 0.53±0.05 mg GAE/g, respectively [24].

Dagnon *et al.* [25] carried out the identification of phenolic compounds present in *Vernonia amygdalina* leaf extract based on ultra high performance liquid chromatography-mass spectrometry (UHPLC-MS/MS). The identification results showed that *Vernonia amygdalina* contains various types of

phenolic compounds, which are neo-chlorogenic acid, chlorogenic acid, 1,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid. Identification of phenolic compounds in *Vernonia amygdalina* leaf extract using LCMS-QTOF has been conducted by Alara *et al.* [2]. These compounds are 3-4-O-dicaffeoylquinic acid, 3,7-dihydroxy-2-4-dimethoxyphenanthrene-3-O-glucoside, 1,3-O-dicaffeoylquinic acid, trans-ferulaldehyde, and 1,3,5-O-tricaffeoylquinic acid.

Determining total flavonoids in the extract and stem of *Vernonia amygdalina* was conducted using aluminium chloride colorimetry method [13]. The principle of this measurement is the reduction-oxidation reaction between the hydroxyl group of flavonoid compounds and $AlCl_3$, which forms a yellow-colored complex that can be measured for its absorbance at a wavelength of 434 nm. The intensity of the yellow color formed is directly proportional to the total flavonoid content. Quercetin is a standard because it is one of the flavonoid compounds of the flavonol group, which consists of a ketone group on the C-4 atom and hydroxyl groups on the adjacent C-3 and C-5 atoms [26]. Quercetin is a compound that is widely found in plants [27].

The total flavonoid content of each sample is significantly different (Figure 3). The highest total flavonoid content was found in chloroform-ethyl acetate fraction (CELF), 21.128±0.577 mg QE/g DW, while the lowest was in stem crude extract (SCE), 9.28±0.176 mg QE/g DW (Figure 3). These values are higher than the total flavonoid content of *Vernonia amygdalina* ethanol extract obtained by microwave-assisted extraction conducted by Alara *et al.* [2], which was 10.29 mg QE/g DW. Wang *et al.* [28] reported that the total flavonoid content of *Vernonia amygdalina* ethanol and aqueous extracts obtained by ultrasonication were 3.393 mg QE/g and 0.347 mg QE/g, respectively. These differences indicate that the extraction method, solvent type, extraction time, and yield extract can influence the total flavonoid and phenolic content [18].

The leaf parts' total phenolic and flavonoid content is higher than the stem's (Figures 2 and 3). This is because the leaf parts are more exposed to sunlight and light [29]. Phenylalanine ammonia-lyase (PAL) is an important enzyme in the biosynthesis of phenolic acids, and its activity increases when induced by high light intensity. Therefore, the increase in the production of phenolic acids may be attributed to the increased activity of the PAL enzyme [30], [31]. The higher content of flavonoids and phenolics in leaves compared to stems is due to the higher amount of epidermal tissue, mesophyll, parenchyma, and secretory glands (which are storage places for secondary metabolites) present in the leaves, in contrast to the stems that mainly consist of transport tissues such as xylem and phloem [32]. Furthermore, environmental conditions such as CO2 levels, temperature [33], plant origin, age, environmental conditions of the growing place [34], and the maturity level of the plant [35].

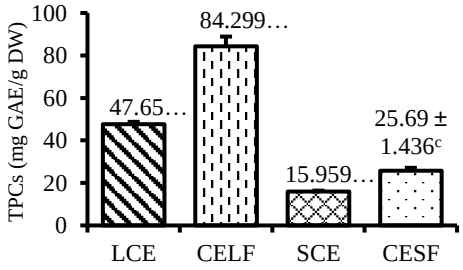

Figure 2. Total phenolic contents of LCE, CELF, SCE, and CESF. Different letters notation indicated that there was a significant difference between the groups analyzed by ANOVA with Tukey's test at a 95% confidence level

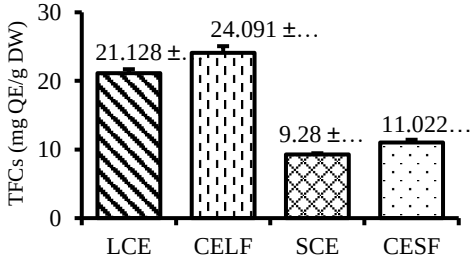

Figure 3. Total flavonoid contents of LCE, CELF, SCE, and CESF. Different letters notation indicated that there was a significant difference between the groups analyzed by ANOVA with Tukey's test at a 95% confidence level

3.3. Diphenylpicrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) antioxidant activity

One of the methods for testing antioxidant activity is the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay [36]. The principle of this measurement is based on the color-fading process of the purple DPPH radical due to the hydrogen donor from antioxidant molecules, which neutralizes the DPPH radical into a yellow color (Figure 4). This fading reaction can be quantified using a spectrophotometer at a wavelength of 517 nm. The higher the intensity of the resulting yellow color, the higher the antioxidant activity [22]. Antioxidant activity is expressed in trolox equivalents in units of μmol TE/g dry weight [13]. Trolox is a standard in antioxidant testing because it has high antioxidant activity and carbonyl groups in its structure, making it soluble in polar solvents. The trolox equivalent value is directly proportional to the antioxidant activity, meaning that antioxidant activity will increase as the trolox equivalent value increases [37].

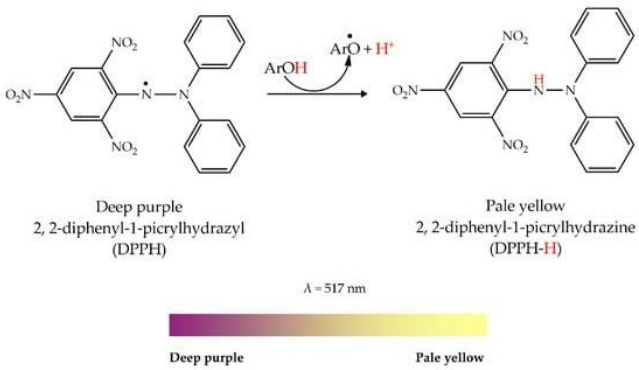

Figure 4. Reaction mechanism of DPPH antioxidant assay [20]

The DPPH antioxidant activity of each sample is presented in Figure 5. The highest antioxidant activity was found in chloroform-ethyl acetate leaf fraction (CELF) at 33.881±0.106 μmol TE/g DW, while the lowest value was in stem crude extract (SCE) with an average of 4.561±0.004 μmol TE/g DW. DPPH antioxidant activity was much higher in leaves than stems due to the higher yield obtained from leaf extracts and fractions than stem extracts (Figure 1). The high yield indicates the extract's total flavonoid and phenolic content [38]. The total content of flavonoids and phenolics is directly proportional to their antioxidant activity, which means that antioxidant activity will increase as the total content of flavonoids and phenolics increases [39], [40].

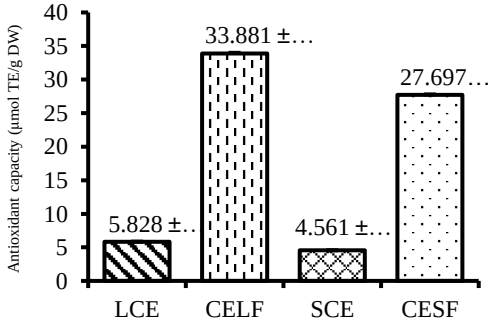

Figure 5. The antioxidant capacity of LCE, CELF, SCE, and CESF. Different letters notation indicated that there was a significant difference between the groups analyzed by ANOVA with Tukey's test at a 95% confidence level

Another method for measuring plant antioxidant activity is the FRAP assay. The mechanism (Figure 6) of this assay involves the reduction of $[Fe(TPTZ)_2]^{3+}$ (which is orange in color) to $[Fe(TPTZ)_2]^{2+}$ (which is blue) under acidic conditions [41]. The higher the concentration of Fe3+-TPTZ that the sample reduces to Fe2+-TPTZ, the higher the antioxidant activity in the sample [42]. This color change reaction can be quantified using a spectrophotometer at a wavelength of 593 nm [22].

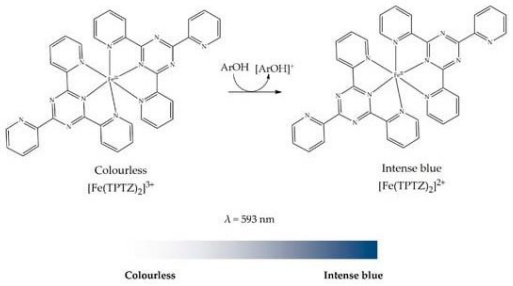

Figure 6. Reaction mechanism of FRAP antioxidant assay [22]

The FRAP antioxidant activity of each sample is presented in Figure 7. The highest antioxidant activity was found in chloroform-ethyl acetate leaf fraction (CELF), which was 312.022±1.745 µmol TE/g DW, while the lowest value was observed in stem crude extract (SCE), which was 104.215±1.538 µmol TE/g DW. These values are much higher compared to the antioxidant activity values obtained using the DPPH method, which was 33.881±0.106 µmol TE/g DW for chloroform-ethyl acetate leaf fraction (CELF) and 104.215±1.538 µmol TE/g DW for stem crude extract (SCE). This suggests that the bioactive compounds in the leaves and stems of the vernonia amygdalina have potential as antioxidants and tend to work through a single electron transfer (SET) mechanism, which is commonly used in the FRAP assay. On the other hand, the DPPH assay tends to work through a hydrogen atom transfer (HAT) mechanism [43].

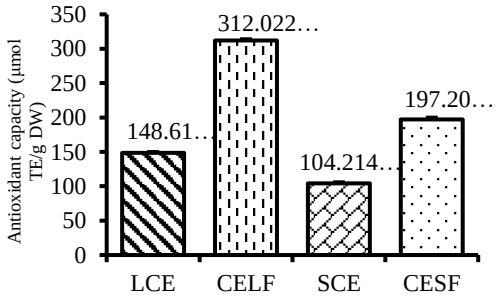

Figure 7. The antioxidant capacity of LCE, CELF, SCE, and CESF. Different letters notation indicated that there was a significant difference between the groups analyzed by ANOVA with Tukey's test at a 95% confidence level

3.4. Inhibition activity of α-glucosidase

Colorimetric assay is one of the methods to test the in vitro inhibition activity of α-glucosidase. The principle of this testing is based on the enzymatic hydrolysis reaction of the substrate p-NPG by α-glucosidase, which produces α-D-glucose and *p*-nitrophenol that appears yellow [44]. The intensity of the yellow color formed, measured at a wavelength of 405 nm, is inversely proportional to the inhibition activity of α-glucosidase. Therefore, the clearer the yellow color produced, the less glucose and p-nitrophenol are formed. The low amount of p-nitrophenol formed indicates that the enzymatic hydrolysis reaction is less active, which means that the inhibition activity of α-glucosidase is high [45].

Acarbose is a positive control in the inhibition activity of the α-glucosidase assay. It is a commercial α-glucosidase inhibitor that has been tested as a synthetic drug for type II diabetes mellitus patients [46]. Acarbose has a chemical structure similar to the substrate α-D-glucopyranoside, resulting in competitive competition between the substrate and acarbose, inhibiting the activity of α-glucosidase [47]. Acarbose plays a crucial role in directly absorbing carbohydrates into the bloodstream and indirectly helps optimize glucose metabolism through insulin secretion adaptation [48].

Based on Figure 8, statistical tests showed that the inhibition activity of α-glucosidase from *Vernonia amygdalina* had various IC_{50} values and significant differences. The chloroform-ethyl acetate leaf fraction (CELF) had the highest inhibition activity with the lowest mean IC_{50} value of 1.23±0.025 mg/mL. In contrast, the stem crude extract (SCE) had the lowest inhibition activity with a mean IC_{50} value of 1.795±0.030 mg/mL. The inhibition activity of α-glucosidase in the leaves and stems of *Vernonia*

amygdalina extract was much lower than acarbose, with a mean IC_{50} value of $1.4x10^{-4}$ mg/mL. This is because acarbose is a single compound that effectively inhibits α-glucosidase. At the same time, other compounds in the leaves and stems of *Vernonia amygdalina* can decrease the inhibition activity [49]. Furthermore, the obtained value is lower than the mean IC_{50} values of 12.17 μg/mL, 59.83 μg/mL, 12.93 μg /mL and 13.59 μg /mL obtained from the methanol extract, n-hexane, dichloromethane, and aqueous fraction, respectively, in the study conducted by Igbindu dan Nimenibo [9].

The inhibition activity of α-glucosidase from the leaves and stems of *Vernonia amygdalina* extract is due to its secondary metabolite content. Among the other secondary metabolites, flavonoids have a greater influence on the inhibition activity of α-glucosidase [50]. This is consistent with the data obtained in this study, where the higher the level of flavonoids, the higher the inhibition activity with lower IC_{50} values [17]. Flavonoid glycoside structures are similar to acarbose, which plays a role in the inhibition activity of α-glucosidase. This inhibition activity is due to hydrogen bonding formed from hydroxyl groups at C3' and C4' in the B and C3 rings of the C ring with the active site of the α-glucosidase enzyme [51], [52].

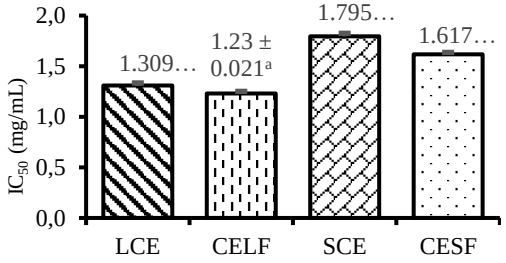

Figure 8. IC_{50} of α-glucosidase inhibition of LCE, CELF, SCE, and CESF. Different letters notation indicated that there was a significant difference between the groups analyzed by ANOVA with Tukey's test at a 95% confidence level

4. CONCLUSION

Based on statistical tests, leaf crude extract (LCE), chloroform-ethyl acetate leaf fraction (CELF), stem crude extract (SCE), and chloroform-ethyl acetate stem fraction (CESF) have significantly different antioxidant and α-glucosidase inhibition activities. The total flavonoid and phenolic content of *Vernonia amygdalina* are higher in the leaf part than in the stem. Chloroform-ethyl acetate leaf fraction (CELF) has the highest total flavonoid and phenolic content, DPPH and FRAP antioxidant activities, and the lowest IC_{50} of α-glucosidase compared to the others. Chlroform-ethyl acetate leaf fraction (CELF) effectively increases total flavonoid, phenolic content, antioxidant activity, and α-glucosidase inhibition.

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