






Antioxidant Properties of *Salacca zalacca* (Gaertn.) Voss Peel Ethanolic Extract Compared to Chlorogenic Acid

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Abstract: Oxidative stress from free radicals can cause a variety of chronic and degenerative diseases. The use of antioxidants from natural products is one of the breakthroughs. *Salacca zalacca* (Gaertn.) Voss is one of the tropical fruits that have biological activities that are important for human health. This study aims to determine total phenol content (TPC) and flavonoid content (TFC), also the antioxidant activity of *Salacca zalacca* peel ethanolic extract (SEE) compared with chlorogenic acid (CGA). **METHODS:** The total phenolic and flavonoid content of SEE were measured and followed by 2,2'-azinobis-3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS), H₂O₂, NO, OH scavenging, and ferric reducing antioxidant power (FRAP) assay to determine the antioxidant properties. The TPC of SEE value is 6.97 µg GAE/mg extract and the TFC value is 3.92 µg QE/mg extract. The IC₅₀ value of ABTS, H₂O₂, NO, OH scavenging activity of SEE were 57.71; 103.84; 38.09; 27.77 µg/mL compared to CGA 7.76; 13.07; 27.15; 13.71 µg/mL respectively. The FRAP activity of SEE, CGA respectively 240.08; 399.21 µm Fe (II)/µg at the highest concentration (50 µg/mL). SEE and Chlorogenic acid as its compound have antioxidant activity through ABTS, H₂O₂, NO, OH and ferric reducing antioxidant power (FRAP) scavenging activities.


1 INTRODUCTION


Free radicals can be the cause of oxidative stress, which leads to a variety of chronic and degenerative diseases. Oxidative stress can be caused by free radicals. Free radicals are very reactive and unstable because the electrons do not pair with the outermost atomic orbitals. Free radicals react by binding molecules in cells, which cause the oxidation of


nucleic acids, proteins, fats, and DNA (Halliwell & Gutteridge, 2015).


Sources of free radicals can originate from normal metabolic processes in the human body or external exposure (Widowati et al., 2016). The body needs antioxidants as oxidation inhibitors to overcome the negative effects of free radicals. Antioxidants work by reacting to reactive free radicals to form relatively stable reactive substances. Thus, the antioxidant supply was needed for the human body to prevent oxidative stress (Rusmana et al., 2017).

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Antioxidants are divided into two types based on their source, synthetic, and natural antioxidants. Synthetic antioxidants can be carcinogenic if consumed continuously. Therefore, natural antioxidant needs to continue to increase because they have fewer side effects. In addition to having fewer side effects, natural antioxidants also protect the body from damage caused by free radicals and inhibit degenerative diseases (Xu et al., 2021) (Laintonjam, 2012). Several studies have shown that plant extract has the potential compound to be active antioxidants. Among all of the natural compounds, phytochemicals are best known for their various biological activities, such as antiaging, antioxidants, and anti-inflammatory (Widowati et al., 2016)(Girsang et al., 2020a)(Girsang et al., 2020b).

Secondary metabolites from non-edible fruits can be source of antioxidants, because they are rich in polyphenols (Vijayalaxmi et al., 2015). Some tropical and subtropical fruits have a protective effect on health. Salak or snake fruit (*S. zalacca*) is one of the tropical fruit that has biological activities that are important for human health. This fruit which has antioxidant potential is widely cultivated in the Southeast Asia region (Dembitsky et al., 2011). *S. zalacca* has active compounds in the form of polyphenols, chlorogenic acid, ferulic acid, gallic acid, and catechins (Hlásná Čepková, et al., 2021). Previous study stated that *S. zalacca* fruit has strong antioxidant activity, which was evaluated by 2,2'-azino-bis-3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazil (DPPH) scavenging assays (Saleh et al., 2018) (Suica-Bunhez et al., 2016). However, the inhibitory effects of *S. zalacca* peel active compounds on specific radical species have not been widely presented. In the present study, free radical scavenging activity of *S. zalacca* peel ethanolic extract (SEE) compared with its compounds chlorogenic acid, such as H₂O₂, NO, OH, ABTS scavenging activity, and FRAP activity were evaluated as well as total phenolic and flavonoid were measured. Thus, SEE which has been a waste can be utilized as an antioxidant agent derived from natural products.

2 METHODS AND MATERIALS

2.1 Plant Material Preparation and Extraction

Dried salak peels was obtained from Kampung Rahayu Cicadas, Ciampea, Bogor, West Java, Indonesia. The phytochemical compound and

chlorogenic acid (CGA) were obtained from Chengdu Biopurify (Biopurify Phytochemical Ltd, BP0345). Identification of the salak plants performed by a staff of herbarium, Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The salak plant was identified as *S. zalacca* (Gaertn.) Voss. The extraction method used in this study is the maceration method using 70% ethanol as the solvent.

The filtrate is collected every 24 hours until the colorless filtrate. After that, the filtrate is evaporated using a rotary vacuum evaporator at a temperature of 50°C until the extract becomes a paste-shaped extract. Then, the *S. zalacca* extract was stored at -20°C (Widowati et al., 2018)(Widowati et al., 2017)(Lister et al., 2019).

2.2 Total Phenol Assay

Briefly, 15 µl standard gallic acid (Sigma 398225) solution in 6 concentration level (50.00; 25.00; 12.50; 6.25; 3.13; 1.56 µg/ml) and sample of SEE in concentration of 2000; 1000; and 500 µg/ml were prepared for total phenol assay. Each standard and sample was mixed with 60 µl of Na₂CO₃ 7.5% (Merck A897992745) and 75 µl Folin-Ciocalteu reagent 10% (Merck 1.090.010.500) in the microplate.

The solution was incubated at 50°C for 10 minutes, then the absorbance was measured at a wavelength of 760 nm using a microplate reader (Multiskan Go Reader, Thermo Fisher Scientific 1510). Analysis of the phenol content was carried out based on the gallic acid (Sigma Aldrich, G7384) linear regression equations ($y = 0.0429x + 0.152$) (Rusmana et al., 2017)(Widowati et al., 2018)(Nurhayati et al., 2018)(Utami et al., 2019).

2.3 Total Flavonoid Assay

The total flavonoid content was measured with an AlCl₃ colorimetric assay with minor modification (15). A 75 µl standard quercetin (Sigma Q4951) solution in 7 concentration level (500.00; 250.00; 125.00; 62.50; 31.25; 15.60; and 7.80 µg/ml) and SEE in concentration of 2000 and 1000 µg/ml were added to microplate and mixed with 75 µl AlCl₃ 2% (Merck 449598). Using a microplate reader (Multiskan Go Reader, Thermo Fisher Scientific 1510) the absorbance was measured in 415 nm of wavelength. The linear regression equation ($y=0.0095x+0.037$) was created based on the quercetin standard (Sigma Aldrich, Q4951). The analysis of the flavonoid content of the sample was

performed based on each of standard linear regression equation (Prahastuti et al., 2019).

2.4 ABTS-reducing Activity

Antioxidant capacity of SEE and CGA were measured using the 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) (Sigma Aldrich, A1888) diammonium salt-free radical assay. ABTS^{•+} was produced by reacting 14 mM ABTS^{•+} and 4.9 mM potassium persulfate (Merck 1.05091.0250). The final concentration of the mixture is 7 mM ABTS^{•+} in 2.45 mM potassium persulfate. After that, the mixture was incubated at the darkroom temperature for 16 h. Using 5.5 mM PBS (pH 7.4) the ABTS^{•+} solution was diluted then the absorbance of the solution was measured with a microplate reader at 745 nm, resulting in the absorbance of 0.70±0.02. Then, a sample about 2 µl was added of ABTS^{•+} solution 198 µl. The solution was incubated at 30°C for 6 min and the absorbance was measured at a wavelength of 745 nm. The ABTS radical inhibition percentage (%) was calculated based on the ratio of ABTS^{•+} absorbance reduction of the sample relative to a negative control (Rusmana et al., 2017)(Widowati et al., 2018).

2.5 FRAP Activity Assay

Briefly, 10 ml of 300 mM acetate buffer (pH 3.6 adjusted with the addition of acetic acid) was mixed with 1 mL of 20 mM ferric chloride hexahydrate (Merck 1.03943.0250) and 1 ml of 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, T1253) to prepare the FRAP reagent. A 142.5 µl FRAP was mixed with 7.5 µl samples (SEE, CGA) in a microplate and incubated for 6 min at 37°C. Using a microplate reader the absorbance of the solution was measured in 593 nm of wavelength (Rusmana et al., 2017)(Widowati et al., 2018)(Prahastuti et al., 2019).

2.6 Hydrogen Peroxide (H₂O₂) Scavenging Activity Assay

Hydrogen peroxide scavenging activity was measured using a method described by Utami et al. (2017) and Prahastuti et al. (2019) with minor modifications (Prahastuti et al., 2019)(Utami et al., 2017). The mixture was made, then transferred into a microplate and incubated for 5 minutes at room temperature, then 75 µl 1,10-phenanthroline (Sigma 131377) was added to the mixture and incubate the mixture for 10 min at room temperature. The

absorbance was measured using a spectrophotometer at 510 nm. The result was depicted as a scavenging percentage that calculated using the following formula:

$$\% \text{ scavenging activity} = A/C \times 100\%$$

when A is sample absorbance and C controls absorbance.

2.7 Nitrogen Oxide Scavenging Activity Assay

Sodium nitroprusside (SNP) 10 mM (Sigma Aldrich, 71780) in phosphate buffer saline (PBS) (Gibco, 1740576) was mixed with several concentrations (2.08-66.67 µg/mL) of SEE and CGA. The mixture was then incubated for 2 hours at room temperature. Furthermore, the mixture was added Greiss reagent containing 1% Sulphanilamide (Sigma Aldrich, S9251), 2% H₃PO₄ (Merck, 100573), N-(1-naphthyl) ethylenediamine dihydrochloride (Sigma Aldrich, N9125). The absorbance was measured at 546 nm wavelength (Multiskan GO Reader, Thermo Fisher Scientific 1510) (19). The antioxidant activity of SEE and CGA in the experiment was determined as follows:

$$\% \text{ scavenging activity} = (A_c - A_s) / A_c \times 100$$

A_c: negative control absorbance

A_s: sample absorbance

2.8 Hydroxyl Radical (OH) Scavenging Activity Assay

The reaction mixture contained 30 µL of different concentrations of a sample (0.83 – 26.67 µg/mL), 10 µL of FeCl₃ 25 mM-EDTA, 5 µL of 20 mM H₂O₂ (Merck, 1.08597), 5 µL of 1 mM L-Ascorbic acid (Sigma Aldrich, K3125), 10 µL of 28 Mm Deoxyribose (Sigma-Aldrich, 121649), and 70 µL phosphate buffer. The mixture was incubated at 37 °C for 30 min and then 25 µL of 5% TCA (Merck, 100807), and 1% TBA (Sigma-Aldrich, T5500) were added to be further incubated at 80 °C for 30 min. The absorbance was measured at 532 nm wavelength using a spectrophotometer (Multiskan GO Reader, Thermo Fisher Scientific 1510) (Irwan et al., 2020).

The antioxidant activity of SEE and CGA in the experiment was determined as follows:

$$\% \text{ scavenging activity} = (A_c - A_s) / A_c \times 100$$

A_c: negative control absorbance

A_s: sample absorbance

2.9 Statistical Analysis

The result data were expressed as mean ± standard deviation and the data were analyzed using One-way ANOVA followed by Tukey’s HSD Post-hoc test. Statistical analysis was performed using SPSS software (version 20.0), with P < 0.05 as the significant value of the data.

3 RESULTS AND DISCUSSION

Salak (*S. zalacca*) is a species of palm tree group originating from Malaysia and Indonesia. This fruit is known as the 'snake fruit' because it has skin that is reddish-brown and scaly. Researchers believe that residues from plants can still be used because they have the potential as a source of antioxidants, and they are rich in polyphenols (Vijayalaxmi et al., 2015). The previous studies showed that *S. zalacca* peel extract had better antioxidant potential through inhibition of DPPH compared to other tropical fruits such as Matoa (*Pometia pinnata*), Papaya (*Carica papaya* L.), Soursop (*Annona muricata*), Chlorine (*Baccaurea racemosa*), and Rambai skin and seed extract (*B. motleyana*) (Fitri et al., 2016).

Phenol is one of the most contained compounds in plants and has been widely studied due to its biological activities such as anti-mutagenic, anticarcinogenic, anti-aging, and antioxidant (Cetin et al., 2014). In the present study, the result of total phenol and total flavonoid SEE has result with a value of $6.97 \pm 0.55 \mu\text{g GAE/mg}$ and $3.92 \pm 0.78 \mu\text{g QE/mg}$ extract, respectively (Table 1).

Table 1: The average total phenol and flavonoid level concentration of *S. zalacca* peels extract.

| Sample | Total Phenolic Content ($\mu\text{g GAE/mg}$ extract) | Total Flavonoid Content ($\mu\text{g QE/mg}$ extract) |
|--------|--|--|
| SEE | 6.97 ± 0.55 | 3.92 ± 0.78 |

*SEE: *S. zalacca* peel ethanolic extract

In other study, *S. zalacca* peel extract has total flavonoid content is $124.9 \pm 0.004 \text{ mg/g}$ Catechin and total phenolic content is $946.61 \pm 0.042 \text{ mg/g}$ Gallic acid (Suica-Bunghez et al., 2016). The value of total phenol and flavonoids is influenced by the level of fruit maturity, the young fruit has the highest total phenol value which is the highest compared to the ripe fruit (Mokhtar et al., 2014). *S. zalacca* peel

extract contains phenolic compounds that have an antioxidant activity such as chlorogenic acid, rutin, protocatechuic acid, and caffeic acid (Girsang et al., 2019)(Girsang et al., 2020).

The ABTS-reducing activity assay assesses an antioxidant’s ability to scavenge the ABTS generated. The long-wave absorption spectrum is used to quantify the reduction of blue-green ABTS radical colored solution by hydrogen-donating antioxidant (Widowati et al., 2016).

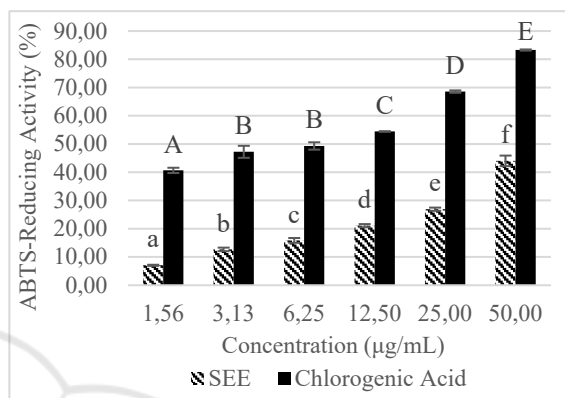


Figure 1: Effect variety concentrations of SEE, CGA toward ABTS-reducing activity.

*ABTS-reducing activity (%) of SEE, CGA were diluted in DMSO to reach the final concentration of 1.56; 1.13; 6.25; 12.50; 25.00; 50.00 ($\mu\text{g/mL}$). Different small letter (a,b,c,d,e,f) shows significant differences among concentration of SEE and different capital letter (A,B,C,D,E) among concentration of CGA toward ABTS-reducing activity based on Tukey HSD post hoc test ($p < 0.05$).

Table 2: IC₅₀ Value ABTS-reducing Activity of SEE and CGA.

| Sample | Linear Equation | R ² | IC ₅₀ (μM) | IC ₅₀ ($\mu\text{g/ml}$) |
|--------|------------------------|----------------|------------------------------------|---------------------------------------|
| SEE | $y = 0.6954x + 9.8705$ | 0.97 | - | 57.71 |
| CGA | $y = 0.8407x + 43.473$ | 0.97 | 21.90 | 7.76 |

*Linear equations, coefficient of regression (R²), and IC₅₀ of each sample were calculated. IC₅₀ of SEE was presented in $\mu\text{g/ml}$, while CGA was presented in μM and $\mu\text{g/ml}$.

The results showed that both SEE and chlorogenic acid possess high ABTS-reducing activity, with chlorogenic acid (CGA) as the highest ABTS-reducing activity value. The average percentage of ABTS-reducing activity of chlorogenic acid shown in Table 2 was higher compared to the ABTS-reducing activity of SEE. The results of ABTS-reducing activity of SEE between concentrations 1.56-50 $\mu\text{g/ml}$ was the concentration-dependent manner and chlorogenic acid compounds between concentrations

1.56-50 $\mu\text{g/ml}$ were concentration-independent manner (Figure 1). The value of IC_{50} of SEE and chlorogenic acid in reducing the ABTS free radical in Table 2 revealed that SEE has a high value of IC_{50} rather than CGA. This assured that CGA exhibited effective antioxidant activity. CGA is one of the most available phenolic acid compounds, and it is widely distributed in plants (Meng et al., 2013). The antioxidant effects of phenolic acids such as CGA has been reported in various plant extracts. Extract from the *Hypericum hircinum* L., a plant that containing CGA have been shown to have antioxidant properties that can inhibit free radicals (Mandrone et al., 2015).

Hereinafter, FRAP reducing power was assessed to indicate the efficiency of the extract to reduce the oxidized intermediates of the lipid peroxidation process.

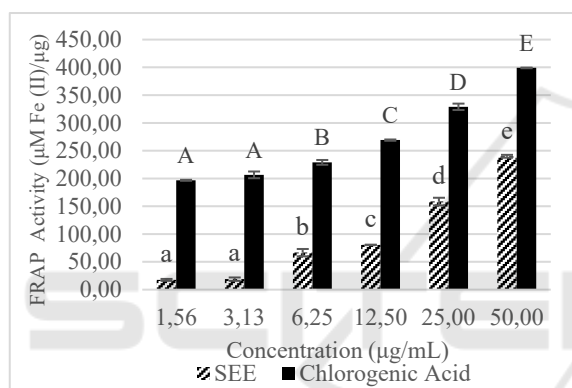


Figure 2: Effect variety concentrations of SEE, CGA toward FRAP activity.

*FRAP activity ($\mu\text{M Fe (II)/}\mu\text{g}$ sample) of SEE, CGA were were diluted in DMSO to reach the final concentration of 1.56; 1.13; 6.25; 12.50; 25.00; 50.00 ($\mu\text{g/mL}$). Different small letter (a,b,c,d,e,f) shows significant differences among concentration of SEE and different capital letter (A,B,C,D,E) among concentration of CGA toward FRAP activity based on Tukey HSD post hoc test ($p < 0.05$).

The FRAP activity in this study showed that both SEE and CGA were increased significantly between concentration 6.25-50 $\mu\text{g/ml}$ ($p < 0.05$) and showed in a concentration-dependent manner, in which higher concentration increased FRAP activity (Figure 2). The CGA indicated high FRAP activity at the highest concentration (50 $\mu\text{g/ml}$) with value ($399.21 \pm 0.59 \mu\text{M Fe (II)/}\mu\text{g}$) which indicates high antioxidant capacity, while SEE shows the lowest activity with value ($240.08 \pm 2.65 \mu\text{M Fe (II)/}\mu\text{g}$). CGA is considered as well-known antioxidant agents (Yun et al., 2012), and also known as antidiabetic, anti-obesity, anti-hypertension, and anti-inflammatory (Naveed et al., 2018).

The H_2O_2 scavenging activity of SEE and CGA of various concentrations were measured to determine the antioxidant activity.

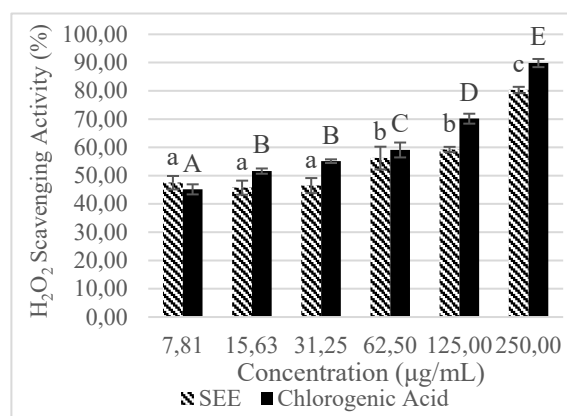


Figure 3: Effect variety concentrations of SEE, CGA toward antioxidant activities.

* H_2O_2 scavenging activity (%) of SEE, CGA were were diluted in DMSO to reach the final concentration of 7.81; 15.63; 31.25; 62.50; 125.00; 250.00 ($\mu\text{g/mL}$). Different small letter (a,b,c) shows significant differences among concentration of SEE and different capital letter (A,B,C,D,E) among concentration of CGA toward H_2O_2 scavenging activity based on Tukey HSD post hoc test ($p < 0.05$).

Both SEE and chlorogenic acid expressed high H_2O_2 scavenging activity. The highest concentration (250 $\mu\text{g/mL}$) of chlorogenic acid was slightly higher compared to the scavenging activity of SEE, however at the lowest concentration (7.81 $\mu\text{g/mL}$) SEE was slightly higher than CGA. The results of H_2O_2 scavenging activity of SEE and CGA between concentrations 7.81-250 $\mu\text{g/mL}$ was concentration-dependent manner (Figure 3). The IC_{50} value of CGA was lower (13.07 $\mu\text{g/mL}$) than the IC_{50} value produced by SEE (103.84 $\mu\text{g/mL}$) (Table 4). These results showed that the potency of CGA as an H_2O_2 scavenging agent was better than SEE. Based on *in vivo* study, SEE has potential as an antioxidant and anti-inflammatory activities through suppressed of intracellular ROS levels and decrease $\text{TNF-}\alpha$, and increase of IL-10 in lead-induced human fibroblast cells (Girsang et al., 2020). *S. zalacca* has polyphenols are protocatechuic acid and ferulic acid, both of them have the ability as H_2O_2 scavenger with value 42.25 $\mu\text{g/mL}$ and 73.37 $\mu\text{g/mL}$, respectively (Girsang et al., 2020). The antioxidant activity of phenolic compounds in *S. zalacca* depend on the amount of hydroxyl group contained in the chemical structure, that hydroxyl group will react with radical species such as hydrogen peroxide (H_2O_2) (Girsang et al., 2020).

Table 4: IC₅₀ Value of H₂O₂ Scavenging Activities of SEE and CGA.

| Sample | Linear Equation | R ² | IC ₅₀ (µM) | IC ₅₀ (µg/ml) |
|--------|----------------------|----------------|-----------------------|--------------------------|
| SEE | y = 0.1399x + 44.473 | 0.97 | - | 103.84 |
| CGA | y = 0.1716x + 47.757 | 0.98 | 36.89 | 13.07 |

*Linear equations, coefficient of regression (R²), and IC₅₀ of each sample were calculated. IC₅₀ of SEE was presented in µg/mL, while CGA was presented in µM and µg/mL

Nitric oxide (NO) is a free radical belonging to reactive nitrogen species (RNS) (Utami et al., 2018). The NO scavenging activity of SEE and CGA can be seen in Figure 4D.

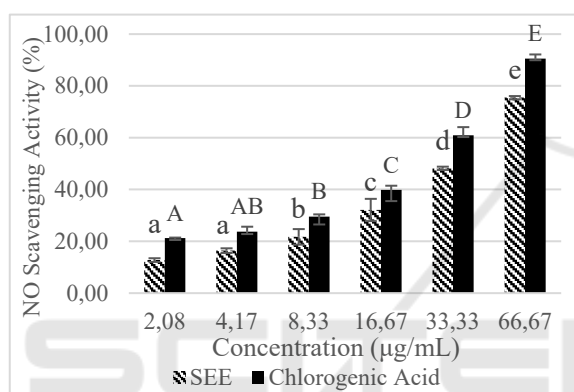


Figure 4: Effect variety concentrations of SEE, CGA toward antioxidant activities.

*NO scavenging activity (%) of SEE, CGA were were diluted in DMSO to reach the final concentration of 2.08; 4.17; 8.33; 16.67; 33.33; 66.67 (µg/mL). Different small letter (a,b,c,d,e) shows significant differences among concentration of SEE and different capital letter (A,AB,B,C,D,E) among concentration of CGA toward NO scavenging activity based on Tukey HSD post hoc test (p<0.05)

Table 5: IC₅₀ Value of NO Scavenging Activities of SEE and CGA.

| Sample | Linear Equation | R ² | IC ₅₀ (µM) | IC ₅₀ (µg/ml) |
|--------|----------------------|----------------|-----------------------|--------------------------|
| SEE | y = 1.4726x + 9.1012 | 0.99 | - | 27.77 |
| CGA | y = 1.5876x + 28.231 | 0.99 | 38.66 | 13.71 |

*Linear equations, coefficient of regression (R²), and IC₅₀ of each sample were calculated. IC₅₀ of SEE was presented in µg/mL, while CGA were presented in µM and µg/mL

SEE and CGA expressed high NO scavenging activity. The highest concentration (66.67 µg/mL) CGA was slightly higher compared to SEE. The results of NO scavenging activity of SEE and CGA with concentrations 2.08-66.67 µg/mL were in a

concentration-dependent manner (Figure 4). The IC₅₀ values showed that the IC₅₀ value of CGA was lower (27.15 µg/mL) than SEE (38.09 µg/mL) (Table 5), thus indicated CGA was better than SEE. Based on another study showed that CGA has antioxidant activity caused the hydroxyl groups that responsible as a positive group for its antioxidant properties (Naveed et al., 2018). OH group molecule has play role in ·OH radical scavenging mechanism (Tremel & Karelšmejkal, 2016)(Irwan et al., 2020).

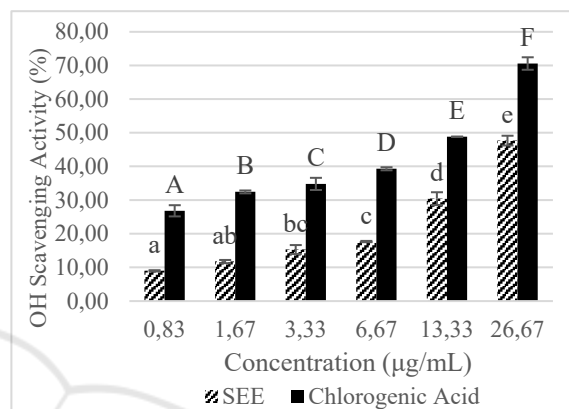


Figure 5: Effect variety concentrations of SEE, CGA toward OH Scavenging Activity.

*OH scavenging activity (%) of SEE, CGA were were diluted in DMSO to reach the final concentration of 0.83; 1.67; 3.33; 6.67; 13.33; 26.67 (µg/mL). Different small letter (a,ab,bc,c,d,e) shows significant differences among concentrations of SEE and different capital letter (A,B,C,D,E,F) among concentration of CGA toward OH scavenging activity based on Tukey HSD post hoc test (p<0.05).

Table 6: IC₅₀ Value of OH Scavenging Activities of SEE and CGA.

| Sample | Linear Equation | R ² | IC ₅₀ (µM) | IC ₅₀ (µg/ml) |
|--------|----------------------|----------------|-----------------------|--------------------------|
| SEE | y = 0.959x + 13.471 | 0.99 | - | 38.09 |
| CGA | y = 1.0826x + 20.606 | 0.99 | 76.56 | 27.15 |

*Linear equations, coefficient of regression (R²), and IC₅₀ of each sample were calculated. IC₅₀ of SEE was presented in µg/mL, while CGA were presented in µM and µg/mL

The present study has resulted, SEE and CGA expressed high OH scavenging activity. The highest concentration (26.67 µg/mL) CGA was higher compared to SEE. The results of OH scavenging activity of SEE and CGA with concentrations 0.83-6.67 µg/mL was a concentration-dependent manner (Figure 5). The IC₅₀ values showed that the IC₅₀ value of CGA was lower (13.71 µg/mL) than SEE (27.77 µg/mL) showed that CGA was more active compared

to SEE (Table 6). In Suica-Bunghez et al. (2016) study was reported that *S. zalacca* fruit and peel extract has antioxidant properties through DPPH scavenging activity with value 82.68% and 73.14% (Suica-Bunghez et al., 2016). In other side, CGA also can scavenge free radicals and promote antioxidant enzymatic activities based on *in vivo* and *in vitro* studies (Shi et al., 2016).

However, all antioxidant potent (ABTS, H₂O₂, NO, OH scavenging activity) of CGA had IC₅₀ value < 50 µL categorized as highly active, and SEE had IC₅₀ value < 50 µL for OH, NO (13.71 and 27.15 µg/mL) scavenging activities categorized highly active and the IC₅₀ value of ABTS scavenging activity of SEE 57.71 µg/mL was categorized active and H₂O₂ scavenging activity of SEE 103.84 was categorized moderate (Marjoni & Zulfisa, 2017). Antioxidant activity that is strong enough in SEE may be related to various active compounds in plants, including flavonoid and phytochemical polyphenols (Mazumdar et al., 2019). However, the factor that causes CGA to show more active in free radical scavenging activities is the number of OH groups possessed by CGA. The more hydroxyl groups possessed by active compounds affect the amount of free radicals that can be scavenged (Mathew et al., 2015).

4 CONCLUSIONS

In conclusion, *S. zalacca* peels extract (SEE) contains phenolic and flavonoid content, which is potential as an antioxidant. SEE and chlorogenic acid have an antioxidant-activities against ABTS, FRAP, H₂O₂, NO, and OH in oxidative stress parameters. Therefore, *S. zalacca* peel extract and its compound can be a potential source of antioxidants compound.

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