Journal of Natural Sciences and Mathematics Research
J. Nat. Scien. & Math. Res. Vol. 10 No. 1 (2023) 46-55
ISSN: 2460-4453 (Online); 2614-6487 (Printed)
Available online at http://journal.walisongo.ac.id/index.php/jnsmr

Antioxidant activity assay of Roselle (*Hibiscus sabdariffa*) seeds Ethanol extract with DPPH radical scavenging using UV-Vis Spectrophotometer

Muhammad Alwi Syahara [1,*], Nunuk Helilusiatiningsih[2], and Titik Irawati [2]

[1]Chemistry Department, Faculty of Agriculture, Universitas Islam Kadiri, Indonesia
[2]Agrotechnology Department, Faculty of Agriculture , Universitas Islam Kadiri, Indonesia

*Corresponding author's e-mail: alwisyahara@uniska-kediri.ac.id

ABSTRACT
Hibiscus sabdariffa (Roselle) is known to have a strong antioxidant compound. Thus, the calyx becomes the most important area to research. Another part of this plant that was starting to be known for its benefits is rosella seeds. Roselle seeds are a source of fat-soluble antioxidants. The aim of this study was to investigate the antioxidant activity of Roselle Seed Ethanol Extract (RSEE) with the DPPH (1,1-Diphenyl-2 Picrylhidrazyl) radical scavenging method. This research begins by doing the extraction of Roselle seeds using ethanol (96%) as a solvent and then assessing the antioxidant activity (Radical Scavenging Activity/RSA) of the extract, which is compared with the ascorbic acid standard at several concentrations (5-200 ppm). Antioxidant activity was assessed with DPPH Radical Scavenging using UV-Vis spectrophotometer and IC50 value parameters. The results of this study were the absorbance of DPPH from spectrophotometer at 517 nm was 0,824 (purple color). The absorbance of RSEE and ascorbic acid at 5 ppm to 200 ppm, respectively 0.523-0.124 and 0.594-0.112. The antioxidant activity (RSA) of the RSEE and ascorbic acid, respectively was 57.93% and 59.24%. The IC50 value of RSEE was 30.158 µg/ml and as comparison is ascorbic acid, the IC50 value was 26.948 µg/ml. Thus, the antioxidant activity of roselle seeds ethanol extract (RSEE) belongs to a very strong category (<50 µg /ml). This is due to the presence of several phenolic compounds and unsaturated fatty acid derivatives.

Keywords:
Antioxidants activity; DPPH Radical Scavenging; IC50; Roselle seeds; UV-Vis Spectrophotometer;

Introduction

Hibiscus sabdariffa is included in the Malvaceae family, also known as roselle, a plant that can grow in tropical and subtropical areas, such as Indonesia, India, Saudi Arabia, Malaysia, and Sudan. This plant has very useful plant parts. Rosella calyx is most known to have antioxidant, anti-inflammatory, antilipidemic, antidiabetic, antibacterial, diuretic, and anti-cholesterol properties, lowering blood pressure and digestive disorders (Fathimah et al., 2020; Ghosh et al., 2023; Singh et al., 2021; Suradji et al., 2016). It is the most researched part of the plant because of its antioxidant content (Adinda et al., 2023; Amperawati et al., 2019; Djaeni et al., 2017; Rupaddatu, 2022). Another part of this plant that was starting to be known for its benefits is rosella seeds. Several researchers have tried to use roselle seeds as an additional flour ingredient in bread (Ayo-Omogie et al., 2023; Nguyen et al., 2018; Nyam et al., 2014; Zarringhalami et al., 2021), cakes (Sayed & Mohamed, 2019; U.D. Chavan, 2017), and mayonnaise (El-Deab & Ghamry, 2017). The addition of rosella seeds can increase the protein content and food quality significantly ($p<0.05$). Previous research revealed that the protein content in rosella seeds added to cakes can replace the protein needs of vegetarians (Sayed & Mohamed, 2019).

Roselle seeds contain nutrients in the form of carbohydrates (26.92%-41.48%), fiber (15.75%-40.61%), protein (27.06%-30.40%), fat (13.09%-19.70%), the macro and micro

nutrient content in roselle seeds such as Ca, K, P, Mg, Zn, Fe, Mn, and Cu were found to have relatively high value (Ayo-Omogie et al., 2023; Ghosh et al., 2023; Phewphong et al., 2023; Sayed & Mohamed, 2019). Furthermore, roselle seeds contain oil with a content of 21.85 – 27.78%. Roselle seed oil contains high levels of essential fatty acids, including linoleic acid (39.16%), oleic acid (31.84%), palmitic acid (21.15%) (Eltayeib et al., 2014; Le et al., 2020; Mokhtari et al., 2018; Zarringhalami et al., 2021). In addition, roselle seed oil is a source of fat-soluble antioxidants, which are dominated by gamma-tocopherol (74.5%) (El-Deab & Ghamry, 2017; Nurnasari et al., 2019). Phewpong's (Phewphong et al., 2023) research noted that antioxidant activity assay of rosella seeds showed total phenol levels equivalent to gallic acid of 18.80-23.65 mg GAE/100g, with free radical inhibition of 44.70% - 56.95%. This high antioxidant activity means that rosella seeds can be used as an additional ingredient as well as a natural preservative in food (El-Deab & Ghamry, 2017; Mohd-Esa et al., 2010; Nyam et al., 2014; Sayed & Mohamed, 2019; Tounkara et al., 2014; U.D. Chavan, 2017) and to prevent cancer (U.D. Chavan, 2017).

The antioxidant activity assay of compounds in roselle seeds has been carried out using several extraction methods, including the Soxhlet method (Eltayeib et al., 2014; Hagr & Adam, 2020), the supercritical CO2 method (Naeem et al., 2019; Peng et al., 2020) and the maceration with solvents method (Cissouma et al., 2013; El-Deab & Ghamry, 2017; Mohd-Esa et al., 2010; Zarringhalami et al., 2021). The maceration method is easy to carry out because it is considered easy, and cheap, does not contain materials that easily expand in the filter fluid and is suitable for the sample. Previous research reported that the test results of 30% ethanol extract had the highest hydroxyl radical scavenging ability value compared to methanol and acetone solvents (Cissouma et al., 2013). Further research needs to be carried out regarding increasing the concentration of ethanol solvent on the results of testing the antioxidant activity of the extracted compounds.

Antioxidant activity assay can use several methods, including β-carotene bleaching method (Mohd-Esa et al., 2010), Fe^{3+} ion reduction test (FRAP) (N et al., 2022a), 2,2-azinobis 3-ethylbenzthiazoline-6-sulfonic acid radical ($ABTS^{\cdot+}$) (Zarringhalami et al., 2021), superoxide anion ($O2^{\cdot-}$) radical removal experiment (Tounkara et al., 2014), and the most widely used is the DPPH radical inhibition test method (Andzi Barhé & Feuya Tchouya, 2016; Ghosh et al., 2023; Hagr & Adam, 2020; Naeem et al., 2019; Nyam et al., 2014; Phewphong et al., 2023; Tounkara et al., 2022). Most of these methods use similar principles and techniques, namely detecting the occurrence of electronic transitions in compound molecules which are measured using an appropriate spectrophotometer.

DPPH Radical Scavenging is used fairly accurately because DPPH contains a stable radical structure. This stability is due to the steric effect that surrounds the first-order divalent N atom and the impact of the "push-pull" effect that is applied between the second-order diphenylamine group (electron donor) and picryl (electron acceptor). As a result, there is a $\pi-\pi^*$ transition which produces a fairly wide distance between the two color bands and shows a dark purple color. This color will slowly disappear when the DPPH molecule is reacted with a molecule that can donate hydrogen to become DPPH-H. The formation of hydrazine (DPPH-H) causes the distance between the two bands to disappear and is visible by changing the color of the solution from purple to pale yellow. The intensity of the color change from this reaction can be easily recorded by the instrument spectrophotometer UV- Vis (Gulcin & Alwasel, 2023). The reaction that occurs between DPPH radicals and antioxidant compounds can take place quickly (± 30-45 minutes) at room temperature. Other methods require quite a long time to react, such as ABTS requiring an incubation time of 12-16 hours, while the FRAP method requires an incubation time of 30 minutes but must be carried out at a temperature of 37-50°C (Shah & Modi, 2015). This causes many researchers to prefer using the DPPH method for testing antioxidant activity because it is considered one of the easiest, fastest and most accurate methods (Gulcin & Alwasel, 2023; Phewphong et al., 2023). Moreover, it is most generally used for in vitro samples, and also is a simple method, fast and uses little chemicals and samples, low cost, reproducibility, as well as automation possibilities (Fardani et al., 2023; Firmansyah et al., 2017; Munteanu & Apetrei, 2021)

The dark purple color that appears on the DPPH radical molecule indicates that there is a maximum absorption at 517 nm. This absorption will decrease along with the reduction in the number of radicals in DPPH by antioxidants. This absorption intensity (absorbance) will be the activity parameter antioxidant or inhibition radical free. Determination of activity antioxidants needs a different concentration of antioxidants that captures 50% of the initial DPPH radicals in certain time intervals. This concentration is called as "EC50", abbreviation of "concentration efficient " or sometimes as "IC50", abbreviation of " inhibitory concentration ". The accepted term, in a way wide, is "IC50" to show the practicality of testing antioxidants using DPPH Radical Scavenging (Gulcin & Alwasel, 2023) . This research aims to determine the antioxidant activity of roselle seed ethanol extract with the DPPH Radical Scavenging using a UV-Vis spectrophotometer.

Methods

This research is experimental research. The materials used in the research were roselle seeds taken from Blitar, ascorbic acid (Merck), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich), distilled water, ethanol 96% (Merck), Whattman filter paper no. 1

Work procedures

Roselle Seed Preparation

Rosella seeds are removed from impurities by washing and air drying. After that, the rosella seeds are crushed and filtered using a 50-mesh sieve.

Roselle Seed Extraction

Rosella seeds were extracted using 96% ethanol. A total of 5 grams of rosella seeds were added in 100 ml ethanol 96%. The mixture is heated at 45°C using a hot plate and stirred with a magnet stirrer at 500 rpm for 2 hours to extract the polyphenol. The extract obtained is centrifuged for 20 minutes and filtered using paper strain Whattman no. 1. Supernatant is added to a rotary evaporator to obtain a dry extract of roselle seed ethanol extract (RSEE) (Singh et al., 2021). Dried extract dissolved in 10 ml into a volume flask to get 1000 ppm of RSEE. The solution was diluted to several concentrations of 5, 10, 25, 50, 100, and 200 ppm.

Preparation of DPPH Solution

DPPH 0.004% solution was made by weighing 3.95 mg DPPH and dissolved until homogeneous in 100 mL ethanol in a volumetric flask. The solution was then protected from light using aluminum foil (Agustiarini & Wijaya, 2022; Gulcin & Alwasel, 2023)

Antioxidant Activity Assay

The antioxidant activity of each extract was measured using the modified method, as reported by Mishra. A total of 300 µL sample and 300 µL DPPH solution (0.004% w/v in ethanol) is included to in cuvette. After incubating in the dark for 45 minutes, the next uptake solution was recorded at 517 nm with UV-Vis Spectrophotometer (Mishra et al., 2012; Sayed & Mohamed, 2019). Radical Scavenging Activity (RSA)/ inhibition of free radical DPPH (%) was calculated using **Error! Reference source not found.**.

$$RSA(\%) = \frac{Ac - As}{Ac}\ x\ 100\% \qquad (1)$$

Where RSA (%) was Radical Scavenging Activity/ Inhibition Activity, Ac was Absorbance of control, As was Absorbance of sample.

From the RSA/inhibition of DPPH graph versus extract concentration, the extract concentration that has the ability to 50% radical scavenging activity/inhibition of radical was calculated. The incline of the line curve enables the IC50 of the extract can be determined (Andzi Barhé & Feuya Tchouya, 2016; Keyata et al., 2023).

Making comparison solution

Ascorbic acid concentration of 1000 ppm was made with 1 mg of acid ascorbate, dissolved in 10 mL of distilled water, and then shaken until homogeneous. Then, the solution was diluted to several concentrations of 5, 10, 25, 50, 100, and 200 ppm. For every concentration, take 2 mL and add 0.004% (40 ppm) DPPH solution 2ml and shake until homogeneous and incubated at the temperature room for 45 minutes. Absorption from solution measured in wave-length 517 nm (Agustiarini & Wijaya, 2022).

Results and Discussions

Antioxidant Activity Assay

The results of DPPH 40 ppm in UV- Vis spectrophotometer show an absorbance of 0.824 at 517 nm. This result is in accordance with several studies that also showed absorption at a wavelength of 517 nm (Agustiarini & Wijaya, 2022; Mishra et al., 2012; Sayed & Mohamed, 2019). Furthermore, it is appropriate to Gulcin and Alwasel's statement that theoretical absorbance on DPPH molecules happens at a wavelength of 517 nm (Gulcin & Alwasel, 2023). DPPH is radical, which is stable in solution and looks colored purple when absorbed at 517 nm in ethanol. This assessment shows that DPPH absorbs the reduction molecule, that is, atom hydrogen (H), from the antioxidant molecule, so that there is the reduction of DPPH to DPPH-H, converting color from purple to pale yellow, all at once lower uptake at 517 nm. The color change is monitored in a way spectrophotometry and used to determine antioxidant characteristics (Gulcin & Alwasel, 2023; Mishra et al., 2012).

All sample extracts (5, 10, 25, 50, 100, and 200 ppm) were evaluated for antioxidant activity with the inhibition radical DPPH test, and ascorbic acid was used as a comparison. The antioxidant ability of ascorbic acid has been used as a comparison of previous research (Agustiarini & Wijaya, 2022; Andzi Barhé & Feuya Tchouya, 2016; Fardani et al., 2023; Horozić et al., 2023; Iqbal et al., 2021), this because ascorbic acid has free hydroxyl group which role as antioxidant, capable catch various free extracellular radical and preventing chain reactions, as well own polyhydroxy group which increase the antioxidant activity (Iqbal et al., 2021). Moreover, a comparison with ascorbic acid done because in roselle seed there is found ascorbic acid around 35.91 - 38.35 mg/100 g between other organic acids (Da-Costa-Rocha et al., 2014; Ghosh et al., 2023).

Table 1. Test Results of Free Radical Scavenging Activity/Inhibition

Sample	Concentration (ppm)	Absorption (absorbance)	RSA/Inhibition (%)
RSEE*	5	0.523	36,529
	10	0.470	42,961
	25	0.399	51,577
	50	0.312	62,135
	100	0.252	69,417
	200	0.124	84,951
Ascorbic Acid**	5	0.594	37,539
	10	0.531	44,164
	25	0.458	51,840
	50	0.369	61,198
	100	0.262	72,450
	200	0.112	88,222

*Standard Deviation of RSA = 17.90099
**Standard Deviation of RSA = 18.82184

The assessment of antioxidant activity from rosella seed ethanol extract (RSEE) was conducted after reacting with 40 ppm DPPH solution and incubating for 45 minutes, then

measuring its absorbance at 517 nm wavelength. It is incubated for 45 minutes so that DPPH reacts with antioxidant compounds completely and is protected from light, which can interfere with the reaction process (Gulcin & Alwasel, 2023). The results of the assessment were that the absorbance (Table 1) at a concentration of 5 ppm was 0.523; at 10 ppm, it was obtained at 0.470 and decreased as the antioxidant concentration increased, as in Figure 1. The absorbance at 100 ppm was obtained at 0.252, and the lowest absorbance obtained at a concentration of 200 ppm was 0.124. This decrease in absorbance indicates a reaction between DPPH and higher levels of antioxidant compounds. The higher antioxidant concentration will be marked with a change in color from purple to pale, as stated in another previous research (Agustiarini & Wijaya, 2022; Gulcin & Alwasel, 2023; Mishra et al., 2012), as in seen on Figure *2*. This reaction occurs when the DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule (N et al., 2022b).

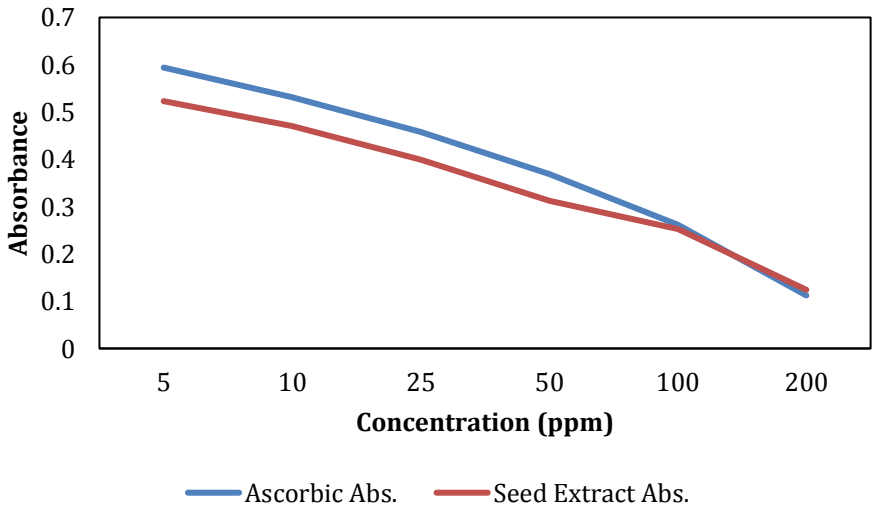

Figure 1. Absorbance curve of Ascorbic Acid and RSEE

The results of the ascorbic acid samples that were used as a comparison show that the highest absorbance at a concentration of 5 ppm is 0.594, and the lowest concentration at 200 ppm is 0.112, as seen in Figure 1. This decrease in absorption proves that ascorbic acid has good antioxidant activity. Ascorbic acid is considered a good standard because of its higher DPPH radicals inhibition efficiency (IC50 value is 0.03 mg/mL) (Horozić et al., 2023).

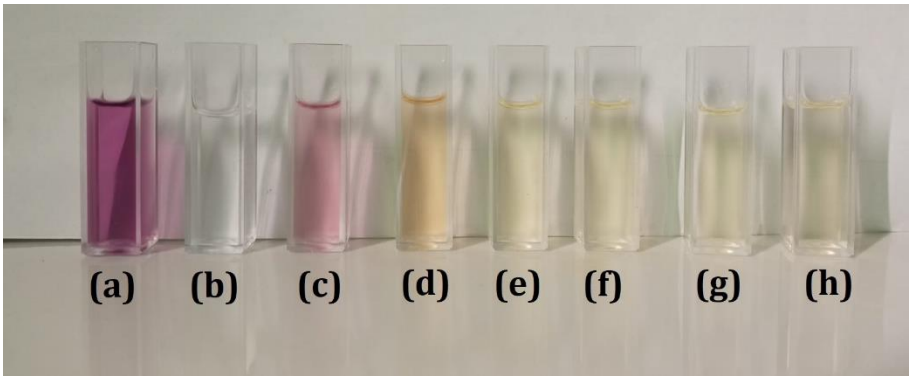

Figure 2. Results of changes in DPPH molecules and compounds antioxidant . From left (a) DPPH 40 ppm, (b) ethanol, (c) DPPH + 5 ppm antioxidant, (d) DPPH + 10 ppm antioxidant, (e) DPPH + 25 ppm antioxidant , (f) DPPH + 50 ppm antioxidant , (g) DPPH + 100 ppm antioxidant , (h) DPPH + 200 ppm antioxidant

Meanwhile, the antioxidant activity in the form of radical scavenging activity (RSA) of DPPH on RSEE as is in Figure 2. Obtained concentration of 5 ppm is 36.529%, 10 ppm is 42.96% and increase along with enhancement concentration with radical scavenging at 100 ppm was 69,417%. The highest RSA at 200 ppm was 84.95%. From the RSA, the average data showed that the antioxidant activity of RSEE was 57.93%. This shows that the RSEE has good activity in inhibiting free radicals. These results are higher than the results of Phewpong's, et al which showed that crude rosella seed extract had antioxidant activity of around 46.28% (Phewphong et al., 2023). Mean-while, according to Ghosh, et al. Rosella seed has antioxidant activity between 56.44-63.82% (Ghosh et al., 2023). This difference is possible due to the origin of the seeds and different environmental conditions, as previous research which compare results extracts from various areas show results vary (Le et al., 2020; Phewphong et al., 2023).

Meanwhile, in the comparison sample of ascorbic acid, the lowest RSA was obtained at 37.539% for a concentration of 5 ppm, and the increase, like the value of the highest RSA, was 88.222% at a concentration of 200 ppm. So, the antioxidant activity of ascorbic acid was 59.24%. This is in line with the results obtained on RSEE samples, so it can be concluded that the antioxidant capacity of RSEE has antioxidant activity similar to ascorbic acid. Ascorbic acid is known as a compound that has many hydroxyl groups (Iqbal et al., 2021). It proved that RSEE has many compounds that consist of hydroxyl groups that will act as a scavenger of various free radicals.

IC50 Parameter

The test results can seen in Table 1; from these data, the percentage of the radical scavenging and a linear line curve were made, so the equation line y = 0.2275x + 43.139 was obtained. As seen in Figure 3, there is a good linear connection (R^2 = 0.9015) between the free radical scavenging rate and con-centration of roselle seed extract 5-200 µg / mL (heavy dry seed base). It showed that the R^2 score is close to or equal to 1, so the regression analysis is considered to be the most dependable. A number with a range of 0 to 1 that shows how well the approximate value for the regression analysis matches the real data is called the coefficient of determination (Firmansyah et al., 2017).

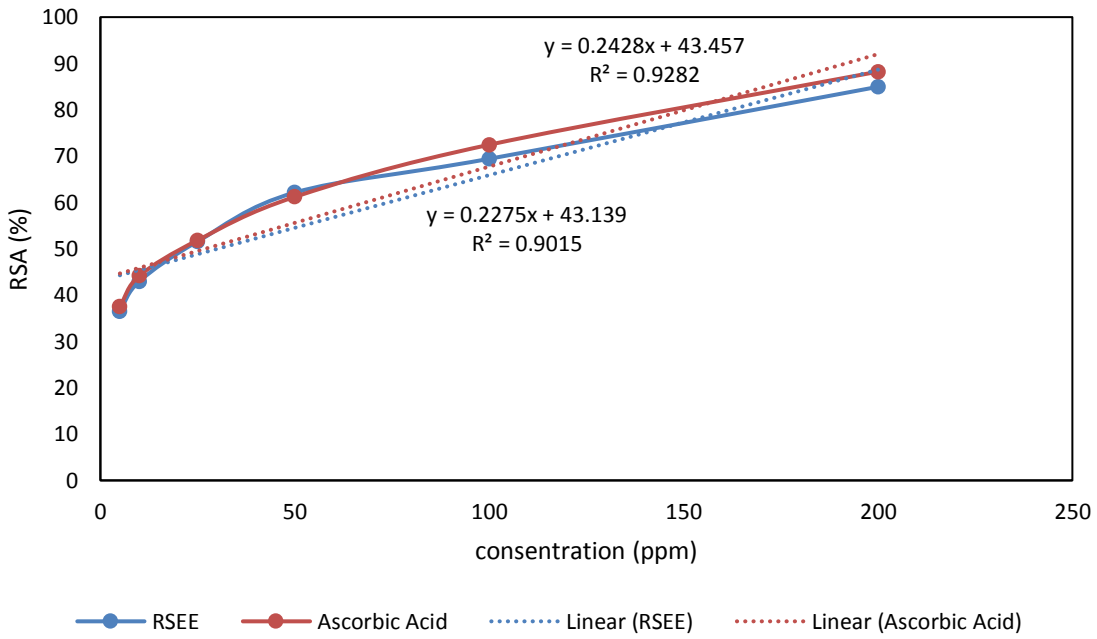

Figure 3. Regression linear curve of RSEE and Ascorbic acid

Next , antioxidant activity can determined in a significant way with calculation IC50 parameters. IC50 is a number that shows the sample concentration that is able to inhibit the oxidation process by 50%. The lower the IC50 value, the higher the antioxidant activity of the sample. Antioxidant activity was very strong compound category if the IC50 value was less than 50 µg /ml, strong category if the value was 50–100 µg /ml, moderate category if the value was 100–150 µg /ml, weak category if the value is 150-200 µg /ml and is very weak category if worth more than 200 µg /ml (Fardani et al., 2023). Based on data obtained from the antioxidant activity (RSA) of RSEE, the line equation y = 0.2275x + 43.139 (R^2 = 0.9015) was obtained, while the line equation for ascorbic acid was y = 0.2428x + 43.457 (R^2 = 0.9282)(Figure *3*). Calculation results of IC50 value from the line equation obtained IC50 of RSEE amounted to 30.158 µg / ml. Whereas, as a comparison, in ascorbic acid, the IC50 value was 26.948 µg /ml. This shows that both samples have very strong antioxidant activity (<50 µg/ml).

The high antioxidant activity is also influenced by the content of phenolic compounds contained therein such as anthocyanidins, phenolic acids, chlorogenic acid, caffeic acid, routine and flavonoids (Ghosh et al., 2023; Horozić et al., 2023; Mohd-Esa et al., 2010). According to previous studi, total phenol contained in Roselle found approx. (39.47–37.35 mg/100 g)(Ghosh et al., 2023). Phenolic compounds are found in plants, and their antioxidant activity is mainly due to their redox properties, which act as reducing agents and hydrogen donors. Phenol will reduce and stabilize the radicals by donating the hydrogen. The greater the phenolic content, the greater the antioxidant activity (Sa'adah et al., 2023). So, the reaction of the DPPH radical atom with a suitable reducing agent will result in a color change, and the solution loses some of its color due to electron pairing with the hydrogen donor (Hadad & Husni, 2019). According to previous research, another chemical compound that contributes to antioxidant activity on roselle seed is the unsaturated fatty acid derivatives (linoleic acid, oleic acid, and palmitic acid). These fatty acid esters are involved in human physiology and pathology and play a role in wound healing (Hagr & Adam, 2020; Mokhtari et al., 2018).

These results indicate that roselle seeds can be considered as a potential source of antioxidants for the food and beverages industry also other industries. Antioxidants are known as substances that have the ability to counteract the deleterious consequences of free radicals, which arise from oxidative metabolism or reactive oxygen species. Even more, natural antioxidant has the ability to obstruct degenerative disease, and obstruct lipid peroxide in food as a treatment for cancer and immune system disorders (Bangalino et al., 2017; Syafa'atun & Marisa, 2020). Further research is needed regarding antioxidant mechanisms and evaluation of the safety of roselle seeds. It is necessary to compare the extracts with various extraction methods to determine the content of rosella seed oil.

Conclusion

The result of this research was the absorbance of RSEE at 5 ppm to 200 ppm, respectively 0.523-0.124. The antioxidant activity (RSA) of the RSEE was 57.93%. The IC50 value of RSEE was 30.158 µg/ml. In the comparison sample, the absorbance of ascorbic acid at 5 ppm to 200 ppm, respectively, 0.594-0.112. The antioxidant activity (RSA) of ascorbic acid was 59.24%. And the IC50 value of ascorbic acid was 26.948 µg/ml. The antioxidant activity of roselle seeds ethanol extract (RSEE) same as the ascorbic acid belong to very strong category (<50 µg /ml). This high antioxidant activity value is obtained from the phenolic compounds and the unsaturated fatty acid derivatives contained therein, so roselle seeds can be considered a potential source of antioxidants for the food and beverages industry and other industries. Further research is needed to determine the chemical compound of rosella seed oil from various extraction methods.

Acknowledgments

We would like to thank all parties and the Faculty of Agriculture UNISKA for their valuable support in this research. We also thank the reviewers for their valuable advice for this article.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

Agustiarini, V., & Wijaya, D. P. (2022). Uji Aktivitas Antioksidan Ekstrak Etanol-Air (1:1) Bunga Rosella (Hibiscus Sabdariffa L.) Dengan Metode DPPH (1,1-Difenil-2-Pikrilhidrazil). *Jurnal Penelitian Sains*, *24*(1), Article 1. Https://Doi.Org/10.56064/Jps.V24i1.679

Andzi Barhé, T., & Feuya Tchouya, G. R. (2016). Comparative Study Of The Anti-Oxidant Activity Of The Total Polyphenols Extracted From *Hibiscus Sabdariffa* L., *Glycine Max* L. *Merr.*, Yellow Tea And Red Wine Through Reaction With DPPH Free Radicals. *Arabian Journal Of Chemistry*, *9*(1), 1–8. Https://Doi.Org/10.1016/J.Arabjc.2014.11.048

Ayo-Omogie, H. N., Oluwajuyitan, T. D., Okorie, E. I., Ojo, O. O., & Awosanmi, N. D. (2023). A Study On The Use Of Sorrel Seed Flour (Hibiscus Sabdariffa) For Improving Functionality Of Wheat Flour Bread. *Heliyon*, *9*(7), E18142. Https://Doi.Org/10.1016/J.Heliyon.2023.E18142

Bangalino, W. W., Lestario, L. N., & Riyanto, C. A. (2017). The Influence Of Alginate And Cacl2 Concentration Towards Betacyanin Content And Antioxidant Activity Of Red Dragon Fruit (Hylocereus Polyrhizus) Restructuring Product. *Journal Of Natural Sciences And Mathematics Research*, *2*(2), Article 2.

Cissouma, A., Tounkara, F., Nikoo, M., Na, Y., & Xu, X. (2013). Physico Chemical Properties And Antioxidant Activity Of Roselle Seed Extracts. *Advance Journal Of Food Science And Technology*, *5*, 1483–1489. Https://Doi.Org/10.19026/Ajfst.5.3371

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). *Hibiscus Sabdariffa* L. – A Phytochemical And Pharmacological Review. *Food Chemistry*, *165*, 424–443. Https://Doi.Org/10.1016/J.Foodchem.2014.05.002

El-Deab, S., & Ghamry, H. (2017). Nutritional Evaluation Of Roselle Seeds Oil And Production Of Mayonnaise. *International Journal Of Food Science And Nutrition Engineering*, *7*, 32–37. Https://Doi.Org/10.5923/J.Food.20170702.02

Eltayeib, A. A., Elaziz, A. A., & Department Of Chemistry, Faculty Of Science, University Of Kordofan, Elobeid, Sudan. (2014). Physicochemical Properties Of Roselle (Hibiscus Sabdariffa L.) Seeds Oil (Elrahad-1) In North Kordofan, Sudan. *Journal Of Scientific And Innovative Research*, *3*(6), 578–582. Https://Doi.Org/10.31254/Jsir.2014.3606

Fardani, R., Rodiatullah, R., Halid, I., & Atfal, B. (2023). Uji Aktivitas Antioksidan Ekstrak Bunga Rosella (Hibiscus Sabdariffa) Menggunakan Metode 1,1-Difenil-2-Pikrilhidrazyl (DPPH). *JSN : Jurnal Sains Natural*, *1*(4), Article 4. Https://Doi.Org/10.35746/Jsn.V1i4.411

Fathimah, R. N., Setyaningsih, W., Carrera, C., & Palma, M. (2020). Microwave-Assisted Extraction Of Phenolics From Hibiscus Sabdariffa Flowers: Method Development And Validation. *Proceedings*, *70*(1), Article 1. Https://Doi.Org/10.3390/Foods_2020-07681

Firmansyah, S. B., Firmansyah, R. A., & Hayati, N. (2017). Antioxidant Activity And Antibacterial Seaweed Methanol Extract (Sargassum Duplicatum J. Agardh) And Its Potential As A Natural Preservative Alternative To Salted Eggs. *Journal Of Natural Sciences And Mathematics Research*, *2*(1), Article 1.

Ghosh, P., Priyadarshini, S., Munshi, M., Beula, P., Atthoti, L., Kruthi, G. S., Sravan, B. S., Irshaan, S., Rana, S. S., & Abdullah, S. (2023). Physicochemical Characterization And Mass Modelling Of Roselle (Hibiscus Sabdariffa L.) Calyx And Seeds. *Food Chemistry Advances*, *3*, 100400. Https://Doi.Org/10.1016/J.Focha.2023.100400

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, *11*(8), Article 8. Https://Doi.Org/10.3390/Pr11082248

Hadad, N. D., & Husni, P. (2019). Review: Penentuan Kandungan Senyawa Antioksidan Dalam Rosella (Hibiscus Sabdariffa L.). *Farmaka*, *17*(1), Article 1. Https://Doi.Org/10.24198/Jf.V17i1.22148

Hagr, T., & Adam, I. (2020). Phytochemical Analysis, Antibacterial And Antioxidant Activities Of Essential Oil From Hibiscus Sabdariffa (L) Seeds, (Sudanese Karkadi). *Progress In Chemical And Biochemical Research*, *3*(3), 194–201. Https://Doi.Org/10.33945/SAMI/PCBR.2020.3.2

Horozić, E., Kolarević, L., Bajić, M., Alić, L., Babić, S., & Ahmetašević, E. (2023). Comparative Study Of Antioxidant Capacity, Polyphenol And Flavonoid Content Of Water, Ethanol And Water-Ethanol Hibiscus Extracts. *European Journal Of Advanced Chemistry Research*, *4*(2), Article 2. Https://Doi.Org/10.24018/Ejchem.2023.4.2.130

Iqbal, M., Aliyah, A., & Alam, G. (2021). Efek Antioksidan Ekstrak Bunga Rosela (Hibiscus Sabdariffa L.) Dengan Metode Spektrofotometri UV-Vis. *Jurnal Farmasi Dan Bahan Alam : FARBAL*, *9*(1), Article 1.

Keyata, E. O., Tola, Y. B., Bultosa, G., & Forsido, S. F. (2023). Bioactive Compounds, Antioxidant Capacity, Functional And Sensory Properties Of Optimized Complementary Weaning Flour Processed From Sorghum, Soybean, And Karkade (*Hibiscus Sabdariffa* L.) Seeds. *Scientific African*, *19*, E01457. Https://Doi.Org/10.1016/J.Sciaf.2022.E01457

Le, B. A., Okitsu, K., Imamura, K., Takenaka, N., & Maeda, Y. (2020). Ultrasound Assisted Cascade Extraction Of Oil, Vitamin E, And Saccharides From Roselle (Hibiscus Sabdariffa L.) Seeds. *Analytical Sciences*, *36*(9), 1091–1097. Https://Doi.Org/10.2116/Analsci.20P073

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation Of Antiradical Properties Of Antioxidants Using DPPH Assay: A Critical Review And Results. *Food Chemistry*, *130*(4), 1036–1043. Https://Doi.Org/10.1016/J.Foodchem.2011.07.127

Mohd-Esa, N., Hern, F. S., Ismail, A., & Yee, C. L. (2010). Antioxidant Activity In Different Parts Of Roselle (Hibiscus Sabdariffa L.) Extracts And Potential Exploitation Of The Seeds. *Food Chemistry*, *122*(4), 1055–1060. Https://Doi.Org/10.1016/J.Foodchem.2010.03.074

Mokhtari, Z., Zarringhalami, S., & Ganjloo, A. (2018). Evaluation Of Chemical, Nutritional And Antioxidant Characteristics Of Roselle (Hibiscus Sabdariffa L.) Seed. *Nutrition And Food Sciences Research*, *5*(1), 41–46. Https://Doi.Org/10.29252/Nfsr.5.1.41

Munteanu, I. G., & Apetrei, C. (2021). Analytical Methods Used In Determining Antioxidant Activity: A Review. *International Journal Of Molecular Sciences*, *22*(7), Article 7. Https://Doi.Org/10.3390/Ijms22073380

N, S., Burle, S. S., Kosalge, S. B., & Bansode, M. N. (2022a). Antimicrobial And Antioxidant Activity Of Hibiscus Sabdariffa. Linn (Roselle). *International Journal Of Pharmacy Research & Technology (IJPRT)*, *12*(1), Article 1. Https://Doi.Org/10.31838/Ijprt/12.01.04

N, S., Burle, S. S., Kosalge, S. B., & Bansode, M. N. (2022b). Antimicrobial And Antioxidant Activity Of Hibiscus Sabdariffa. Linn (Roselle). *International Journal Of Pharmacy Research & Technology (IJPRT)*, *12*(1), Article 1. Https://Doi.Org/10.31838/Ijprt/12.01.04

Naeem, M. A., Zahran, H. A., & Hassanein, M. M. M. (2019). Evaluation Of Green Extraction Methods On The Chemical And Nutritional Aspects Of Roselle Seed (Hibiscus Sabdariffa L.) Oil. *OCL*, *26*, 33. Https://Doi.Org/10.1051/Ocl/2019030

Nguyen, N. T. T., Le, H. A. V., Pham, D. A., & Tran, T. N. Y. (2018). Evaluation Of Physical, Nutritional And Sensorial Properties Cookie Supplied With Hibiscus Sabdariffa L. Seed Powder (Without Shell). *International Food Research Journal*, *25*, 1281–1287.

Nurnasari, E., Anggraeni, T. D. A., & Nurindah, N. (2019). Profil Minyak Biji Dari Empat Varietas Rosela Herbal (Hisbiscus Sabdariffa Var. Sabdariffa) Indonesia. *Buletin Tanaman Tembakau, Serat & Minyak Industri*, *11*(1), 8. Https://Doi.Org/10.21082/Btsm.V11n1.2019.8-15

Nyam, K.-L., Leao, S.-Y., Tan, C.-P., & Long, K. (2014). Functional Properties Of Roselle (Hibiscus Sabdariffa L.) Seed And Its Application As Bakery Product. *Journal Of Food Science And Technology*, *51*(12), 3830–3837. Https://Doi.Org/10.1007/S13197-012-0902-X

Peng, W. L., Mohd-Nasir, H., Setapar, S. H. M., Ahmad, A., & Lokhat, D. (2020). Optimization Of Process Variables Using Response Surface Methodology For Tocopherol Extraction From Roselle Seed Oil By Supercritical Carbon Dioxide. *Industrial Crops And Products*, *143*, 111886. Https://Doi.Org/10.1016/J.Indcrop.2019.111886

Phewphong, S., Roschat, W., Namwongsa, K., Wonam, A., Kaisri, T., Duangpakdee, P., Leelatam, T., Moonsin, P., & Promarak, V. (2023). Evaluation Of The Nutritional, Minerals, And Antioxidant Potential Of Roselle (Hibiscus Sabdariffa Linn.) Seeds From Roi Et Province In The Northeastern Region Of Thailand. *Trends In Sciences*, *20*(6), Article 6. Https://Doi.Org/10.48048/Tis.2023.6664

Sa'adah, S. M., Putri, F. R., Ibtisam, A. A., Arrohmah, R. S., & Fitriyah, F. (2023). Phytochemical Analysis Of Secondary Metabolite Compounds Of Pandanwangi Leaf Extract (Pandanus Amaryllifolius). *Journal Of Natural Sciences And Mathematics Research*, *9*(2), Article 2.

Sayed, E., & Mohamed, W. (2019). Nutritional Evaluation Of Roselle (Hibiscus Sabdariffa L.) And Its Application In Biscuit Supplementation. *Arab Universities Journal Of Agricultural Sciences*, *27*(5), 2563–2572. Https://Doi.Org/10.21608/Ajs.2020.20818.1138

Shah, P., & Modi, H. (2015). *Comparative Study Of DPPH, ABTS And FRAP Assays For Determination Of Antioxidant Activity*.

Singh, M., Thrimawithana, T., Shukla, R., & Adhikari, B. (2021). Extraction And Characterization Of Polyphenolic Compounds And Potassium Hydroxycitrate From Hibiscus Sabdariffa. *Future Foods*, *4*, 100087. Https://Doi.Org/10.1016/J.Fufo.2021.100087

Suradji, S. I., Najib, A., & Ahmad, A. R. (2016). Studi Komparasi Kadar Flavonoid Total Pada Bunga Rosella Merah (Hibiscus Sabdariffa L.) Asal Kabupaten Luwu Utara Provinsi Sulawesi Selatan Dan Kabupaten Kediri Provinsi Jawa Timur. *Jurnal Fitofarmaka Indonesia*, *3*(2), Article 2. Https://Doi.Org/10.33096/Jffi.V3i2.219

Syafa'atun, T., & Marisa, N. (2020). Antioxidant And Organoleptic Test Of Soursop (Annona Muricata Linn.) Leaf Tea In Herbal Tea Production Process. *Journal Of Natural Sciences And Mathematics Research*, *6*(2), Article 2.

Tounkara, F., Bashari, M., Le, G.-W., & Shi, Y.-H. (2014). Antioxidant Activities Of Roselle (Hibiscus Sabdariffa L.) Seed Protein Hydrolysate And Its Derived Peptide Fractions. *International Journal Of Food Properties*, *17*(9), 1998–2011. Https://Doi.Org/10.1080/10942912.2013.779700

Tounkara, F., Togola, I., Konare, M. A., & Fane, M. M. (2022). Identification Of Antioxidative Peptides From Roselle (*Hibiscus Sabdariffa* Linn) Seeds Protein Hydrolysates. *Journal Of Food And Nutrition Sciences*, *10*(4), Article 4. Https://Doi.Org/10.11648/J.Jfns.20221004.11

U.D. Chavan, K. B. R. (2017). Antioxidant Activity And Nutritional Value Of Roselle Seeds Flour. *International Journal Of Current Microbiology And Applied Sciences*, *6*(4), 2654–2663. Https://Doi.Org/10.20546/Ijcmas.2017.604.309

Zarringhalami, S., Ganjloo, A., & Mokhtari Nasrabadi, Z. (2021). Optimization Xanthan Gum, Roselle Seed And Egg White Powders Levels Based On Textural And Sensory Properties Of Gluten-Free Rice Bread. *Journal Of Food Science And Technology*, *58*(3), 1124–1131. Https://Doi.Org/10.1007/S13197-020-04626-9