

Characterization and Antioxidant Activity Assay of Essential Oil Parts of Rosella (*Hibiscus sabdariffa* L.)

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ABSTRACT

Rosella (Hibiscus sabdariffa L.) is known as a plant whose calyx contain high levels of antioxidant bioactive compounds and good inhibitory power against free radicals. However, other parts of the rosella plant have not yet been utilized for their antioxidant content and activity. The aim of this research is to compare the profiles of essential oils of parts of the rosella plant (seeds, leaves and flower petals) and their antioxidant activity. This research method begins with the process of extracting essential oils using the water-steam distillation method. Furthermore, the profile of the essential oil is known from the results of yield calculations and characterization using the GC-MS instrument. The antioxidant activity was tested using the DPPH method. The yield of essential oils obtained was 0.0107% for seeds, 0.0087% for leaves and 0.0136% for flower petals. GC-MS characterization shows that the most abundant chemical components contained in the essential oils of seeds, leaves, and flower petals are fatty acid compounds (2-propanoic acid, butanoic acid, hexanoic acid) and esters (neopentyl isobutyrate). The results of the antioxidant activity test showed the IC₅₀ value for each essential oil, namely, seeds: 30.15 µg/ml, leaves: 171.27 µg/ml and flower petals: 126.58 µg/ml. The compound characteristics obtained showed the best results for rosella seed essential oil, this was supported by the fairly high antioxidant activity test results IC₅₀ <50 µg/ml

1. INTRODUCTION

Rosella or roselle (*Hibiscus sabdariffa* L.) can grow well in tropical climates such as in Blitar and Kediri Regencies. This plant has flower petals or calyx which are known to have potential as an antioxidant ingredient which is widely used in health drinks (Nurnasari & Khuluq, 2018). This is obtained mainly from flower petals or calyx which are often used as natural dyes that are rich in antioxidants and function as free radical scavengers (Alshami & Alharbi, 2014). Rosella flower petals are known to contain total phenolic compounds, flavonoid, and anthocyanin which are equivalent to 10.44-19.75% gallic acid, 5.8-42.57 mg catechin, and 4.45-5.39 mg cyanidin-3- glucoside so that it can be a strong antioxidant and scavenger of free radicals. Apart from that, there are also organic acid compounds, such as hibiscus acid, hydroxycitric, ascorbic, citric and tartaric acids (Singh *et al.*, 2021). Based on the facts of its content, many people use rosella flower petals extract as an anti-bacterial, antioxidant, anti-inflammatory, herbal medicine in the treatment of colds, coughs, hypercholesterolemia, hypertension, diabetes and digestive problems (Malinda & Syakdani, 2020; Nurnasari & Khuluq, 2018). The color contained in rosella flower petals is widely used as a natural coloring in food (Fauziati & Sampepana, 2016; Handarini, 2016) and as an additive to inhibit premature aging (Gustiarani & Triastuti, 2021). Several previous studies used only flower petals, while other parts of the plant such as roots, stems, seeds and leaves have not been used.

Most rosella farmers process the harvest of rosella flower petals into dry products for steeping tea, syrup and jam (Setyawan *et al.*, 2019), while other plant parts (roots, stems, seeds and leaves) are discarded or become waste. Rosella waste needs to be researched so that it can be reused. Previous research (Helilusiatiningsih *et al.*, 2023) showed that the results of the hedonic test for rosella flower petals essential oil were 60% highly liked by the panelists, 40% liked it, while in the aroma test the oil was rated at around 55% as very liked and 45% liked the color. Essential oil extraction can be done in various ways or methods. Putri & Zamrudy (2021) have succeeded in comparing the results of patchouli essential oil using the water distillation, water-steam distillation and water bubble distillation methods, and it was found that the oil yield produced by the water-steam distillation method was higher than the other distillation method used. This is supported by the opinion (Cahyati *et al.*, 2016), that the yield of essential oils from water-steam distillation (2.38%) is higher than water distillation (0.35-0.37%) and steam distillation (0.6 %). Pratimasari (2023) also showed that the chemical content of palmarosa essential oil resulting from steam-water distillation is higher than that of water distillation.

Based on the explanation above, extracting essential oils from other parts of the rosella plant is expected to produce good results. Therefore, the aim of this research is to determine the comparison of yields, the composition of chemical compounds contained in essential oils and the antioxidant activity of parts of the rosella plant including flower petals, leaves and seeds.

2. MATERIALS AND METHODS

The materials used include leaves, seeds and rosella flower petals purchased from CV Huday, Blitar Regency. Aquades, alcohol, chemicals supporting chemical analysis were obtained from the Chemistry Lab. of Universitas Islam Kadiri (UNISKA), Kediri. The tools used include steam distillation equipment, UV-Vis spectrophotometer, analytical balance, computer, measuring cup, bottles, scissors, knives, stove, LPG, Erlenmeyer, magnetic stirrer, GC-MS analyzer, essential oil bottles.

2.1. Time and Place of Research

The research time is from August to December 2023. This research activity is carried out in collaboration with partners who can help to obtain oil and analyze the data, namely the essential and biochemical laboratory located on the Brawijaya University Malang campus. Antioxidant activity testing was carried out by UNISKA, Kediri.

2.2. Research Procedures

2.2.1. Essential Oil Extraction using the Water-Steam Distillation Method

Each ingredient (seeds, leaves and rosella flower petals) is weighed as much as 1 kg of dry material, then put into the distillator and record the amount of ingredients on the distiller form, then close the distillator tightly, turn on the burner. Distillation for 4.5 hours for each production batch, holding each distillation result and collecting the distilled oil in an oil container bottle (SOP from the Atsiri Lab, FTP, UB Malang).

2.2.2. Characterization of Essential Oil Chemical Compounds

Characterization of essential oils was aimed at determining chemical compound components and samples were analyzed for chemical content using gas chromatography–mass spectrometry (GC-MS) using a 680 Perkin Elmer Clarus (Perkin Elmer, Inc. United States) with a fused silica column and a capillary column (length 30 m × diameter 250 µm × thickness 0.25 µm). The carrier gas used is pure helium (99.99%). The ionization energy method was used to detect GC-MS spectra with a high ionization energy of 70 eV, 0.2 seconds for scanning time, and 40-600 m/z for the fragment range. The injection split ratio was 10:1 with a quantity of 1 µL at a constant temperature of 250°C. The composition of essential oils was identified based on the reference mass spectrum (Willey Lib) (Suniarti *et al.*, 2022).

2.2.3. Antioxidant Activity Assay

The extract was used as several samples with concentrations of 5 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm and 200 ppm using 96% methanol. The procedure for testing antioxidant activity is by adding 1 mL of DPPH (1,1-Diphenyl2-

picrylhydrazyl) 40 ppm in methanol solution with 2.5 mL of extract solution then leaving it at room temperature for 30 minutes to react (Hadam & Husni, 2019). Then the absorbance was measured at 518 nm using a UV-Vis spectrophotometer. The absorbance results were used to calculate antioxidant activity with the following equation.

$$\text{DPPH Inhibition (\%)} = \frac{\text{Absorbansi control} - \text{Absorbansi sample}}{(\text{Absorbansi control})} \times 100\% \quad (1)$$

2.2.4. Preparation of Ascorbic Acid Comparison Solution

A comparison solution of 100 ppm ascorbic acid was made by weighing 10 mg of ascorbic acid powder and dissolving it with methanol p.a in a volumetric flask to 100 ml. The 100 ppm stock solution was diluted to concentrations of 5 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm and 200 ppm. Then the antioxidant activity was tested like the essential oil samples (Iqbal *et al.*, 2021).

2.3. Data Analysis

2.3.1. Calculation of Essential Oil Yield

Yield analysis calculated the average weight obtained from 3 repetitions of distillation and then calculated the results in the form of % distillation yield using the following equation (adapted from Juliarti *et al.*, 2020):

$$\text{Yield (\%)} = \frac{\text{Oil mass (kg)}}{\text{Sample mass (kg)}} \times 100\% \quad (2)$$

2.3.2. IC50 Calculation

Calculation of the effectiveness of DPPH inhibition was carried out by creating a linear regression equation between the percentage of inhibition and the concentration of each sample. IC50 can be determined through the linear regression equation obtained (Fardani *et al.*, 2023):

3. RESULTS AND DISCUSSION

The yield results from 3 types of samples with an extraction duration of 4.5 hours and a material weight of 1 kg each showed in Table 1. Based on the research results, it shows that the yield of essential oil from the rosella plant parts (flower petals, leaves and seeds) are very small. In table 1 it can be seen that the yield of flower petals is greater than seeds and leaves. This shows that flowers contain more essential oil than others. The different yield of flowers and other parts of the plant are common occurs, Zaituni's research results show that the results of distilling essential oil from lemongrass leaves are greater than those from the stem (Zaituni *et al.*, 2016). The same results were also reported by Kapelle *et al.*, (2023) that the yield of clove flower essential oil was greater than the yield of clove stems. Helilusiatiningsih *et al.* (2023) explains that the yield of essential oils in flower petals is 0.0136% in 1 kg of dry material, the results are relatively small in general, but when compared to the levels found in leaves and seeds, the flower petals were the highest. Rosella's flower petals contain many bioactive compounds and natural dyes as well as vitamins A and C, so it is thought that their influence produces a higher yield of essential oils than other organs.

Figure 1 show that the largest component of rosella seeds essential oil is fatty acid (2-propanoic acid, butanoic acid). Apart from that, there are ester compounds (neopentyl isobutyrate) and aldehyde (2-ethyl 2 hexenal). Hagr & Adam (2020) stated that the characterization of rosella seed essential oil contain fatty acid components, namely hexadecanoic acid, octadecanoic acid and esters (methyl stearate) as well as other organic compounds.

Table 1. Essential oil yield obtained from different parts of rosella

Sample	Method	Time	Sample mass	Oil mass	Yield (%)
Seed	Water-steam distillation	4.5 hour	1000 g	0.1408 g	0.0107 %
Leaf	Water-steam distillation	4.5 hour	1000 g	0.0869 g	0.0087 %
Petal	Water-steam distillation	4.5 hour	1000 g	0.1494 g	0.0136 %

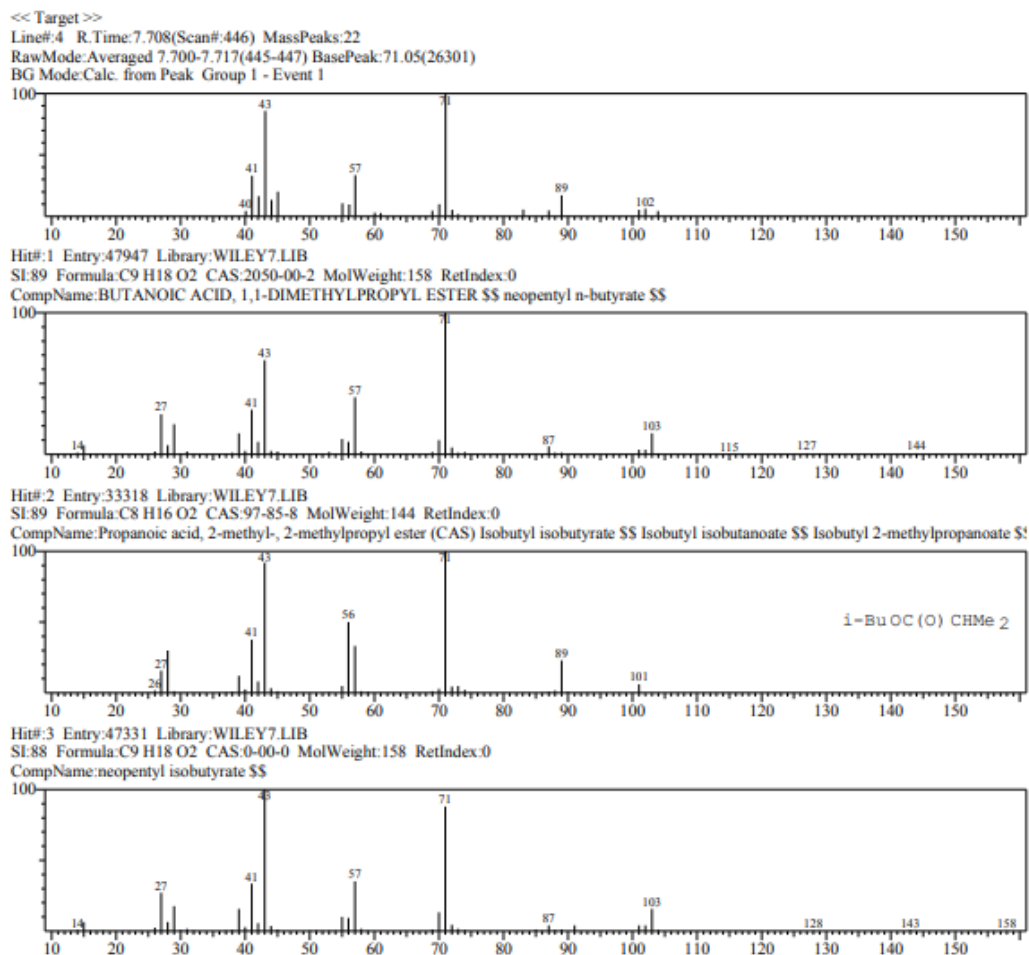


Figure 1. MS spectra results of fatty acids contained in rosella seed essential oil

The results of the characterization of rosella leaves essential oil show that there are components in the form of fatty acids (tetracosanoic acid, hexanoic acid, butanoic acid), which can be seen in Figure 2. There are also detected many compound such as alkane compounds (undecane, hexadecane, pentadecane), ketones (4 octanone, 3 pentanone, 4 heptanone) and phenol (2-tert-Butyl-4-(2,4,4-trimethylpent-2-yl)phenol). These results are almost the same as Amlashi's research, which shows that the presence of compounds in rosella leaf essential oil mostly consist of alkanes (n-heneicosane, n-pentacosane), β -caryophyllene, anethole and dihydro aromadendrene (Amlashi et al., 2020).

The results of the analysis show that the largest component of the rosella flower petals essential oil is fatty acid (2-propanoic acid), which can be seen in Figure 3. Apart from that, there are also aldehyde compounds (acetaldehyde), Tetrahydrofurfuryl alcohol, butoxy 2-propanol, and other compounds. This is similar to the results of Shen's research (Shen et al., 2016) which obtained fatty acids and esters as the main components of rosella flower petal essential oil. Inikpi et al. (2014) reported that most of the components of flower petal essential oil compounds are fatty acids, namely hexadecanoic acid and linoleic acid. Ibrahim et al. (2022) also reported that the essential oils, apart from containing fatty acids, also contained aldehydes, namely furfural.

The antioxidant activities assay of essential oil samples was carried out using the DPPH method and using ascorbic acid as a comparison, which can be seen in Table 2. The seed antioxidant activity testing data shows that the DPPH inhibition value for sample concentrations of 5-200 ppm increased, from 36.52% to 84.95%. The results of DPPH inhibition in the leaves also resulted in an increase, from 25.50% to 51.40%. Rosella flower petals essential oil also increased as the concentration increased from 30.56% to 57.46%. Our previous study also shows that rosella flower petals have an inhibitory power ranging from 50-60% toward DPPH free radicals (Helilusiatiningsih et al., 2023). As a

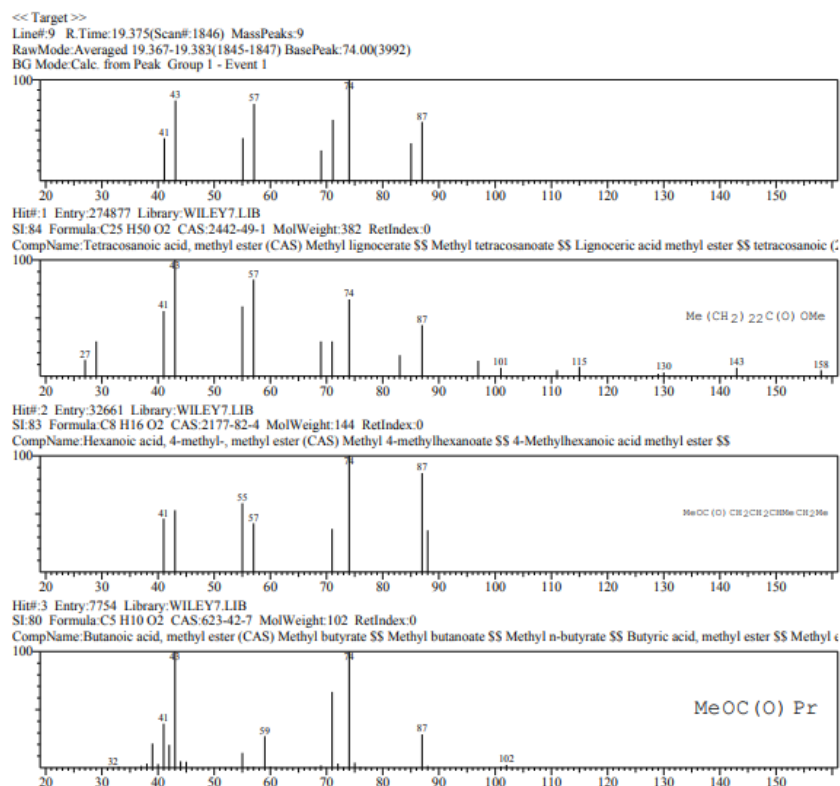


Figure 2. MS spectra results of fatty acids contained in rosella leaf essential oil

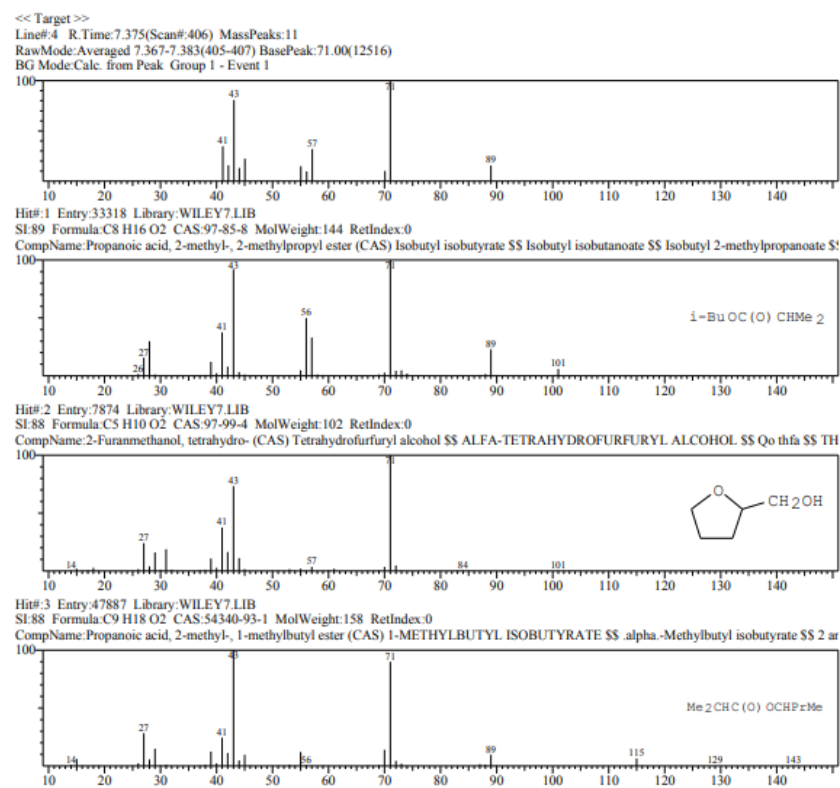


Figure 3. MS spectra results of fatty acids contained in the essential oil of rosella flower petals

Table 2. Inhibition test data against DPPH (1,1-Difenil2-pikrilhidrazil) free radicals

Sample concentration (ppm)*	DPPH [#] inhibition (%)			
	Seed	Leaf	Flower petals	Ascorbic Acid
5	36.52	25.50	30.56	37.53
10	42.96	29.70	33.71	44.16
25	51.57	34.90	39.78	51.84
50	62.13	42.13	43.75	61.19
100	69.41	44.50	48.25	72.45
200	84.95	51.40	57.46	88.22

*ppm : part per million = µg/ml

comparison, ascorbic acid shows almost the same results as rosella seed essential oil, namely 37.53% to 88.22%. This similarity is shown by the percentage of DPPH inhibition with the same range (30%-90%).

The results of the inhibition percentage are then used as a linear regression equation with the sample concentration to calculate the IC₅₀ value. In Table 3 were the results of the linear regression equation and the IC₅₀ value for each sample. The IC₅₀ value is the effectiveness value of the sample concentration containing antioxidant compounds in inhibiting 50% of the concentration of free radicals (DPPH) in solution (Fardani *et al.*, 2023). An antioxidant compound has several categories, a compound is said to have very strong antioxidant activity if it has an IC₅₀ of 50 µg/ml, strong if 50–100 µg/ml, medium if 100–150 µg/ml, weak if 150-200 µg/ml ml and very weak if the value is more than 200 µg/ml (Iqbal *et al.*, 2021).

Table 3. Calculation results of the linear regression equation for antioxidant activity

Sample	Linear Regression Equation	R ²	IC ₅₀ (µg/ml)
Seed	Y = 0.2275x + 43.139	0.9015	30.15
Leaf	Y = 0.1214x + 29.207	0.9069	171.27
Petal	Y = 0.1258x + 34.076	0.9104	126.58
Ascorbic acid	Y = 0.2428x + 43.457	0.9282	26.94

IC₅₀ : Efficiency of concentration 50%

The results of calculating the IC₅₀ value of ascorbic acid as a comparative antioxidant compound showed a value of 26.94 µg/ml. This value provides the conclusion that ascorbic acid as an antioxidant compound has very strong antioxidants category. The IC₅₀ value for the seed sample was 30.15 µg/ml indicating that the antioxidant content in the seed essential oil was included in the very strong category. This shows that roselle seeds contain high levels of bioactive compounds, such as antioxidants (Phewphong *et al.*, 2023). Antioxidant compounds in rosella seeds can be anthocyanidins, phenolic acids, flavonoids (Ghosh *et al.*, 2023), as well as saturated and unsaturated fatty acids (Hagr & Adam, 2020; Le *et al.*, 2020). This is in accordance with the results of the GC-MS analysis that has been carried out where roselle seeds contain fatty acid compounds, namely 2-propanoic acid, butanoic acid.

The IC₅₀ results for leaves and flower petals were 171.27 µg/ml and 126.58 µg/ml, making these antioxidant compounds fall into the weak category, because they had a value of 150-200 µg/ml. This is in line with our previous study that rosella leaves are included in the weak category because they have an IC₅₀ value of between 150-200 µg/ml (Helilusiatiningsih *et al.*, 2024). The results of the antioxidant activity of the three parts of the rosella plant show that apart from flower petals, other rosella plants parts also have potential as antioxidants. This is confirmed by the opinion of Hadad & Husni, (2019), explaining that rosella flower petals contain phenolic compounds whose function is as an antioxidant. The results of the antioxidant activity assay can be clarified from the GC-MS results of rosella leaves and flower petals that each has fatty acid antioxidant compounds, namely in the leaves there is 2-propanoic acid while the rosella flower petals contain butanoic acid. The differences in the structure of the fatty acid compounds contained in the essential oils cause differences in the antioxidant activity categories of the three samples.

Rosella plants have bioactive compounds which can be antioxidant compounds such as phenols, alkaloids, tannins, flavonoids, saponins and organic acids (Nurnasari & Khuluq, 2018). In the opinion of Ingrid & Santoso, (2014), antioxidant compounds can help capture or bind free radicals or Reactive Oxygen Species (ROS) so that they can

increase immunity and prevent dangerous diseases in the body. The presence of antioxidant compounds in parts of the rosella plant has the potential to be used as a food coloring, additive for the food and beverage industry and the pharmaceutical industry (Hagr & Adam, 2020).

4. CONCLUSION

The research activities that have been carried out provide several conclusions including: 1) The yield of essential oils from seeds are 0.0107%, leaves are 0.0087%, and flower petals are 0.0136%. 2) The characterization results show that the largest chemical compound components in the three samples (seeds, leaves and flower petals) are fatty acids (2-propanoic acid, butanoic acid) and esters (neopentyl isobutyrate, methyl stearate), these compounds that acts as an antioxidant. 3) Antioxidant activity shows the highest IC 50 value in rosella seeds, namely 30.15 µg/ml, which is almost the same as ascorbic acid. Meanwhile, leaves and flower petals have an IC 50 value of 100-150 µg/ml which is included in the weak antioxidant category.

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