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Submission date: 05-Jun-2020 06:06AM (UTC+0700)

Submission ID: 1337936531

File name: Salni_2020_IOP_Conf._Ser.__Mater._Sci._Eng._857_012002.pdf (954.77K)

Word count: 2924

Character count: 15238

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To cite this article: Salni *et al* 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* **857** 012002

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Antioxidant Activities Bioactive Compound of Ethyl Acetate Extracts from rose myrtle Leaves (*Rhodomyrtus tomentosa* (Ait.) Hassk.)

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Abstract. One of the plants traditionally used as medicine is rose myrtle (*Rhodomyrtus tomentosa* (Ait.) Hassk.). Rose myrtle has the potential as an antioxidant because it has secondary metabolites including flavonoids, phenols, terpenoids, tannins, saponins, and steroids. The purpose of this study was to determine the fraction that has antioxidant activity and obtain antioxidant compounds from ethyl acetate extract of rose myrtle leaves. The research methods included extraction by Soxhletasi, fractionation by vacuum liquid chromatography, purification of compounds with gravity columns, and antioxidant activity test using the DPPH method. The results obtained 32% yield of ethyl acetate extract which was then followed by fractionation. The fractionation results obtained 13 fractions. The fractions that have antioxidant activity namely fractions 5-6, 7, and 8. The fraction was continued to purification and obtained by compound E.1, compound E.2 from the essential oil, and compound E3 allegedly rhodomyrtone. The bioactive compound determined by IC₅₀ value obtained that E1, E2 and E3 that have IC₅₀ value of 218, 207 and 90 µg/mL, respectively. The bioactive compounds of ethyl acetate extract have strong antioxidant activity in E3 and very weak in E1 and E2 compounds.

Keywords: Antioxidant, *Rhodomyrtus tomentosa*, essential oils, free radical, DPPH

1. Introduction

Free radical compounds are one of the factors in DNA damage. If the damage is not too severe, the DNA system can repair it. However, if damage has occurred in various places, cannot be repaired by a DNA system so that cell division will be disrupted. This damage causes abnormal changes in the gene and can cause cancer [1]. Antioxidants are compounds that can bind free radicals and molecules that are very reactive. Antioxidants will give hydrogen atoms that are oxidant so that cell damage in the body can be inhibited. This compound has a small molecular weight and can inactivate the development of oxidation reactions by preventing radical formation [2].

2 Natural antioxidants derived from plants are safer than synthetic antioxidants such as propyl gallate, citric acid, butylated hydroxyanisole, and butylated hydroxytoluene which can cause hypoxic and carcinogenic [3]. Synthetic antioxidants such as BHA (butylated hydroxy aniline) and BHT (butylated hydroxy toluene) have great side effects including causing liver damage.



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On the other hand, nature provides an effective and relatively safe source of antioxidants such as flavonoids, vitamin C, beta carotene and others. This encourages more exploration of natural materials as a source of antioxidants [4].

One of the plants in Indonesia that is traditionally used as medicine is rose myrtle plants (*Rhodomyrtus tomentosa* (Ait.) Hassk.). According to other research [3] that rose myrtle leaf extract has strong antioxidant activity in vitro and in vivo. Therefore, rose myrtle extract can be used as an antioxidant supplement in food products to prevent food oxidation and also as an alternative antioxidant therapy in the pharmaceutical industry to prevent free radical damage. Further research is needed to evaluate the toxicity and antioxidant activity of each phytochemical compounds to develop complete supplement/antioxidant therapy. The ethyl acetate fraction from rose myrtle leaf extract has antioxidant activity with IC₅₀ value of 70.12 µg/mL [5]. The important compound in antioxidant activity in the fraction is phenols and flavonoids. In this study, was to isolate antioxidant compounds from ethyl acetate fraction of rose myrtle leaves.

2. Material and methods

2.1. Materials

The material used is *Rhodomyrtus tomentosa* leaves obtained from the flat land of West Sumatra Sungayang district. The chemicals used include ethyl acetate, methanol, n-hexane, DMSO, DPPH (1,1-Diphenyl-2-picrihidrazil), Silica gel 60, and silica gel 60 F254 from Merck.

2.2. Extraction

Extraction using Soxhlet with ethyl acetate. 150 g of simplicia powder wrapped in filter paper put into Soxhlet, added with ethyl acetate 1 L. The liquid ethyl acetate extract obtained was evaporated with a rotary evaporator at a temperature of 80°C until thick extracts were obtained.

2.3. Fractionation

Fractionation was carried out using the VLC (Vacuum Liquid Chromatography) method, with a stationary phase in the form of silica gel and various stages of the mobile phase of solvents. Silica gel was inserted into the VLC column with 5 cm silica height. The solvents used were n-hexane, ethyl acetate, and methanol with a n-hexane ratio (%) of 100:0; 95:5; 90:10; 85:15; 80:20; 75:25; 70:30; 65:45; 60:40; 55:55; 50:50, 100% ethyl acetate, and 100% methanol as much as 200 mL.

2.4. Antioxidant test of fraction

The fraction of the ethyl acetate extract was bottled on the TLC plate using a capillary pipette, then inserted into the chamber containing n-hexane: ethyl acetate (8:2) eluent. Furthermore, the eluent was allowed to propagate to the TLC plate boundary. TLC was left in the air to dry. The dry TLC was sprayed using DPPH 0.5 mM. Color changes in TLC were observed. Spots from the fraction that have antioxidant activity will turn yellow with a purple background. Spots that appear then calculated the RF (Retardation Factor) value.

2.5. Compounds purification

Purification of compounds from the active fraction was carried out by gravity column chromatography with the stationary phase of silica gel, while the mobile phase was a mixture of n-hexane and ethyl acetate and methanol. The polarity was increased gradually. Elution was carried out by eluent solvent mobile phase which corresponds to the elution rate of 17 drops per minute, with the volume of the fraction collected being 10 mL. The pure compounds obtained were then tested for antioxidant activity and determined IC₅₀ values.

2.6. Antioxidant activity

Pure compounds tested antioxidant activity using the DPPH method. DPPH solution is made with a concentration of 0.5 mM, as much as 1.98 mg DPPH is dissolved in 100 mL of methanol. 1 mg pure compound dissolved in 1 mL DMSO (1000 µg/mL). The solution was diluted with concentrations (500, 250, 125, 62.5, 31.25, and 15.625) µg/mL. 0.2 mL of each concentration was added 3.8 mL of DPPH 0.5 mM solution, the mixture was homogenized and left for 30 minutes in a dark place. The absorbance value was determined by UV-VIS spectrophotometer at λ max 517 nm. Positive control is used ascorbic acid. Determination of antioxidant activity is determined calculating the formula (1) as follows :

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

3. Results and Discussions

3.1. Extraction and fractionation

The extraction of 150 g of simplicia was obtained by 48 g extract with a yield of 32%. The extract was fractionated by the VLC (Vacuum Liquid Chromatography) method using a solvent ratio that varied in degree of polarity. The fractionation results obtained 13 fractions, the results of the antioxidant activity test with DPPH obtained 3 active fractions namely 5-6, 7 and 8 fractions characterized by yellow spots on the VLC plate. The RF value of antioxidant compounds in 2-5 fractions was 0.51; fraction 6 was 5.54 while fraction 8 was 0.33 with n-hexane eluent: ethylacetate (8: 2).

The RF value of the antioxidant compounds obtained has the same value as the research of [5] The value of Rf spotting that has antioxidant activity in the ethylacetate extract of rose myrtle leaves is 0.54; 0.51; 0.33 with n-hexane eluent: ethylacetate (8: 2). Fractions containing antioxidant compounds, 5-6, 7 and 8 fractions were purified using the gravity column chromatography method.

3.2. Antioxidant activity of active compounds

The purification of antioxidant compounds found 3 bioactive compounds, namely compounds E1, E2, and E3. The antioxidant activity of compounds E1, E2, E3, and ascorbic acid can be seen in Figures 1 and 2. In Figure 2 there is a discoloration of DPPH solution with the addition of E1 (A) compounds, E2 (B) compounds, E3 (C) compounds, and ascorbic acid (D) with concentrations (500 µg/mL; 250 µg/mL; 125 µg/mL; 62.5 µg/mL; 31.25 µg/mL; 15.125 µg/mL).

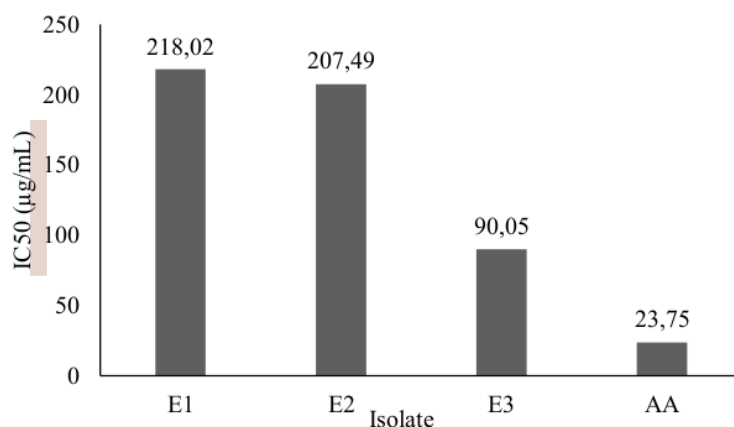


Figure 1. The antioxidant activity of compounds E1, E2, E3, and ascorbic acid (AA).

Figures 1 and 2 obtained that E1 compound have an IC_{50} value 218 $\mu\text{g/mL}$ so that it is categorized as having very weak antioxidant activity. E2 compound had an IC_{50} value of 207.49 $\mu\text{g/mL}$, it was categorized as having very weak antioxidant activity. E3 compound has an IC_{50} value of 90.05 $\mu\text{g/mL}$ classified as a strong antioxidant activity. The IC_{50} value of the three isolates has IC_{50} under ascorbic acid. Ascorbic acid has very strong antioxidant activity with IC_{50} 23.75 $\mu\text{g/mL}$. Comparing to others [6], it shows that antioxidant activity can be classified into several categories: under 50 $\mu\text{g/mL}$ is very strong, between 51-100 $\mu\text{g/mL}$ is strong, 101-150 $\mu\text{g/mL}$, between 151-200 $\mu\text{g/mL}$ is strong and greater than 200 $\mu\text{g/mL}$ is very weak.

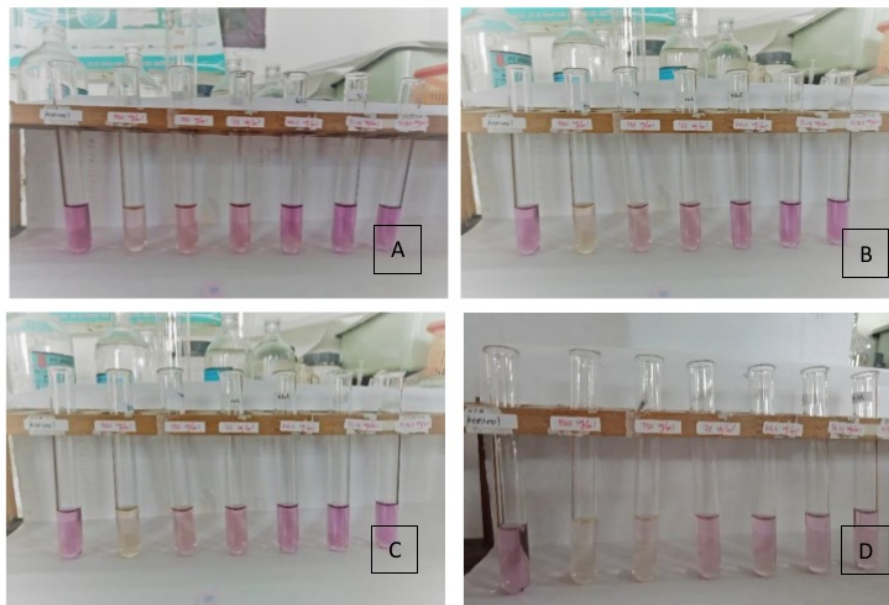


Figure 2. Change the color of the solution (A) E1, (B) E2, (C) E3 compounds and (D) ascorbic acid.

The characterization of E1 and E2 bioactive compound with GC-MS are known to be essential oils. The chromatogram of E3 compounds is thought to be rhodomertone. Some types of plants have essential oils that have antioxidant activities, including: antioxidant activity of 423 essential oils from 48 plant species obtained 73 essential oils showing IC_{50} concentration of 1.25 mg/mL . IC_{50} of the 73 most active oils ranges from 4 to 20 $\mu\text{g/mL}$. Essential oils which have IC_{50} of fewer than 300 $\mu\text{g/mL}$ of 20 samples. Essential oils from the Lamiaceae and Myrtaceae families are the most effective antioxidants. Thymol and carvacrol are the main compounds in most essential oils of the Lamiaceae and eugenol families and terpenes are the main compounds in all essential oils of the Myrtaceae family [7]. Essential oil from *Origanum vulgare* L. with main components as thymol (37.13%), gamma-terpinene (9.67%), carvacrol (9.57%), carvacrol methyl ether (6.88), cis-alpha-babolabolen (6.80%), eucalyptol (3.82%), p-cymene (3.58%) and elemol (2.04%) [8].

Antioxidant activity of essential oils of E1 and E2 compound from plants rose myrtle is still under the antioxidant activity of red betel essential oil (*Piper crocatum* Ruiz & Pav.) With IC_{50} value 136,947 $\mu\text{g/mL}$. Essential oils of red betel leaves were analyzed by GC-MS to determine the constituent components of the oil. The chemical components of red betel leaf essential oils which have the largest percentage based on area percent are sabinene (33.35%), β -mycenae (20.18%), β -caryophyllene (7.07%), linalol (5.41%) and germakren (4.96%). The results of GC-MS analysis of red betel leaf essential oil showed that the main constituents of essential oils were monoterpene and sesquiterpene groups [9].

3.3. Characterization of E1 isolates

Isolate E1 in the form of a yellow paste is thought to be an essential oil. Chromatogram of E1 compound is shown in Figure 3. The E1 isolate obtained that 31 peaks spectra which showed there were 31 types of compounds contained in the essential oil. Isolate E1 contained six main compounds namely peak 8.08 semimyrtycommulone compound (15.63), peak 8.10 semimyrtycommulone (6.97), peak 12.91 caryophyllene (13.12), peak 13.17 menthol (29.49), peaks of 13.20 menthol (11.31) and peaks of 13.38 cubenol (10.63), menthol compounds were the compounds with the highest E1 isolates of 29.49%.

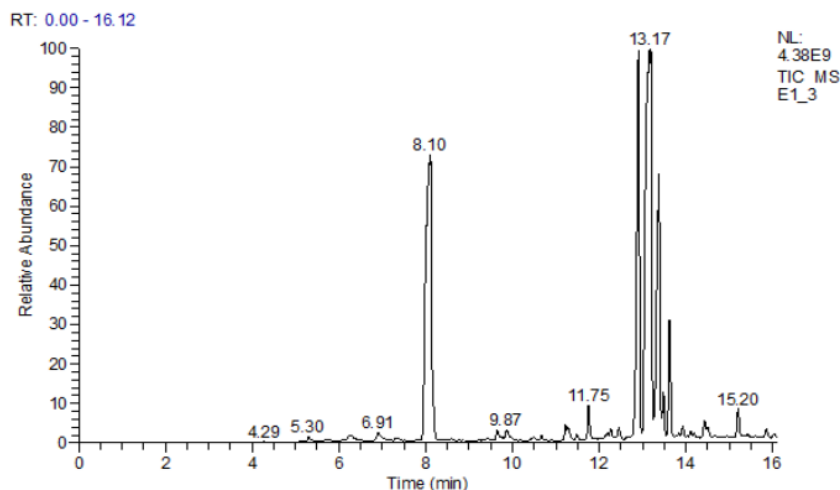


Figure 3. Chromatogram of E1 compound.

3.4. Characterization of E2 isolate

E2 isolate in the form of a reddish colored paste. E2 isolate, is thought to be an essential oil. The determined E2 isolate by GC-MS shown in Figure 4. The E2 isolate contained 30 compounds of five major compounds namely Peak 8.08 semimyrtycommulone (15.63), peak 8.10 semimyrtycommulone (6.97), peak 12.91 caryophyllene (13.12), peak 13, 17 menthol (29.49), peak of 13.20 menthol (11.31), peak of 13.38 cubenol (10.63), and menthol compound is the most abundant E1 isolate is 29.49%

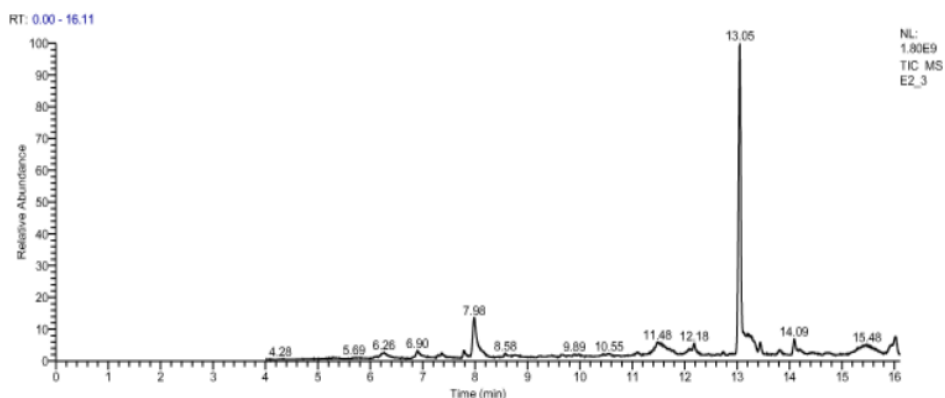


Figure 4. Chromatogram of E2 compound.

3.5. Characterization of E3 isolate

E3 isolate in the form of white crystal, chromatogram E3 isolate using thin layer chromatography (TLC) method with mobile phase n-hexane: ethyl acetate (8: 2) obtained rf value of 0.24, after spraying 10% H₂SO₄ there are yellowish-orange spots which indicate that these antioxidant compounds belong to the class of phenol compounds. The Rf value and the color of the isolate E3 are the same as the rhodomyrtone compound [10, 11]. Isolate E3 is thought to be the Rhodomyrtone compound, Rhodomyrtone including the phenol group, phenol compounds have antioxidant activity because they can bind to free radicals. According to Sari et al. [12], phenol compounds play a significant role in antioxidant activity, the greater the content of phenol groups, the greater their antioxidant activity. This is due to the presence of a hydroxyl group in the phenol group that is capable of capturing free radicals.

4. Conclusions

The fractionation of ethylacetate extract produces 13 fractions, which fractions 5-6, 7, and 8 have antioxidant activity characterized by yellow spots with a purple background. The fractions of 5-6 obtained by E1 compound have IC₅₀ value of 218 µg/mL with very weak antioxidant activity, from fraction 7 obtained E2 compound which has IC₅₀ value of 207 µg/mL very weak antioxidant activity. The fraction 8 obtained by the antioxidant E3 compound which has an IC₅₀ value of 90 µg/mL with strong antioxidant activity. The IC₅₀ value of 3 compound has IC₅₀ value under ascorbic acid. The E1 and E2 isolates are essential oils, while the E3 isolates are thought rhodomyrtone compounds.

Acknowledgment

This research was funded from the Kompetitif Universitas Sriwijaya grant in 2019

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