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ANALYSIS OF FLAVONOIDS ON ETHANOL EXTRACT OF *RHIZOPHORA MUCRONATA* FRUIT

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Abstract - Mangrove species *Rhizophora mucronata* fruit has the potential to be utilized in a variety of processed foods. This study aimed to analyze the flavonoid compounds from the ethanol extract of fruit powder mangrove *Rhizophora mucronata*. The study conducted through several stages, extraction using ethanol solvent, phytochemical screening, FT-IR (Fourier Transform Infrared Spectroscopy), the creation of a standard solution and eluent preparation, isolation of flavonoids using TLC (Thin Layer Chromatography) densitometric method. The results of the analysis of phytochemical screening showed positive results containing flavonoids. Determination of Rutin at 0.32 to 0.46 Rf range obtained rutin an average grade 13.29% ± 0.43. Infrared spectrum analysis showed absorption OH phenol in number 3260 cm^{-1}, C = C aromatic ring in the area from 1650 to 1450 cm^{-1}, and the absorption at 1660 cm^{-1} indicating a carbonyl group C = O.

INTRODUCTION

The world of mangrove ecosystems was reported in 124 countries located between 30ˆ(North) and 30ˆ (South) latitude (Giesen *et al*., 2007; FAO, 2010). Closure of mangrove world estimated at 15,000,000 ha (FAO, 2010; Giri *et al*., 2011). The extent of mangrove forests in Asia (42%), followed by Africa (20%), North and Central America (15%), Oceania (12%) and South America (11%), while in Indonesia breadth is 3,112,989 ha which is a mangrove forest the widest in the world (Arobaya and Wanma, 2006; Giri *et al*., 2011). Mangrove forests are one of the tropical forests that store millions of benefits both ecologically, socio-economically, and social-cultural which are very important; for example, safeguarding coastal stability from abrasion, sources of fish, shrimp and other biodiversity, sources of fuelwood and building wood, and having functions of conservation, education, ecotourism and cultural identity (Setyawan, 2006). Mangrove species are often found in Asia, including in Indonesia, among others: *Acanthus ebracteatus, Acanthus ilifolius, Acrostichum aureum*, *Bruguiera sp, Bruguiera hainessii* and *Rhizophora* (Mesta *et al.*, 2014; Umroh *et al*., 2016).

One of the mangrove species found in Indonesia is *Rhizophora mucronata* a mangrove plants from the family *Rhizophoraceae*. These plants contain bioactive compounds such as alkaloids, carbohydrates, glycosides, tannins, proteins, and amino acids, flavonoids, saponins, sterols, acidic compounds, resins, peroxide, and polyuronides. Bioactive compounds are components derived from natural materials. Bioactive compounds used in the health sector among others to prevent cancer (Das *et al.*, 2013), as antioxidants (Ravikumar, 2012), antibacterial (Ramasubburayan, 2015), antidiabetic (Hardoko, 2016), antimicrobial (Saad, *et al* ., 2012) as well as anti-inflammatory (Chakraborty, 2016). One of the chemical constituents *R. mucronata* that is important is flavonoids. Flavonoids including one secondary metabolite (Harborne, 1984; Pietta *et al*., 2003; Kevin and Carolyne, 2006; Joel, 2010; Cahyani, 2016; Obidallah, 2018), which is uniformly dispersed in the vegetable kingdom, and includes one of the largest natural phenols.

Interest in food and pharmaceutical industries increased to flavonoids, as it has antioxidant compounds. Based on the literature search of *R.*

mucronata flavonoid compounds have been studied from the leaves (Behbahani *et al*., 2018), and roots (Asha, 2011), but for fruit, Rhizophora has not widely researched. The initial approach to determine the levels of flavonoids in fruits Rhizophora conducted qualitative phytochemical screening, followed by TLC detection and quantitative measurement of flavonoids using standard solutions quercetin and Rutin.

Rutin (quercetin-3-O-rutinoside) is a flavonoid commonly found in nature. At first, it was known as a mixture of flavonoids extracted from plants with the name "vitamin P" (Kalinova and Dadakova, 2009). Rutin can reduce capillary fragility and permeability, retinal hemorrhage and venous insufficiency (such as varicose veins, hemorrhoids, diabetic vascular disease) and improve vascular blood flow (tired legs, cramps) (Fathiazad *et al*., 2006). Rutin is also beneficial for the prevention and treatment of colorectal cancer and can prevent the pain induced by antitumor drugs (Azevedo *et al*., 2013). Currently, Rutin used as a single compound with chemical formulas, and its biological activity is essential, so it has the potential to be studied further. This study aims to analyze the flavonoid compounds of the ethanol extract of mangrove flour from *R. mucronata* using quercetin and rutin standards using the Thin Layer Chromatography (TLC)-densitomeric method.

MATERIALS AND METHODS

Collection and identification of fruits. Sample pieces of mangrove *R. mucronata* taken in the village of Kalianyar, Bangil, Pasuruan, East Java, Indonesia. Chemicals used was 96% ethanol, HCl, Magnesium, H_2SO_4, and other chemical materials that are reagent grade.

Preparation of fruit. This experiment started with the process of making flour *R. mucronata* includes sorting fruit, fruit leather stripping, immersion in fresh water and change water regularly until quite clear, boiling for 15 minutes, lower temperature drying between 50-70 °C to achieve 10-11% moisture, smoothing milled flour with tools Willey mill mesh sieve size 60. After that dried fruit flour for 10 minutes at a temperature of 70 ° C, to prevent the powder into a dry and sour

Sample extraction. Extraction is done using solvent ethanol, *R. mucronata* fruit flour weighed 10 grams and supplemented with 96% ethanol, 250 mL. Sample and the solvent extracted with speed extractor utilizing a pressure of 100 bar and a temperature of 70 °C, and 3x more repetitions to obtain the filtrate. Concentrated using a vacuum rotary evaporator temperature of 40 °C, 60 rpm, and a pressure of 200 mBar until no solvent drips.

Phytochemical screening. The preliminary phytochemical analysis of the plant extracts was performed using a standard protocol given by Harborne (1984) to identify the presence of flavonoids. Test flavonoid phytochemical active compound content of ethanol extract of *Rhizophora* done three times repetition. Extract *R. mucronata* put in a test tube is then dissolved in 1-2 mL of hot 50% methanol. After that, added Mg metal and 4-5 drops of concentrated HCl. The red or orange colored solution formed indicate flavonoids.

FT-IR analysis. Extract of *R.mucronata* then analyzed by FT-IR to measure the molecules of complex compounds.

Preparation of standard solution and eluent preparation. Raw carefully weighed 2 mg quercetin (Sigma Q4951), then diluted with 10 mL of ethanol as a stock solution. Created dilution Quercetin with concentration of 0.100; 0.125; 0.150; 0.175 and 0.200 µg/ µL as the reference solution. The stationary phase Silica gel 60 F254 (Merck) with the sample volume of 1.0 µL and standard 1 µL. Eluent preparation for the determination of quercetin is Ethyl acetate: formic acid: Aquadest (85:10:15), while for the determination Rutin was Chloroform: Ethyl Acetate: Format acid (5: 4: 1). Distance elution 9 cm. After completion of the elution plate TLC is dried. Visual detection of the UV lamp 366 nm and detection wavelength TLC densitometry at 365 nm (Camag TLC Scanner 3). Interpretation of the sample contains Quercetin at Rf range from 0.85 to 0.90, and Rutin at Rf: 0.30 -0.40.

Total flavonoid content measurement. Flavonoids total ethanol extract of *R. mucronata* sample calculated by linear regression of the calibration curve that has been measured quercetin previously.

RESULTS

Phytochemical screening and Determination of Quercetin levels using TLC-Densitometry

The test results showed that the ethanol extract of *R. mucronata* showed a positive reaction to the presence of flavonoids characterized by color changes to

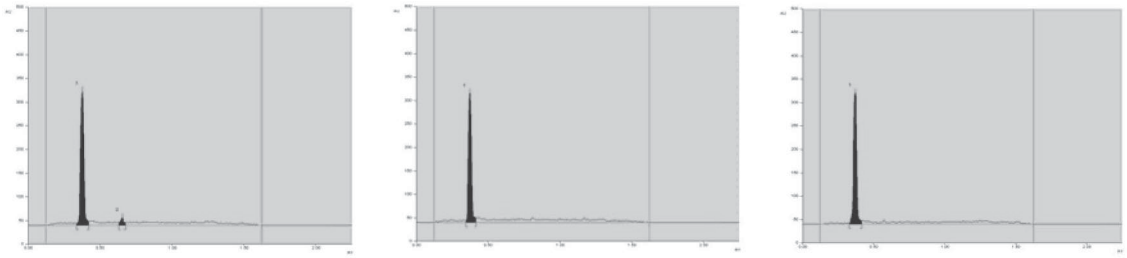


Fig. 1. Peak display of TLC-densitometric on Fruit Quercetin levels *R. mucronata* using ethanol solvent

orange. Results of analysis using standard flavonoid quercetin showed negative results in Figure 1.

The results of the ethanol extract of *R. mucronata* Rf shown in Figure 2

The Rutin measurement results are shown in Table 1

Table 1. Rutin absorbance value at a wavelength of 366 nm

Levels (µg/µL)	Spot Size (AUC)
0.100	1,688.30
0.125	2,745.20
0.150	3,929.40
0.175	4,787.80
0.200	5,124.70

Results of analysis using standard flavonoid quercetin showed negative results, then the results of the ethanol extract of *R. mucronata* using standard flavonoid quercetin showed Rf 0.34, 0.33 and 0.35. Based on the Rutin absorbance value in Table 1 has an equation: y = bx + a, where b = 35,661.60, a = -1,694.16, R2 = 0968, which is shown in Figure 3. After that equation obtained is used to determine the levels of Rutin found in mangrove fruit,

Analysis of FT-IR (Fourier Transform Infrared Spectroscopy)

The results of FT-IR spectrum shown in Figure 4

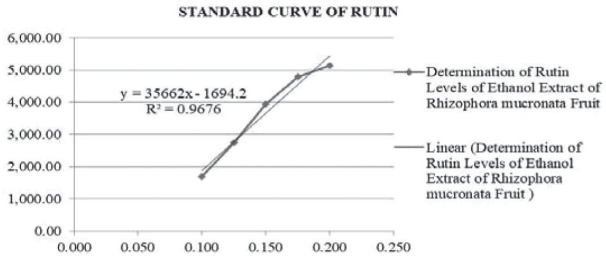


Fig. 3. Determination of standard curve Levels Rutin *R. mucronata* with ethanol

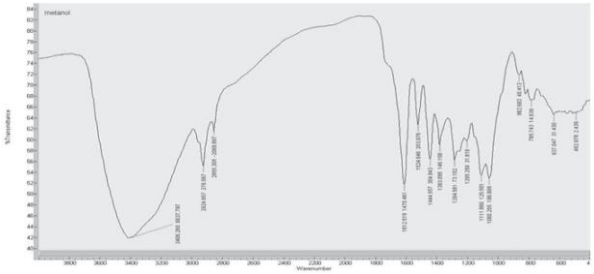
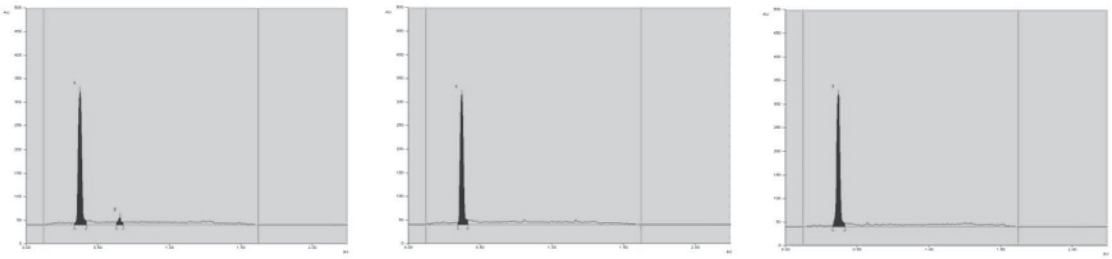


Fig. 4. FT-IR spectrum

DISCUSSION

Phytochemical screening and Determination of Quercetin and Rutin levels using TLC-Densitometry

Phytochemical screening analyzes performed on ethanol, which is polar. The test results showed that the ethanol extract of *R. mucronata* showed a positive reaction to the presence of flavonoids characterized



rf 0:34 to 0:42 rf 0:35 to 0:42 rf 0:33 to 0:42

Fig. 2. Peak display of TLC-densitometric on Fruit Rutin levels *R. mucronata* using ethanol solvent

Table 2. Calculation Rutin levels *R. mucronata* fruit extracts in ethanol

Weighing (g)	Volume added (μL)	Area (AUC)	Content (%)	The mean (%)	standard deviation
10.00	3.00	3179.50	13.67	13.29	0.43
10.00	3.00	2874.90	12.81		
10.00	3.00	3076.80	13.38		

by color changes to orange. Ernawati *et al.* (2019) observed that fruit *R. mucronata* extracted with methanol also showed a positive reaction to the test as flavonoids, so it concluded that the flavonoids in the fruit test *R. mucronata* could be done using both types of such solvents.

Rutin levels of *R. mucronata* fruit ethanol extract produced a value of 13.29% ± 0.43. According to Markham (1988), the levels of flavonoids and other phenolic compounds in plants vary between each part, tissue, and age of plants, and are influenced by environmental factors. These factors are temperature, UV-vis light, nutrition, water availability, and levels of CO_2 in the atmosphere.

Analysis of FT-IR (Fourier Transform Infrared Spectroscopy)

Infrared spectrum analysis showed the bands in the local catchment area number 3260.1660, 1620, 1520, 1440, 1365, 1285, 1260, 1225, 1200, 1175, 1145, 1125, 1080, 1040, 1010, 940, 860, 835, 780 and 745. Uptake in wave numbers 3260 cm^{-1} is OH absorption phenols that have hydrogen bonding (Silverstein *et al.*, 1991). Bond types are shown in the catchment area 1300-800 cm-1 (C-C, C-O, C-N), 1900-1500 cm^{-1} (CP%O, CP%N, NP%O), 2300-2000 cm^{-1} (Ca"C, Ca"N), and 3000-2200 (C-H, O-H, N-H). Aromatic ring indicated by peaks that appear at 1650-1450 cm^{-1} region, isolated compounds providing a peak around 1620 cm^{-1} and 1520 cm^{-1} which is a strain of C=C aromatic and supported by an absorption band at 860 cm^{-1}, 835 cm^{-1}, 940 cm^{-1} and in the area of 1440 cm^{-1} there is a very strong and sharp band which is an aromatic ring strain.

Isolated compounds showed absorption at wave 1660 cm cm^{-1} which indicated the absorption of carbonyl group C = O, supported by a peak in 1145 cm^{-1}. According to the literature, the characteristics of C = O for flavonoid compounds are 1700-1750 cm^{-1} which is supported by the presence of peaks in the wavenumber region 1158 cm^{-1}. Uptake of carbonyl compounds is smaller isolated because of their conjugation bond. The carbonyl compounds here are esters which are reinforced by peaks in the region of 1300-1000 cm^{-1} (Soliman *et al.*, 2017; Bakkialakshmi, 2017)

CONCLUSION

R. mucronata fruit extract was identified to contain flavonoids in Rutin standard solutions, characterized in FT-IR testing which result strain C = O wavelength 1660 cm^{-1}

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