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DETERMINATION OF TOTAL PHENOLIC CONTENT, ANALYSIS OF BIOACTIVE COMPOUND COMPONENTS AND ANTIOXIDANT ACTIVITY OF ETHYL ASETATE SERI (*Muntingia calabura* L.) LEAVES FROM NORTH SUMATERA PROVINCE, INDONESIA

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Abstract

Seri (*Muntingia calabura* L.) leaves contain various bioactive compounds and have various potential activities. *M. calabura* leaves extraction was carried out by maceration method using ethanol followed by fractionation starting with n-hexane, chloroform, and finally ethyl acetate as solvent. The ethyl acetate fraction was continued for phytochemical screening for the content of bioactive compounds using standard reagents, determination of total phenol content by colorimetric method, determination of antioxidant activity using the DPPH method and analysis of bioactive compounds using gas chromatography mass spectroscopy. The results showed that the ethyl acetate fraction of *M.calabura* leaves was positive for phenolic content which was indicated by the formation of a turquoise color after 5% $FeCl_3$ reagent was added (in ethanol), phenolic content was 0.0727 mg GAE/g dry fraction, indicating antioxidant activity (IC_{50}). amounted to 54.437 including strong categories as antioxidants and the results of GC-MS analysis obtained various kinds of compounds and it is suspected that compounds that provide potential as antioxidants are pyhtol

Keywords: Seri leaves, *Muntingia calabura*, phenolic, antioxidant, and pyhtol

INTRODUCTION

Free radicals play many roles in human life. Free radicals in the body are produced from the metabolism of the ATP production process in the mitochondria. In general, the sources of free radicals are divided into Reactive Oxygen Species (ROS) free radicals and Reactive Nitrogen Species (RNS). Low concentrations of free radicals in the body have beneficial roles, but at high concentrations they can cause oxidative stress and damage cell walls that can trigger the emergence of various chronic and degenerative diseases such as cancer, arthritis, aging, cardiovascular and neurodegenerative (Oliveira et al. , 2017; Gurning). et al., 2021). Minimizing the impact and influence caused by free radicals requires an antioxidant compound. Based on the body's acquisition of antioxidant compounds are divided into endogenous and exogenous. Endogenous are antioxidant compounds produced by the body while exogenous are obtained by the body through consumption of various foods such as vegetables, fruits, nuts, seeds, spices, and oils (Rizzo et al., 2010). One of the potential plants that can be tested for its potency as an antioxidant is seri leaf (Mutingia calabura L.). The content of bioactive compounds include phenolics, flavonoids,

tannins, triterpenes, saponins and alkaloids (Yusof et al., 2013; Mahmood et al., 2014; Buhian et al., 2016). This plant is widely found in Indonesia and grows and breeds on the side of the road and is widely used as a shade tree. Various groups of bioactive compounds contained in the plant *Muntingia calabura* (*M.calabura*) attracted the attention of researchers to determine the quantitative analysis of bioactive compounds, especially phenolics and tested their pharmacological activities as antioxidants with the 2,2-diphenyl-1-dipicrylhydrazil (DPPH) method.

MATERIALS AND METHODS

Materials

The materials used include ethanol (Merck), chloroform (Merck), n-hexane (Merck), ethyl acetate (Merck), Folin-Ciocalteu, Whatman No 1 filter paper, Na_2CO_3 (Merck), standard reagents for phyrochemical filtration, acid gallate (Merck), $FeCl_3$ (Merck), methanol (pa), DPPH (Merck), Vitamin C (Merck) and distilled water.

M.calabura sample preparation

Samples of *M.calabura* leaves were obtained from Namorambe Regency, North Sumatra Province, Indonesia with fresh and green conditions from fruiting trees. The sample was determined and validated by a botanist with a registration number (No.5107/MEDA/2020) at the Medanense Herbarium laboratory, Universitas Sumatera Utara. The samples were cleaned with running water, drained and then dried in a drying cabinet at a temperature of 50°C. The dry samples were ground using a kinetic blender to obtain simplicia powder. Simplicia powder was placed in a storage container and placed in a botanical laboratory before use.

Extraction of *M.calabura* Leaves bioactive compounds

M. calabura simplicia powder was extracted by immersion (maceration) method using ethanol as a solvent at room temperature with occasional stirring with the aim of optimizing the extraction of bioactive compounds for 3 days. Once achieved, then filtered using whatman paper No. 1, so that the liquid *M.calabura* leaves ethanol extract was obtained. The residue was re-macerated 2 times to optimize filtration for 2 days following the previous steps. The ethanol extract of *M. calabura* leaves was concentrated using a vacuum rotary evaporator at 55°C and a thick extract was obtained. The viscous extract was then made graded fractionation based on the difference in solvent polarity, starting with separation with *n*-hexane solvent (the purpose of separating non-polar bioactive compounds), chloroform solvent (separation of semi-polar bioactive compounds), and ethyl acetate solvent (separating low polar bioactive compounds). This fractionation process with the liquid-liquid principle using a separating funnel then obtained the *n*-hexane fraction and ethanol extract residue. Then the ethanol extract residue was fractionated again with chloroform and then with ethyl acetate. For each treatment, the fractionation was repeated 3 times to maximize the fractionation of the bioactive compounds contained based on the polarity of the solvent. The ethyl acetate fraction obtained was concentrated using a vacuum rotary evaporator at 55°C, phytochemical screening, determination of total phenolic content, determination of activity as an antioxidant and analysis of components of bioactive compounds using GC-MS.

Phytochemical screening

Phytochemical screening was carried out as the first step in identifying the group of bioactive compounds contained in the ethyl acetate fraction of *M. calabura* leaves. Identification of bioactive

compounds including flavonoids, alkaloids, saponins, phenolics, tannins, triterpenoids and steroids using standard reagents (Gul et al., 2017; Mahmood et al., 2019; Syahrina et al., 2020; Gurning et al., 2020).

Determination of the group of total phenolic compounds

Determination of total phenolic compounds using a modified colorimetric method using Folin-Ciocalteu reagent, adding Na_2CO_3 with a concentration of 7% and measuring its absorbance at a wavelength of 765 nm by spectrophotometry (Pochapski et al., 2011; Liaudanskas et al., 2017). Determination of phenolic content using standard gallic acid solution with various concentrations of 50 ppm, 75 ppm, 100 ppm, 125 ppm and 150 ppm with methanol as solvent. Each of the concentration variations was taken 200 µL, then added 1 mL of Folin-Ciocalteu and allowed to stand for 5 minutes. Followed by the addition of 4 mL of 7% Na_2CO_3 and distilled water up to 10 mL. The solution mixture was incubated for 30 minutes, then the absorbance was measured at a wavelength of 765 nm. Absorbance measurements were carried out 3 times. The linear regression equation obtained is y=0.0042x-0.0468 with R^2= 0.9793 (Fig. 1). Determination of the phenolic content of the ethyl acetate fraction of M. calabura leaves followed the same procedure with a concentration of 1000 ppm. Phenolic content is expressed in mg by weight of gallic acid equivalent per g dry fraction (mg GAE/g dry fraction).

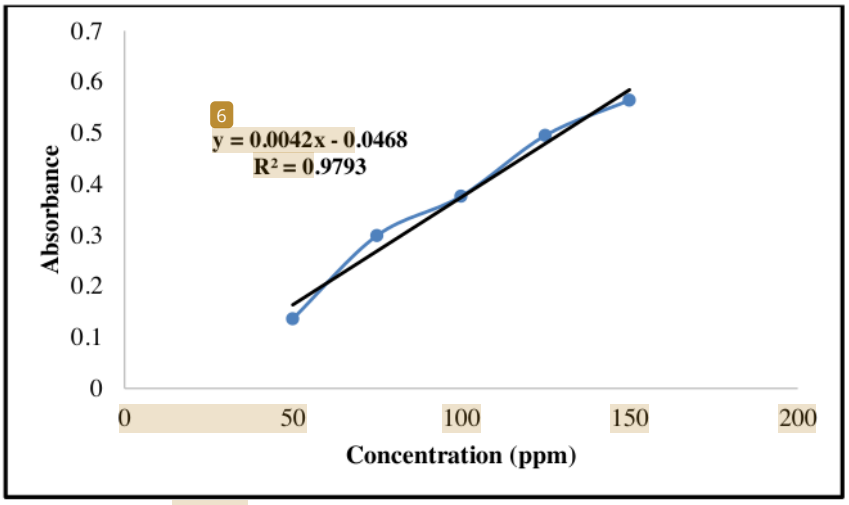

Figure 1. Gallic acid standard solution curve

Determination of antioxidant activity of *M.calabura* leaves fraction

Antioxidant activity of *M.calabura* leaves ethyl acetate fraction by modified DPPH method. Variations in the concentration of the *M.calabura* leaves ethyl acetate fraction used 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm. The volume of each concentration used was 500 µL, then 1 mL of 0.4 mM DPPH was added and ethanol was added up to 5 mL. The mixture was incubated for 30 minutes. After the incubation time was reached, the absorbance was measured at 517 nm (Naz et al., 2013). The same procedure was carried out for vitamin C as a positive control with variations in concentrations of 6.5 ppm, 7.0 ppm, 7.5 ppm, 8.0 ppm, and 8.5 ppm. Negative control was carried out by adding 1 mL of 0.4 mM DPPH and adding ethanol to 5 mL. Absorbance

measurements were carried out 3 times. Determination of free radical inhibition by following the following equation.

$$\text{Inhibition}(\%) = \left[\frac{A_{Blank} - A_{Sample}}{A_{Blank}}\right] x\ 100$$

Linear regression equation in determining the value of antioxidant activity by plotting the percentage of inhibition curve against the concentration variations in order to obtain a linear regression equation. The obtained linear regression equation was used to calculate the value of antioxidant activity (IC_{50}). The IC_{50} value is expressed as the ability of the antioxidant activity of the ethyl acetate fraction leaves to reduce free radicals originating from DPPH by 50% of the initial concentration.

Component analysis of bioactive compounds by GC-MS

The viscous fraction of *M.calabura* leaves ethyl acetate was analyzed using Thermo Scientific Trace 1310 Gas Chromatogrph with column HP-5MS UI operating at an electron collision energy of 70 eV and ISQLT Quadropole Mass Spectrometry specifications. The carrier gas used is Helium with an injection volume of 0.5 µL with a split less injection temperature of 300°C, an ion source temperature of 280°C with a mooring time of 5 minutes starting with a temperature of 100°C to 300°C with a temperature change rate of 5°C. The identified active compounds were compared with peak retention times with the Chromeleon smilirity library MS instrument.

RESULTS AND DISCUSSION

Skrining fitokimia *of ethyl acetate fraction of M.calabura Leaves*

Phytochemical screening aims to obtain initial information on groups of bioactive compounds identified using standard reagents (Table 1). The results of phytochemical screening showed positive groups of flavonoid compounds, saponins, tannins, triterpenoids/steroids and phenolics. The phenolic group was indicated by the formation of a green or blue-green color, but the ethyl acetate fraction leaves of *M, calabura* negative contained alkaloids.

Table 1. Results of phytochemical screening of the ethyl acetate fraction of M.calabura

No.	Compound Group	Reagents	Results
1.	Alkaloids	Dragendrof	-
		Mayer	-
		Libermann Bouchard	-
2.	Phenolic	$FeCl_3$ 5% (at etanol)	+
3.	Flavonoids	Shinoida test	+
4.	Saponins	Foaming test	+
5.	Tannins	$FeCl_3$ 1%	+
6.	Triterpenoids/steroids	Libearmann Bourchard	+

Description: (+) positive contains and (-) negative contains

Determination of total phenolic content *of ethyl acetate fraction of M.calabura Leaves*

The total phenolic content of the ethyl acetate fraction of *M.calabura* leaves from the linear regression equation was obtained y=0.0042x – 0.0468 with R^2 = 0.9793 from 0.0727 mg GAE/g

dry fraction. The quantitative results of this group of phenolic compounds are in line with the phytochemical screening data using $FeCl_3$. Phenolic content was reported to show activity as an antioxidant, anticancer, anti-inflammatory, antibacterial, xanthine oxidase inhibitor (Maria et al., 2018).

Antioxidant activity *of ethyl acetate fraction of M.calabura Leaves*

Antioxidant activity was determined by the DPPH method using ethanol as a solvent and measured at a maximum wavelength of 517 nm DPPH. The selection of the DPPH method in determining antioxidant activity is based on better sensitivity, relatively low cost, and simple and fast processing. The antioxidant activity value of the ethyl acetate fraction of *M.calabura* leaves expressed in Inhibitory Concentration 50 (IC_{50}) from the linear regression equation y= 0.2479x + 36.505; R^2 = 0.9859 (Fig. 2) of 54,437 in the strong category and IC_{50} for vitamin C of 1.657 in the very strong category. The antioxidant activity of ethyl acetate fraction is supported by its ability to release protons to stabilize free radicals from DPPH to DPPH-H so as to produce neutral conditions (Gurning et al., 2021). These results were in line with the presence of phenolic content which is responsible for providing antioxidant activity.

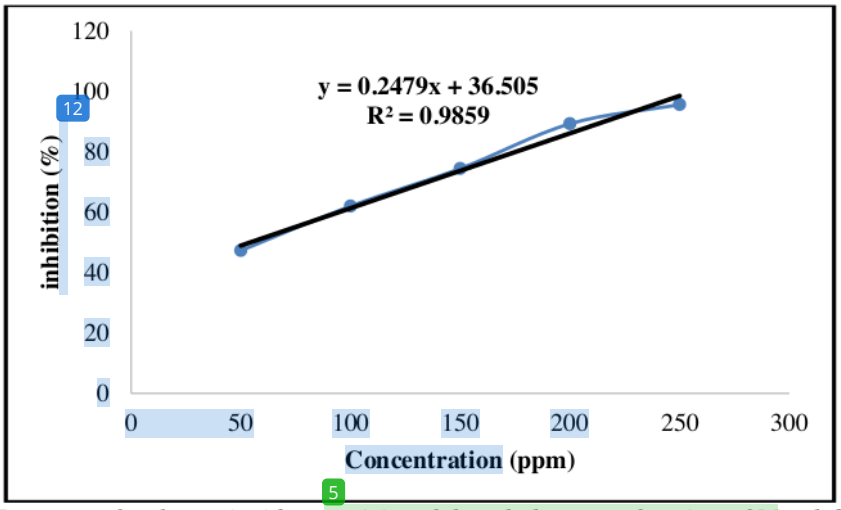

Figure 2. Test curve for the antioxidant activity of the ethyl acetate fraction of M.calabura leaves

GC-MS analysis *activity of the ethyl acetate fraction of M.calabura leaves*

Analysis using GC-MS showed the content of bioactive compounds in the ethyl acetate fraction of *M.calabura* leaves. The results of the GC-MS analysis were shown in Fig.3.

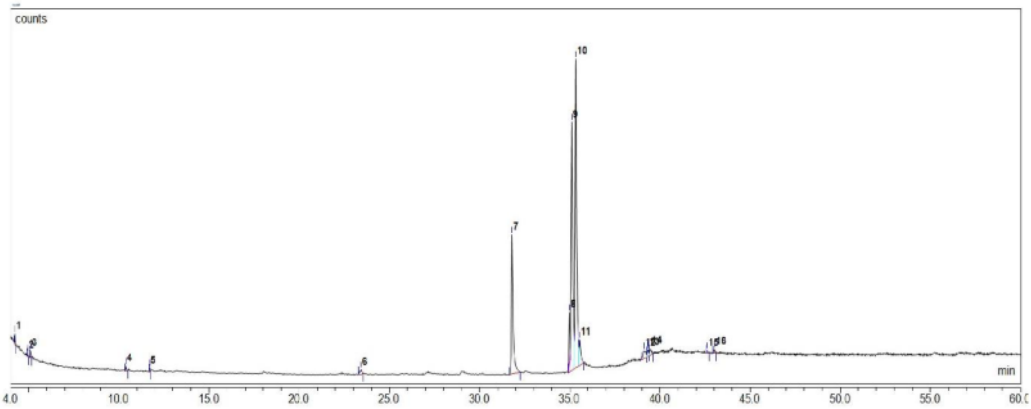

Figure 3. GC-MS Chromatogram M.calabura leaves ethyl acetate fraction

The GC-MS chromatogram showed that there were 16 types of bioactive compounds with the largest retention time span of 4.0-45.0 minutes. The components with the largest percentage reported there were 5 bioactive compounds presented in Table 2.

Table 2. Analysis of bioactive compounds from ethyl acetate fraction of leaves seri with GC-MS

No.	Compound Name	Molecular formula	T_R (menit)	% area	Evidence peak
1.	Phytol	$C_{20}H_{40}O$	35.33	34,84	10
2.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	35.11	3332	9
3.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	31.79	17.77	7
4.	17-Octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	35.55	3.70	11
5.	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	23.43	3.39	8

The largest bioactive compound contained was pyhtol. Pyhtol was reported to have potential activity as antimicrobial, insulin enhancing, anticancer, antidiuretic (Pratama et al., 2019), anti-inflammatory (Vats et al., 2017), antioxidant (Casuga et al., 2016; Thakor et al., 2016; Ishtiaq et al., 2020). Researchers suspect that the content of these compounds provides potential activity as antioxidants, but there were still wide opportunities for other compounds that provide synergism as antioxidants.

CONCLUSION

The ethyl acetate fraction of *M.calabura* leaves has a phenolic content qualitatively with 5% $FeCl_3$ (in ethanol) characterized by the formation of a turquoise color and quantitatively using a colorimetric method of 0.0727 mg GAE/g fraction, has a potential antioxidant activity (IC_{50}) of 54.437 categorized as strong category. The results of the analysis with GC-MS showed the potential as an antioxidant pyhtol.

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