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DETERMINATION OF PHENOLIC, FLAVONOID CONTENT, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SERI (*Muntingia calabura* L.) LEAVES ETHANOL EXTRACT FROM NORTH SUMATERA, INDONESIA

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ABSTRACT

Seri (*Muntungia calabura* L) leaves have various secondary metabolite components and it is necessary to carry out phytochemical screening and determination of their levels as well as in vitro testing of their bioactivity as antioxidants and antibacterials. Extraction was carried out by maceration method with ethanol as solvent. Determination of phenol with Folin-Ciaocalteu reagent was measured at a wavelength of 765 nm, while the levels of flavonoids with $AlCl_3$ reagent were measured at a wavelength of 431 nm using the colorimetric method, antioxidant activity testing with 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured at a wavelength 517 nm using UV-Vis spectrophotometry, and antibacterial activity testing was carried out on the bacteria *Erchericia coli, Staphylococcus aureus*, and *Staphylacoccus epidermidis* using the disc paper diffusion method with various concentrations of 12.5%, 25%, 50%,75%, DMSO10% for control negative and Chlororamphenicol as a positive control by observing the clear zone in mm. Phenolic content equivalent to 2.258±0.008 mg gallic acid (mg GAE/g dw), flavonoid content 2.476±0.019 mg equivalent to quercetin (mg QE/g dw), antioxidant activity of ethanol extract of *M. calabura* L. leaves, leaves was 19.004, antibacterial activity against *Erchericia coli*, *Staphylococcus aureus,* and *Staphylacoccus epidermidis* with a strong category. The ethanol extract of the of Seri (*Muntingia calabura*, L) leaves contains flavonoids, phenolics and shows activity as an antioxidant with a very strong category and a strong antibacterial category

Keywords: *Muntingia calabura* L., Phenolic, Flavonoids, Antioxidant and Antibacterial

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INTRODUCTION

Indonesia stores abundant natural resources to be explored in various utilization activities, especially in the health sector. One of the potential plants, namely seri leaves or known by the Latin name, namely *Muntingia calabura* L (*M. calabura*).[1] This plant contains various secondary metabolites that can be measured and determined by pharmacological activity in various uses in the health sector. *M. calabura* has other parts of roots, stems, skins, fruit, leaves and flowers that contain metabolites that can be determined between and tested for their potential activities in various health fields.[2]

The content of secondary metabolites found in *M. calabura* includes glycosides, flavonoids, tannins, saponins, phenolics, and alkaloids.[3,4,5] Various secondary metabolites are reported to show potential activity including acute toxicity, citotoxic, antiproliferative, quinone reductase, antiplatelet aggregation, antibacterial, antioxidant, anti-insecticide, antimicrobial, antinociceptive, anti-inflammatory, antipyretic, antiulcer, antidiabetic, antihypertensive, and cardioprotective.[4,5,6] The sample in this study was of *M. calabura* leaves. The *M. calabura* leaves were extracted and their secondary metabolites (phenolic and flavonoid groups) were determined and tested for their activity as antioxidants by the 2,2-diphenyl-1-dipicrylhydrazyl (DPPH) method and the antibacterial activity was tested using the paper disc diffusion method.

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EXPERIMENITAL

Materials

Ethanol (Merk), dragendroff reagent, $FeCl_3$ (Merk), gallic acid (Merk), DPPH (Merk), Folin-Ciocalteu reagent, $AlCl_3$ (Merk), Liberman-Burchard reagent, dragendorf reagent, acetic anhydride (Merk), Mg (Merk), HCl (Merk), Mueller Hinton Agar (MHA), Stick L, *Staphylococcus aureus*, *Escherichia coli, Staphylococcus epidermidis,* DMSO, petridish saucer and distilled water

Plant Collection

The *M. calabura* leaves samples used were fresh from plants that had fruit and did not concider the size. Samples were taken from the village of Namorambe, Medan Tuntungan District. Sample identification by a botanist at Herbarium Medanense, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Indonesia (5107/MEDA/2020). The sample was cleaned in running water, drained and dried in an open room which was protected from direct sunlight. The dry sample was mashed to obtain a mixture of *M. calabura* simplicia.

Preparation of *M. calabura* Leaves Extract

The process of preparation *M. calabura* extract uses a standard extraction procedure with a modified maceration method, then evavorated using a vacuum rotary evavorator (Heidolph) at a temperature of 50°C to obtain a crude extract.[7,8]

Phytochemical Screening

Phytochemical screening was used to provide information on the class of secondary metabolite compounds contained in the ethanol extract of *M. calabura* leaves. Phytochemical screening was carried out using standard methods. [9,10]

Determination of Phenolic contents

Total phenolics were determined using a colorimetric method using the Folin-Ciocalteu reagent. Samples and standard solutions were measured using UV-Vis (Genesis 10S UV-VIS) spectrophotometry at a maximum wavelength of 765 nm. The test sample (0.2 mL) was mixed with Folin-Ciocalteu (0.4 mL) and allowed to stand at room temperature for 5 minutes. After 5 minutes, 4 mL 7% Na_2CO_3 were added and distilled water was added to 10 mL. The mixture was centrifuged and the reaction allowed to stand at room temperature for 30 minutes, then measured at 765 nm.[11,12] Phenolic content was calculated and expressed as gallic acid equivalent (GAE/ g dry sample) based on the standard curve of gallic acid (50-150 ppm / $y = 0.0038x + 0.0056$; $R^2 = 0.9531$). All measurements were carried out with three replications.

Determination of Flavonoid Contents

Total Flavonoid were determined by colorimetric method using 10% $AlCl_3$ reagent. Samples and standard solutions were measured using UV-Vis (Genesis 10S UV-VIS) spectrophotometry at a maximum wavelength of 431 nm. The test sample (1 mL) was mixed with 10% (0.2 mL) $AlCl_3$ and added distilled water up to 10 mL. The mixture was centrifuged and the reaction left to stand at room temperature for 30 minutes, then measured at 431 nm.[11,12] Flavonoid content was calculated and expressed as quercetin equivalent (QE/g d.w) based on the standard quercetin curve (25-200 ppm/ $y = 0.0006x + 0.0024$; $R^2 = 0.9977$). All measurements were carried out with three replications.

Determination of Antioxidant Activity with the DPPH Method

The concentration variations of the ethanol extract of *M.calabura* leaves used were 10 ppm; 20 ppm; 30 ppm; 40 ppm; and 50 ppm. Each concentration was taken 1 mL and then added 1 mL of DPPH 0.4 mM, ethanol added to the limit of 5 mL mark. Samples were left at room temperature until they reached operating time for 30 minutes. After reaching the operating time, the absorbance of the maximum wavelength of 517 nm was immediately measured. All measurements were carried out with three replications. Determination of% free radical inhibition with the equation:[13,14,15]

$$\text{Inhibition (\%)} = \left(\frac{A_b - A_s}{A_b}\right) x\ 100\%$$

Where: Ab = absorbance of blank and As = absorbance of test sample. Inhibition (%) was plotted against the concentration and a linear regression equation (y = bx ± a) was obtained to obtain IC_{50} in determining the antioxidant activity of the sample.

Test Bacterial

Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), and *Staphylococcus epidermidis (S. epidermidis)*. Antibacterial activity test of ethanol extract of seri (*Muuntingia calabura* L,) leaves by diffusion method using disc paper. Bacterial culture was carried out at 37ºC for 24 hours with a standard turbidity of 0.5 Macfarland before being inoculated into MHA media. The bacterial medium was dropped 0.1 mL and then flattened using an L rod. Each paper disc was dipped into each of the ethanol extracts of *M. calabura* leaves with a concentration variation of 12.5%; 25%; 50%; and 75%. DMSO 10% was used as a negative control and chloramphenicol as a positive control. Inoculation was carried out for 24 hours at 37ºC and then the clear zone was observed. Repetition was carried out 3 times and the zone of inhibition was measured in mm.[16,17]

Statistical analysis

The results of testing the antibacterial activity of each concentration to observe the distribution of the data were repeated in triplicate expressed by the mean ± standard deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) with multiple comparisons between data using Tukey's real difference test (HSD) using SPPSS software with $P < 0.05$.[18]

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical analysis of the secondary metabolites contained in the ethanol extract of *M. calabura* leaves. The qualitative results show that in the ethanol extract of *M. calabura* leaves there were groups of secondary metabolites, including phenolics, alkaloids, flavonoids, saponins, tannins, steroids and terpenoids. The presence of various secondary metabolites in the ethanol extract of *M. calabura* leaves provides important information as a preliminary tool for testing pharmacological effects in various potential activities.

Total Phenolic Content

The total phenolic total obtained from the ethanol extract of *M. calabura* leaves obtained from the linear regression equation y = 0.0038x + 0.0056 and the calibration curve $R^2 = 0.9531$ is 2.258 ± 0.008 mg equivalent to gallic acid/g dry sample weight (mg GAE/g d.w). The existence of phenolic compounds has a role as antioxidants, this is supported by the presence of a hydroxyl group which allows the nature of redox reactions in deactivating free radicals.[19]

Total Flavonoids Content

The total flavonoids contained in the ethanol extract of *M. calabura* leaves from the linear regression equation were y = 0.0006x + 0.0024; and the calibration curve $R^2 = 0.9977$ is 2.476 ± 0.019 mg equivalent to quercetin/g dry sample weight (mg QE/g d.w). Based on their functional groups, flavonoids have various derivatives including flavones, and flavanols which have free -OH groups which show activity as antioxidants that can be tested in vitro and invivo.[19]

Antioxidants Activity

The selection of the 2,2-diphenyl-1-dipikhirhidrazil (DPPH) method in determining the activity of the ethanol extract of *M. calabura* leaves as an antioxidant was due to its easy working method, fast analysis process, and better sensitivity when compared to other testing methods.[10] The role of DPPH in determining the antioxidant activity of the sample is as a source of free radicals which give the solution a purple color. The ability of the ethanol extract of *M. calabura* leaves as an antioxidant was indicated by the reduction in color from purple to yellow solution. The reduction in yellow color that occurs was indicated by a reduction in radical concentration as seen from the smaller absorbance reading along with the increase in the concentration of the extract measured at the maximum wavelength of DPPH (517 nm) dissolved with ethanol (Fig.-1).The result of the calculation of antioxidant activity is determined from the lineai regression equation from the curve between the concentration (x-axis) and the percent of inhibition

(y-axis). The linear regression equation is y = 1.6928x + 17.83 with R^2 = 0.9708 so that the activity value (IC_{50}) is 19.004. The activity value obtained is in the very strong category as an antioxidant.[20] The possible reaction mechanism to stabilize free radicals from DPPH is by transferring protons from ethanol extract of *M. calabura* leaves.

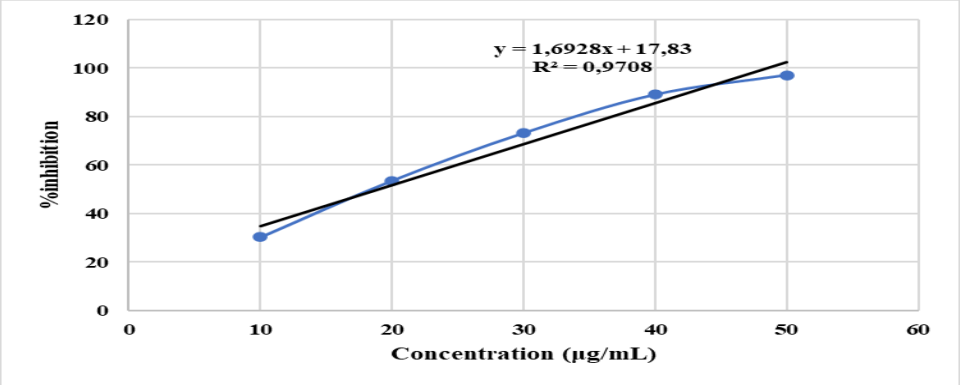

Fig.-1: Measurement Results of %Inhibition of *M.calabura* As An Antioxidant

Antibacterial Activity

The results of the bioactivity of the ethanol extract of *M. calabura* leaves against *E. coli, S. aureus,* and *S. epidermidis* bacteria are shown in Table-1 and Figure-2.

The results of the antibacterial activity test of the ethanol extract of *M. calabura* leaves showed that it has a strong category of activity capability for all bacteria with various concentrations.[21,22] One of the mechanisms of action through the transport of cell membranes of microorganisms causes changes in permeability and leakage of intracellular plasma membranes which can increase efficiency as a antibacterial and mildew.[18]

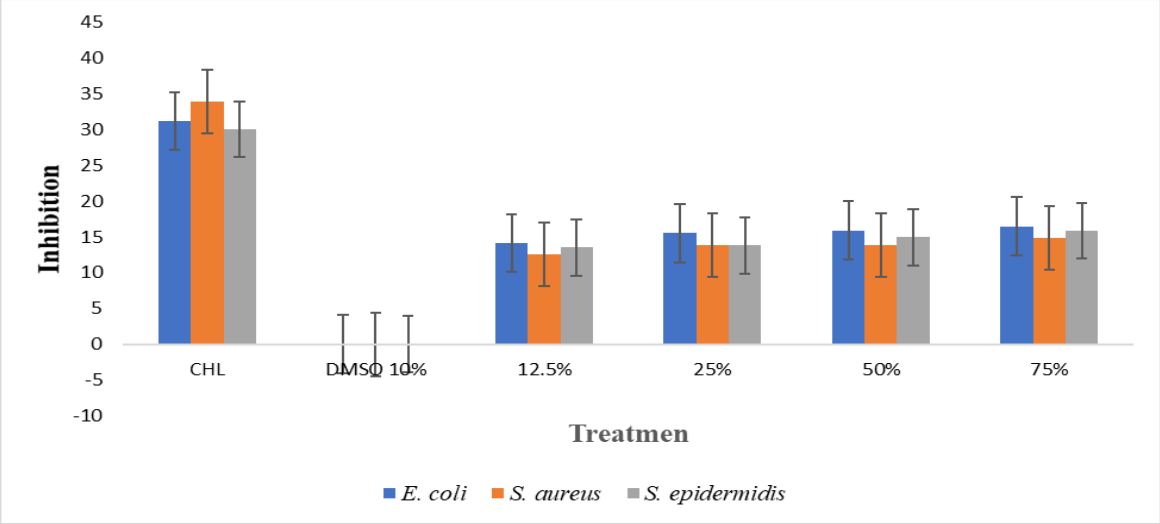

Fig.- 2: The Results of the Antibacterial Activity of the Ethanol Extract of *M. calabura L* Leaves

Table-1: Zona of Inhibition of Ethanol Extracts *M. calabura* Leaves

Ethanol Extracts *M. calabura* Leaves	Bacteria	Treatment					
		Control		extract concentration variations			
		CHL (+)	DMSO10% (-)	12.5%	25%	50%	75%
	E. coli	31.25 ± 2.08	0	14.18 ± 0.96	15.53 ± 0.40	15.92 ± 1.27	16.50 ± 0.52
	S. aureus	33.97 ± 3.19	0	12.62 ± 0.16	13.83 ± 0.78	13.87 ± 0.72	14.82 ± 0.35
	S. epidermidis	30.10 ± 1.01	0	13.53 ± 0.42	13.79 ± 0.37	14.95 ± 0.88	15.90 ± 0.62

Result is expressed as mean ± SD, n = 3

CONCLUSION

The ethanol extract of *M. calabura* leaves showed phenolic content of 2.258 ± 0.008 (GAE mg / g d.w); flavonoids 2.476 ± 0.019 (QE mg/g d.w). The activity value was 19.004 categorized as very strong antioxidant dan shown the strong potential as antibacterial to *E.coli, S. aureus,* dan *S. epidermidis*.

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