Determination of Total Tannins and Antibacterial Activities Ethanol Extraction Seri (Muntingia calabura L.) Leaves

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Abstract. This research aims were to determine the total tannin level and to test the antibacterial activity of the ethanol extract of *Muntingia calabura* L., leaves. The extraction was carried out by maceration method with ethanol solvent. Determination of total tannin content was carried out by colorimetric method which was measured at a wavelength of 745 nm using UV-Vis spectrometry and tannic acid was used as the standard. The antibacterial activity test was carried out by diffusion disc method against, such as *Escherichia coli*, *Salmonella typhi* and *Propionebacterium acnes* bacteria. The variations in the concentration of the ethanol extract of *M. calabura* included 12.5%; 25%; 50%, and 75%. Chloramphenicol was used as positive control and DMSO 10% as negative control. The results showed that the ethanol extract of the *M. calabura* leaves had a total tannin level of 0.0655±0.0002 mg/g d.w (Mean±SD). Antibacterial activity of ethanol extraction by the *M. calabura* leaves against *E. coli* (14.18±0.96; 15.53±0.40; 15.92±1.27; and 16.50±0.52), *S. typhi* (13.37±0.35; 14.47±1.14; 14.97±0.87; and 15.50±0.66), and *P. acnes* (14.13±0.24; 14.60±0.20; 15.52±1.14; and 16.37±0.46). Antibacterial activity of Chloramphenicol against *E. coli*, *S. typhi* and *P. acnes* are 31.25±2.08; 25.15±1.61; and 23.25±4.42.

1. Introduction

The research of testing and finding potential compounds from natural materials become the center of researchers attention on this time. Potential compounds were contained in natural materials have various kinds of bioactivity that can be tested, including as antioxidants [1-3], antibacterial [3,4], uric acid [5], anticancer [6], antimicrobial, hypoglycemic, hypolipidemic, anxiolytic, analgesic, and anti-inflammatory [7]. The plants was have the potential for bioactivity to be tested that's plants of the series leaves (*Muntingia calabura* L.).

M. calabura the potential bioactivity such as antiproliferative, antioxidant, antinociceptive, cardioprotective, antipyretic [8], anti-inflammatory, antibacterial, antidiabetic, and antimicrobial [9]. The content of potential compounds in bioactivity, namely the flavonoid group, sesquiterpenes, chalcanones, phenolics, polyphenols and steroids [9,10]. The based on this description, this study was

aim to test the bioactivity of potential ethanol extract of *M. calabura* leaves against *Escherichia coli*, *Salmonella typhi* and *Propionebacterium acnes* bacteria.

2. Materials and Methods

2.1. Plant colection

M. calabura leaves was collected from Namorambe village, Medan sub-district of Tuntungan , North Sumatra Indonesia and was determined at Herbarium Medanense, Faculty of Mathematics and Natural of Sciences, University of North Sumatera, Indonesia (5107/MEDAN/2020). the leaves was used are fresh, from trees was ever been fruitful and do not pay attention for the leave size. The leaves of *M. calabura* were washed in running water, dried by aerating and avoid direct sun contact, then crushed to obtain simplicia powder.

2.2. Preparation of ethanol extract of M. calabura leaves

The simplicia leaves of *M. calabura* 1,000 g were extracted with ethanol solvent (p.a) by maceration method for five days at room temperature, then filtered to obtain a liquid ethanol extract of *M. calabura* leaves. The ethanol extract was concentrated with a vacuum rotary evaporator at 50°C in order to obtain a crude extract in concentrated conditions [11,12].

2.3. Preliminary phytochemical screening

Phytochemical screening aims to identify the class of compounds contained in the ethanol extract of *M. calabura* leaves. Classes of compounds identified include alkaloids with Dragendroff reagent, flavanodies with Salkowski reagent, saponins with foam testing, tannins with $FeCl_3$, steroids and terpenoids with Salkowski reagent [13].

2.4. Determination of tannin content

Each 200 µL series leaves extract was added with 200 µL of Folin Ciocalteu reagent, then left to stand for 5 minutes. Then add 100 µL of saturated Na_2CO_3 until and add distilled water to 5 mL. then incubated for 35 minutes and the absorbance was measured at 745 nm. Tannic acid is used as standard. Tannin content is expressed as mg of tannic acid equivalent per gram of weight of dried sample used (mg TAE/g d.w).

2.5. Antibacterial assay

The equipment used was sterilized in an oven at 100°C for 2 hours. Activity testing of the ethanol extract of *M. calabura* leaves was carried out by the disc diffusion method using sterile media of Nutrient Agar (NA). The organisms was tested and incubated overnight in NA at 37°C with a turbidity standard of 0.5 McFarland. The determination of antibacterial activity by using NA media was inserted into each sterile petridish as much as 20 mL. The ethanol extract of *M.calabura* was dissolved with Dimethyl Sulfoxide (DMSO) to obtain a concentration of 12.5%; 25%; 50%, and 75%. The negative control used DMSO 10% and the positive control used chloramphenicol. The sterile disc paper with a diameter of 6 mm was smeared with each extract's concentration was dropped in such a way on a petridish dish filled with bacteria. The bioactivity potential of the ethanol extract of *M. calabura* was tested on the bacterium Escherichia coli, Salmonella typhi and Propionebacterium acnes. Incubation was carried out for 24 hours at 37°C and then observed a clear zone around the disc paper that was placed, according to the determination of the activity of the extract's inhibitory zone against antimicrobial activity. The zone of inhibition is measured in mm with three repetitions [14-16].

3. Results and Discussion

3.1. Preliminary phytochemical screening

The results of phytochemical screening contained in the ethanol extract of *M. calabura* leaves are presented in Table 1. The groups of compounds present in the ethanol extract of *M. calabura* leaves contain alkaloids, flavonoids, saponins, tannins, steroids and terpenoids.

Table 1. Phytochemical screening of ethanol extract of *M. calabura* leaves

Phytochemical compounds	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Tannis	+
Steroids/ Terpenoids	+/+

3.2. Total tannins content

Determination of the tannin content contained in the ethanol extract of *M. calabura* leaves using tannic acid as a standard by UV-Vis sepktrophotometry measured at a maximum wavelength of 745 nm. The results of measurements of total tannins contained in *M. calabura* leaves extract are expressed as tannic acid equivalent per gram of weight of dried sample used (mg TAE/g d.w) in Table 2. Tannin content showing the potential for antibacterial activity the higher the content in a sample, the better the potential for antibacterial activity [16,17].

Table 2. Measurement results for the total tannin content of the ethanol extract of *M. calabura* leaves

No.	Absorbance	Tannis Content (µg/mL)	Mean Tannis Content (µg/mL)	Total Tannis Content (mg GAE/g extract ethanolic)
1.	0.260	3.265		
2.	0.261	3.280	3.275±0.0009	0.065 ±0.0002
3.	0.261	3.280		

3.3. Antibacterial test

The test antibacterial bioactivity by ethanol extract of *M. Calabura* leaves was carried out by different bacteria, namely the bacterium *Escherichia coli*, *Salmonella typhi* and *Propionebacterium acnes* using the disc method. These bacterial strains are gram-positive and negative species that often cause infectious diseases. The results bioactivity from the ethanol extract of *M. calabura* leaves against various bacteria are presented in Table 3.

Tabel 3. Zona of inhibition of ethanol extracts *M. calabura* leaves

		Treatment					
		Control		extract concentration variations			
Ethanol Extracts *M. calabura* Leaves	Bacteria	Chloramphenicol (+)	DMSO 10% (-)	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. typhi	25.15±1.61	0	13.37±0.35	14.47±1.14	14.97±0.87	15.50±0.66
	P. acnes	23.25±4.42	0	14.13±0.24	14.60±0.20	15.52±1.14	16.37±0.46

Result are expressed as mean ± SD, n = 3

Based on the inhibition of various bacteria, it showed the ethanol extract of *M. calabura* was increased of bioactivity along with the increase in concentration. The bioactivity of the ethanol extract of *M. calabura* against *E. coli*, *S. typhi*, and *P. acnes* was in the strong category [16]. The activity from

ethanol extract of *M. calabura* leaves is still weak compared to chloramphenicol which is commonly used as an antibacterial drug. The results of phytochemical screening show that there are various classes of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, steroids and terpenoids which have a role in giving. The existence of the existing class of compounds makes it possible to work that is not synergistic between groups in testing various activities against antibacterials. This is because the alkaloid class of compounds has a mechanism of action to inhibit the synthesis of nucleic acid from bacteria, because the enzymes of dihydrofolate reductase and topoisimerase are inhibited. The flavonoids has interact with the membrane, thereby reducing the fluidity of bacterial cells which causes cytoplasmic damage or indirect damage resulting in osmotic lysis [16].

4. Conclusion

The ethanol extract of *M. calabura* leaves had a total tannin level of 0.0655±0.0002 mg TAE/g d.w and showed the strong antibacterial activity against *Escherichia coli*, *Salmonella typhi* and *Propionebacterium acnes*.

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