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Determination of Tannins Content and Antibacteria Activity Test of Ethanol Extract of Sirih Merah (*Piper Crocatum* Ruiz & pav.) Leaf from North Sumatera Province Indonesia

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ABSTRACT

Sirih merah (*Piper crocatum* Ruiz & Pav.) leaves has traditionally been used as a medicinal plant. The content of secondary metabolites contained is assessed to show pharmacological activity. The secondary metabolites in question include tannins, alkaloids, flavonoids, steroids/triterpenoids and saponins. This study aimed to determine the total tannin content and the potential antibacterial activity of the ethanol extract of *P. crocatum* leaves. Extraction process by maceration using ethanol, measurement of total tannin content by colorimetric method using UV-Vis spectrophotometry at a maximum wavelength of 745 nm and determination of antibacterial activity by disc diffusion. The results of this study showed that the ethanolic extract of *P. crocatum* leaves contained about 0.118±0.003 mg TAE/g of dry ethanolic extract. The results of activity testing against *Escherichia coli* and *Staphylococcus aureus* showed activity as antibacterial.

Keywords: Antibacterial, *Escherichia coli*, Sirih merah, *Staphylococcus aureus*, Tannins content.

INTRODUCTION

Diseases caused by microorganisms need to be considered seriously, beside it very contagious, they are also resistant to drugs that cause long-term healing and can cause death[1,2]. The form of transmission of microorganisms can occur directly through skin contact with patients and indirectly through air media. The percentage of contamination by microorganisms around us that causes disease include *Staphylococcus aureus* (11%), *Candida albicans* (13%), *Pseudomonas aeruginosa* (7.1%) and *Escherichia coli* (9.2%)3. Treatment caused by microorganisms generally uses antibiotics. The use of antibiotics, irrationally will cause resistance to drugs, so it is considered less profitable in the

treatment of diseases caused by microorganisms. The need for medicinal compounds that are active in dealing with microbes and do not cause resistance to their use, medicinal plants are assessed as being used[4]. Research on testing and developing potential bioactive compounds as antibacterials continues to be the center of attention of researchers[5,6]. This is because the use of bioactive compounds is considered to have efficiency and does not cause resistance to bacteria such as the use of synthetic drugs. One of the plants that is considered to have potential as an antibacterial is *P. crocatum*[7].

P. crocatum contains various kinds of bioactive compounds including polyphenols, alkaloids, tannins, essential oils, and flavonoids[8,9,10]. Various bioactive compounds have been reported to have various potential uses in medicine, including wound healing in diabetic patients[11], antioxidant[12,13], antidiabetic, anticancer[14,15], antiseptic, anti-inflammatory[16], rheumatoid arthritis[17,18], Antiallergic and antibacteria[19]. Based on this information, this study aimed to determine the total tannin content and to test the antibacterial activity of the leaves of *P. crocatum* extracted with ethanol against *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* bacteria.

MATERIALS AND METHODS

Place and time of research

The research was conducted at the Pharmaceutical Chemistry Laboratory, Sekolah Tinggi Ilmu Kesehatan Senior Medan, which is located on Jl. Jamin Ginting Km 8.1 No. 13 Medan. The research was carried out for 4 months (February–May 2020).

Tools and materials

The tools used were laboratory glassware, scissors, Whattman No. 1 paper, vacuum rotary evaporator, 100µL and 1000µL micro pipettes, test tubes, test tube racks, petri dishes, wire loops, digital caliper, stirring rods, aluminum foil, frosted paper, tweezers, spray bottle, oven, laminary air flow, vortex, spreader/L glass, incubator and UV-Vis spectrophotometry.

The materials used in this study were ethanol(pa), Folin-Ciocalteau, chloramphenicol, aquadest, Na_2CO_3(s), tannic acid (Merck), strains of *Escherichia coli* and *Staphylococcus aureus*, Nutrient Agar Media, alkaloid reagents, tannins, flavonoids, steroids/triterpenoids, saponins and disc paper.

Sample preperation and extraction process

The samples of *P. crocatum* leaves used were obtained from Namorambe District, North Sumatra Province, Indonesia. The Leaves samples used were fresh and good regardless of leaf size. Samples of *P. crocatum* leaves were tested by botanists at the Herbarium Medanense Laboratory, Universitas Sumatera Utara (No. 5113/MEDA/2020). Samples were cleaned in running water to separate and clean from the impurities, then drained and dried in an open room protected from direct sunlight. After drying the samples were mashed with an electric blender and *P. crocatum* leaves simplicia powder was obtained. The simplicia powder obtained was placed in the pharmacognosy laboratory before proceeding to the next stage.

500 g of simplicia powder was extracted with 96% ethanol by maceration method for 3 days in a macerator container with occasional stirring to optimize the extraction of bioactive compounds contained in simplicia powder. After it was reached 3 days ago, it was filtered using Whatman No.1 filter paper and then re-immersed on the filtered residue for 2 days with the same treatment as the previous stage. The first and second extracts were combined and then continued at the solvent separation stage to obtain a thick ethanolic extract of *P. crocatum* leaves.

Phytochemical screening

The ethanol extract of *P. crocatum* leaves was continued for phytochemical screening to identify the components of alkaloids, flavonoids, steroids and triterpenoids, saponins, and tannins using standard reagents.[20,21]

Determination of total tannins content

Determination of total tannin content by colorimetry using Folin-Ciocalteu reagent, saturated Na_2CO_3 and tannic acid as standard solutions. The sample was measured by spectrophotometry UV-Vis at a maximum wavelength of 745 nm standard solution[22]. Making a linear regression curve from variations in the concentration of tannic acid, namely 0.4; 1.0; 1.6; 2.0; 4.0; 8.0; 12.0 and 14.0 g/mL respectively 200 µL then added 100 µL Folin-Ciocalteau and shaken and allowed to stand

for 5 minutes. Then 100 μL of saturated Na_2CO_3 solution was added to each solution and distilled water was added up to the 10 mL mark, homogenized using a vortex at 3000 rpm and then incubated for 35 minutes. All solutions were measured absorbance at 745 nm. Determination of the total tannin content in the sample from a concentration of 1000 ppm ethanol extract of *P. crocatum* leaves by following the same steps in the standard solution. The total tannin content is expressed in mg Tannic Acid Equivalent per g weight of ethanolic extract (mg TAE/g ethanolic extract).

Determination of antibacterial activity

The antibacterial activity test of the ethanol extract of *P. crocatum* leaves was carried out by the paper disc diffusion method and as a medium using sterile Nutrient Agar (NA). All equipment used was first sterilized in an oven for 2 h at 100°C. Dimethyl Sulfoxide (DMSO) was used as a solvent to vary the concentration of 400; 300; 200 and 100 ppm for each test against bacteria *E. coli* and *S. aureus*, while DMSO 10% as a negative control and positive control was chloramphephenicol. The incubation process was carried out for 24 h at 37°C and then the clear inhibit was measured using a digital caliper (mm) with three repetitions[22].

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of the screening analysis of phytochemical compounds contained in the ethanol extract of *P. crocatum* leaves showed various kinds of compounds including alkaloids, flavonoids, steroids/ triterpenoids, tannins and saponins (Table 1).

Table 1: Phytochemical screening of ethanol extract of *P. crocatum* leaves

Phytochemical compounds	Reagent	Results
Alkaloids	Dragendroff	+
Flavonoids	Shinoida test	+
Steroids/triterpenoids	Liberman Bouchard	+
Tanins	$FeCl_3$ 1%	+
Saponins	Forth Methods	+

Total tannis content

Determination of tannin levels in ethanol extract of *P. crocatum* leaves using colorimetric method with Folin Ciocalteu reagent and saturated Na_2CO_3 measured using spectrophotometry at a wavelength of 745 nm. The linear equation obtained was standard, namely y: 0.0695x+0.0321 with R^2=0.9989 (Fig. 1). The calculated total tannin content was expressed in mg of Tannic Acid Equivalent per g weight of ethanolic extract (mg TAE/g ethanolic extract). The total tannin content in the ethanol extract of *P. crocatum* leaves was 0.118±0.003 mg TAE/g ethanolic extract.

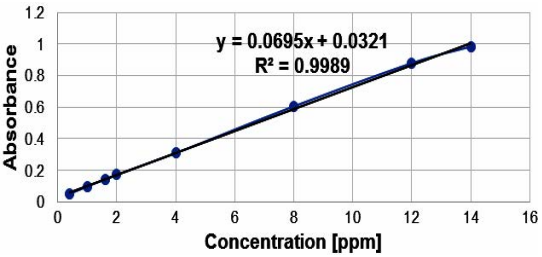


Fig. 1. Tannic Acid Standard Curve

Tannins are polyphenolic compounds that have molecular weights between 120-3000 Da and are soluble in water. Tannins are considered to have pharmacological effects as anti-inflammatory, anticancer, antidiabetic, antioxidant and antimicrobial[23]. In plants, this group of tannins plays a role in providing natural color[23].

Antibacterial activity test

The results of antibacterial bioactivity test ethanol extract of *P. crocatum* leaves contained chemical that can against *E. coli* and *S. aureus* bacteria by disc method. The test results of the ethanol extract of *P. crocatum* leaves containing *E. coli* and *S. aureus* were presented in Table 2 and Figure 2.

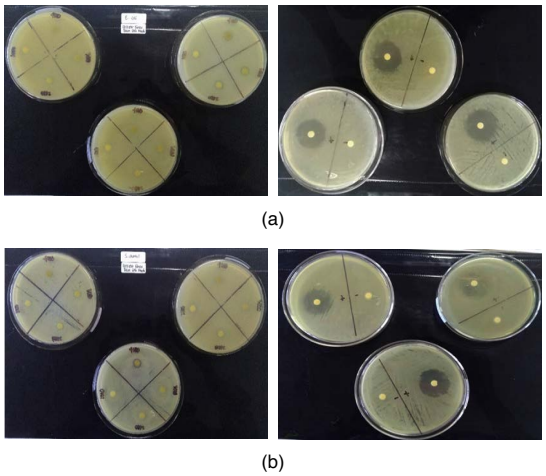
Benning zone measurement results of bacterial growth against *E. coli* and *S. aureus* bacteria from the ethanol extract of *P. crocatum* leaves showed that the antibacterial inhibitory activity was in the moderate category. This can be seen from the increase in the concentration of the ethanolic extract of *P. crocatum* leaves for concentrations of 400; 300; 200 and 100 ppm both showing moderate antibacterial activity against *E. coli* and *S. aureus* bacteria. The data from the phytochemical screening showed qualitative information data that the ethanol extract of *P. crocatum* leaves had various groups of bioactive compounds, including alakaloids, flavonoids, steroids/triterpenoids, tannins and saponins. Previous studies have reported that the tannin content in natural ingredients has

antibacterial, antiviral and antifungal activity. Tannins and polyphenolic compounds are components of potential herbal medicines that have medicinal properties as anti-pathogens, act as pre-biotic products in increasing immunity and tannins have a responsible role as antimicrobials[24].

Table 2: Results of the diameter of the clear inhibit on the antibacterial activity test of the ethanol extract of *P. crocatum* leaves

Extract ethanol of *P. crocatum* leaves	Bacteria	Control		Treatment extract concentration variations [ppm]			
		Chloramphenicol (+)	DMSO [10%]	100	200	300	400
	E. coli	31.25±2.08	0.00	6.27±0.06	6.70±0.10	7.57±0.40	9.60±0.53
	S. aureus	33.97±3.19	0.00	6.27±0.06	6.63±0.06	7.50±0.30	9.27±0.46

The results of testing the activity of the inhibition zone are expressed as mean ± SD, n = 3



(a)

(b)

Fig. 2. The results of testing the activity of the ethanol extract of *P. crocatum* leaves against bacteria n=3; (a) *E. coli* and (b) *S. aureus*

CONCLUSION

The results of this study indicate that the ethanol extract of *P. crocatum* leaves contains tannins with a total tannin content of 0.118±0.003 mg TAE/g ethanolic extract. The test results on *E. coli* and *S. aureus* bacteria with varying concentrations of ethanol extract *P. crocatum* including 400; 300; 200 and 100 ppm showing antibacterial activity of 6.27±0.06; 6.70±0.10; 7.57±0.40; 9.60±0.53 for *E. coli* and 6.27±0.06; 6.63±0.06; 7.50±0.30; 9.27±0.46 for *S. aureus* and was categorized as an moderate antibacterial.

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Conflict of interest

The authors declare that they have no conflict of interest.

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