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Determination of phenolic contents and antioxidant activity test of ethanol extract of Sirih merah (*Piper crocatum* Ruiz & Pav.) leaves using the DPPH method

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Abstract: The research objectives were to identify the secondary metabolite components, total phenolic content and determine the antioxidant activity of the ethanol extract of red betel leaf (Piper crocatum Ruiz & Pav.). The extraction process was carried out by materation using ethanol as a solvent. Determination of total phenolic content was carried out colorimetrically with Folin-Ciocalteu reagent measured at a maximum wavelength of 765 nm. Determination of antioxidant activity using the DPPH method measured by spectrophotometry at a maximum wavelength of 517 nm. The results of phytochemical screening of the ethanolic extract of red betel leaf contain secondary metabolites, including flavonoid, phenolic, tannin, alkaloids, steroids, and triterpenoids. The total phenolic content of the red betel leaf ethanol extract was 0.949±0.003 mg GAE/g d.w. and has antioxidant activity (IC50) 84,656 including strong category as an antioxidant.

Keywords: *Piper crocatum* Ruiz & Pav., Antioxidant, Ethanol extract, Folin-Ciocalteu and DPPH

1. Introduction

Natural resources have a lot of potential tobe used for various purposes, one of which is as an antioxidant. Antioxidants have an important role in deactivating free radicals that trigger various degenerative diseases. Free radicals are atomic or molecular species that are very reactive, causing successive reactions and triggering the emergence of degenerative diseases such as diabetes, tumors, cancer, hypertension and others (Rydlewski et al. 2017). Free radical species generally divided into two group, namely Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Wojcik et al. 2010; Gurning, 2020). The existence of free radicals naturally actually occurs in the body from the work of cell metabolism in

normal amounts. The increase in free radicals in the body can occur because it's influenced by unfavorable environmental conditions and unhealthy lifestyles. The increase in the concentration of free radicals in the body must be balanced by an increase in antioxidants. Antioxidants are divided into two, namely synthetic and natural antioxidants. Synthetic antioxidants in use in the long term can cause side effects so that they are considered unsafe, while natural antioxidants are considered safer in their use for long periods of time. One of the plants that can be used as a source of natural antioxidants is sirih merah (*Piper crocatum* Ruiz & Pav.) leaves. Sirih merah leaves has potential as a natural antioxidant because it is supported by the presence of secondary metabolites such as phenolics and polyphenols, alkaloids, tannins, essential oils, and flavonoids (Sundari et al. 2015). The content of phenolics and polyphenols is believed to have an important role in providing activity as natural antioxidants (Shahidi & Ambigaipalan, 2015).

2. Methods

2.1 Materials

Ethanol (Merk), dragendroff reagent, $FeCl_3$ (Merk), Liberman-Burchard reagent, dragendorf reagent, acetic anhydride (Merk), Mg (Merk), H_2SO_4 (Merk), lead (II) acetate (s), HCl (Merk), Folin-Ciocalteu reagent, gallic acid (Merk), DPPH (Merk), and distilled water.

2.2 Preparation sample

Samples of sirih merah (*Piper crocatum* Ruiz & Pav.) leaves were taken from the District of Namorambe. The leaves samples used did not pay attention to age, but to the condition that was still fresh. Determination of the sample was carried out at the Medannense Herbarium, Department of Biology, FMIPA, Universitas Sumatera Utara (5111/MEDA/2020). The samples were cleaned with running water, drained, and then dried in an open room by airing and avoiding direct contact with sunlight. After drying, the samples were mashed using a blender to obtain sirih merah leaves simplicia powder.

2.3 *Preparation of ethanol extract of* Piper crocatum *Ruiz & Pav Leaves*

500 g of Piper crocatum Ruiz & Pav., leaves simplicia powder was extracted using ethanol as a solvent by maceration method for 2 days. Then filtered using Whatman No. paper. 1, the residue was extracted again using ethanol as a solvent. The extracted filtrate was concentrated using a vacuum rotary evaporator at 45oC to obtain ethanol extract of Piper crocatum Ruiz & Pav leaves in a thick condition. The ethanolic extract of the leaves of Piper crocatum Ruiz & Pav was continued for phytochemical screening, determination of phenolic content and determination of antioxidant activity using the DPPH method.

2.4 Phytochemical screening of ethanol extract Piper crocatum Ruiz & Pav. leaves

Phytochemical screening was carried out to identify the components of secondary metabolites contained in the ethanol extract of sirih merah (*Piper crocatum* Ruiz & Pav.) leaves. Phytochemical screening using standard methods (Haro et al. 2018; Gurning et al. 2020; Gurning et al. 2021).

2.5 Determination phenolic contents

Determination of total phenolics was carried out by colorimetric method using Folin-Ciocalteu reagent measured by UV-Vis spectrophotometry (Genesis 100S UV-Vis) at a

maximum wavelength of 765 nm. *Piper crocatum* Ruiz & Pav., leaves ethanol extract with a concentration of 1000 ppm 0.2 mL mixed with 0.4 mL Folin-Ciocalteu reagent and allowed to stand at room temperature for 5 mm. Then, 4 mL of 7% Na_2CO_3 was added and distilled water was added up to 10 mL. The mixture was centrifuged for 10 minutes and then allowed to stand for 30 minutes. The absorbance of the mixture was measured at a maximum wavelength of 765 nm (Alara et al. 2018; Alara et al. 2019). Calculation of the determination of total phenolic content using the formula (Alara et al. 2019):

$$TPC\ (mg\ GAE/g\ d.w)\ =\ \frac{Conc.\left(\frac{mg}{mL}\right)\ x\ Vol.of\ Solvent\ Used\ (mL)}{Weight\ of\ Dried\ Sample\ Used\ (g)}$$

The phenolic content was calculated and expressed as gallic acid equivalent (GAE) as standard. The same procedure was carried out in measuring and determining the standard curve for gallic acid. The variation of standard gallic acid concentration is 50 ppm, 75 ppm, 100 ppm, 125 ppm and 150 ppm. Measurements were carried out with three repetitions.

2.6 Test of antioxidant activity

Determination of antioxidant activity was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Variations in the concentration of sirih merah ethanol extract (*Piper crocatum* Ruiz & Pav.) leaves used were 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm and a DPPH concentration of 0.4 mM (Gurning et al 2021). As a negative control using ethanol solvent and positive control with vitamin C with various concentrations of 2.5 ppm, 3.0 ppm, 3.5 ppm, 4.0 ppm and 4.5 ppm. The volume of each extract concentration used was 250 L, then 1 mL of 0.4 mM DPPH was added and ethanol was added up to 5 mL. The same treatment was carried out to determine the antioxidant activity of the positive control, while the negative control only used ethanol and DPPH. The mixture was incubated at room temperature for 30 minutes, then the absorbance was measured at a maximum wavelength of 517 nm. Measurements were carried out 3 times. Calculation of% inhibition by using the equation (Gul et al. 2017; Gurning, 2020):

$$\%Inhibition = (\frac{Abs_{Control} - Abs_{sample\ test}}{Abs_{control}})x\ 100$$

where Abs = absorbance; $Abs_{control}$ = Absorbance without sample and Abs_{sample} = Absorbance of the sample tested. IC_{50} of the linear regression equation y = bx + a.

3. Results and Discussion

3.1 Preliminary phytochemical screening

The results of phytochemical screening on the content of secondary metabolites from simplicia and ethanol extract of sirih merah (*Piper crocatum* Ruiz & Pav.) leaves are presented in Table 1.

The results of phytochemical screening carried out on simplicia and ethanol extract of *Piper crocatum* Ruiz & Pav., leaves contained various secondary metabolites. These data support the testing of various potential activities, especially in determining its activity as an antioxidant.

Tabel 1

Phytochemical screening for metabolites secondary of simplisia dan extract ethanol sirih merah (Piper crocatum Ruiz & Pav) leaves

Secondary Metabolites	Reagent	Simplicia	Ethanol Extract
Alkaloids	Mayer	Positive (+)	Positive (+)
	Dragenddroff	Positive (+)	Positive (+)
	Wagner	Positive (+)	Positive (+)
Flavonoids	Shinoda test	Positive (+)	Positive (+)
Phenolic/ polyphenols	$FeCl_3$ 5% (at ethanol)	Positive (+)	Positive (+)
Saponins	Foaming Test	Negative (-)	Negative (-)
Tannins	$FeCl_3$1%	Positive (+)	Positive (+)
Steroids and Triterpenoids	Salkowski Test	Positive (+)	Positive (+)

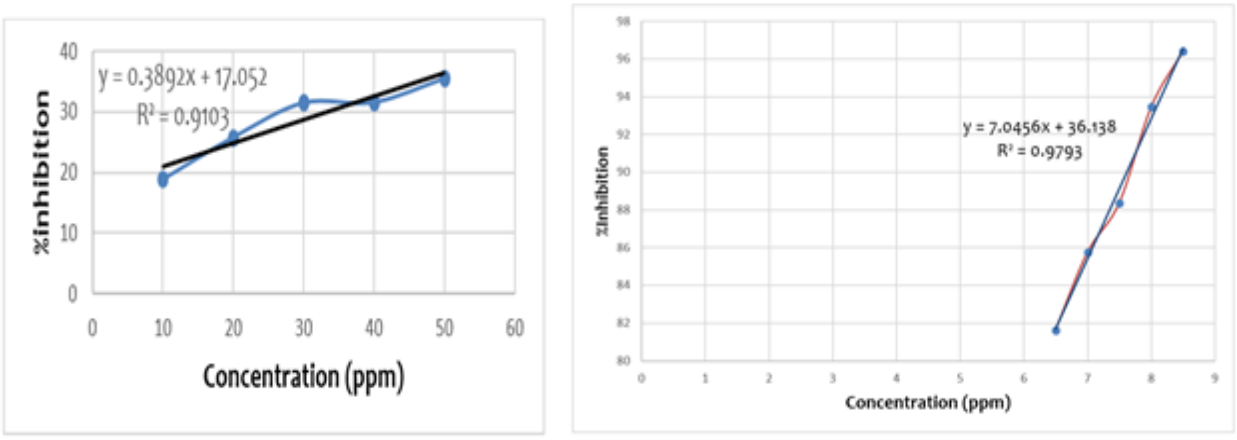
3.2 Total phenolic content

Measurement of total phenolic content of ethanolic extract of sirih merah (*Piper crocatum* Ruiz & Pav.) leaves using colorimetric method using UV-Vis spectrophotometry. The linear regression equation for the linear regression is y= 0.0037x + 0.0078 ; R2 = 0.9514. The total phenolic content in the ethanol extract of sirih merah (*Piper crocatum* Ruiz & Pav.) leaves was 0.949±0.003 mg GAE/g d.w. The presence of secondary metabolites, especially phenolic compounds, has potential activity as an antioxidant capable of deactivating superoxide free radicals, anti-aging and reducing the risk of cancer, diabetes and cardiovascular disease (Ghasemzadeh & Ghasemzadeh, 2011; Kumar & Goel, 2019). Phenolic compounds are included as primary antioxidants in counteracting/deactivating free radicals (Shahidi & Ambigaipalan, 2015). The ability of phenolic compounds as antioxidants is determined by the presence and number of hydroxyl groups contained in a compound (Kumar & Goel, 2019).

3.3 Antioxidant activity test

Determination of antioxidant activity of ethanol extract *Piper crocatum* Ruiz & Pav., leaves by DPPH method using spectrophotometry at a maximum wavelength of 517 nm. Determination using the DPPH method was based on a relatively simple, inexpensive, efficient, fast process and has better sensitivity as an antioxidant. Antioxidants inactivate free radicals from DPPH by involving hydrogen transport (Akar et al. 2017; Sirivibulkovit et al. 2018). The magnitude of the antioxidant activity of the ethanolic extract of the of *Piper crocatum* Ruiz & Pav., leaves is shown in Fig 1a, and the antioxidant activity of vitamin C as a positive control is shown in Fig 1b.

Based on the data obtained that vitamin C has a very stronger activity (IC_{50} = 2.66) than the antioxidant activity of the ethanol extract of the of *Piper crocatum* Ruiz & Pav., leaves (IC_{50} = 84.656). This shows that vitamin C belongs to the category of very strong antioxidant activity, while the ethanol extract *of Piper crocatum* Ruiz & Pav., leaves is included in the strong category as an antioxidant (Marjoni & Zulfisa, 2017).



(1a) **(1b)**

Fig 1. Activity as antioxidants, (a) Ethanol Extract of *Piper crocatum* Ruiz & Pav., Leaves and (b) ascorbic acid

4. Conclusion

The ethanol extract of *Piper crocatum* Ruiz & Pav., leaves had a total phenolic content of 0.949±0.003 mg GAE/g d.w. and has antioxidant activity (IC_{50}) 84.656 which categorized as strong antioxidant.

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