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Total Quantity of Phenol and Isolation Methanol Tannin Extract of Red Betel Leaf (*Piper crocatum*)

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Abstract : Red betel leaf (P. *crocatum*) is a plant-based product has been reported to function as anti-stress, growth promoters, stimulation of appetite, and immunostimulant. It is also acts as antimicrobial because it contains alkaloids, tannins, flavonoids, pigments, phenolics, terpenoids, steroids, and essential oil. This study was aimed to find out the results of phytochemical screening. Total value of phenols and tannins insulation class of compounds was characteristics of the methods of UV-Vis spectrophotometer and FTIR. The extraction method in this research is the method of maceration using methanol for 48 hours with a comparison sample and the solvent of 1: 2.5 (b; v). Qualitative phytochemical test conducted in this study was composed of test alkaloids, flavonoids, steroids, terpenoids, saponins and tannins. Characterization of total phenol red betel leaf is carried out using a UV-Vis spectrophotometer using Folin- Ciocalteu reagent, and its concentration is measured by the standard curve gallic acid. Insulation class compound tannin is using TLC and column chromatography. The test results of phytochemical screening of red betel leaf are a compound containing alkaloids, flavonoids, saponins and tannins. Results of testing the highest value of total phenols contained in fresh betel leaf is 41.29 ± 0.52 GAE / g. Insulation class compound tannin red betel leaves dry with the characteristics of UV-Vis spectrophotometer and FTIR which showed isolated compound is a class of compounds tannins.
Key word : Phytochemicals, Piper crocatum, total phenols, tannins and isolation.

Introduction

Indonesia is rich in biodiversity that potential to be developed as drug or drug raw materials that function as antioxidant **[1]**. *In-vitro* antioxidant study of the methanolic extracts *P. ningrum* of the tested condiments showed significant activity in DPPH method with appreciable low IC50 values **[2]**. Red betel extract has 30,20 % antioxidant potency (IC50) and dose 400µg/ml is toxic to fibroblast cell **[3]**. Ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves show antibacterial activity against MRSA in concentration 20 mg/mL **[4]**.

Red betel leaf (*Piper crocatum*) is a natural resource to be explored as potential drugs. This commodity is containing active compounds such as flavonoids, alkaloids, terpenoids, cyanogenic, glucoside, isoprenoid, nonprotein amino acid and eugenol. While flavonoids and polevenolad is to have the properties as an antioxidant, antidiabetic, anticancer, antiseptic and anti-inflammatory **[5].**

Red betel leaf has been isolated have 677 different compounds from 112 species. Found 190 of alkaloids/amides, 49 of lignans, 70 of neolignans, 97 of terpenes, 39 of phenylpropanoid, 15 of steroids, 18 of kavapyrones, 17 of chalcones / dihydrochalcones, 16 of flavones, flavanones 6, 4 piperolides (cinnamylidone butenolides) and 146 other compounds-that are not included into secondary metabolites **[6].** Craft et al. **[5]** state that the secondary metabolites of red betel leaf are contains flavonoids, alkaloids, terpenoids, cyanogenic, glucoside, isoprenoid, nonprotein amino acid, and eugenol.

Red betel leaf (Piper crocatum) is one alternative medicinal plant that has been widely used by the people. Emrizal et al. **[7]** state that Piper crocatum Ruiz and Pav, showed IC50 values of 2.04; 1.34 and 2.08 ug / mL in n-hexane, ethyl acetate and butanol fractions, it shows that this plant as a potential antitumoral agent. Based on these facts in order to develop the treatment is necessary to study phytochemical test, total phenols and tannins compound insulation class thin-layer chromatography (TLC) of various leaves of P. crocatum conditions.

Materials and Methods

The tools used in this study is a sieve of 40 mesh, grinder, analytical balance, Whatman paper no. 42, TLC, Column, Chamber glass, evaporator, oven, shacker, test tubes, beakers, incubator, erlenmenyer, UV-Vis and FTIR ,

The main ingredient in the research is red betel leaf in the form of fresh powder, powder withered and dry powder derived from Jogjakarta. The chemicals used are methanol PA, Ethyle acetate PA, gallic acid, reagent solution of Folin- Ciocalteu

The extraction of tannins of red betel leaves

The extraction of tannins of red betel leaves were calculated using modification method of Sundang et al. **[8]** and Malik and Ahmad **[9].** While for Thin Layer Chromatography and Chromatography columns using methods of Bigoniya and Singh **[10]** and Vasconcelos et al. **[11]**. Phenolic extraction of red betel leaves is done by dissolving 100 grams of red betel leaf powder to 250 ml of methanol. Methanol extract is then measured at a wavelength of 760 nm using the Folin-Ciocalteu reagent and the concentration measured by the standard curve. Characterization of total phenol red betel leaf is done by using UV-Vis**[12]**[7]**.** Total phenol best results in terms of quantity and then dissolved in 0.5 ml of ethanol; ethyl acetate (1: 8, v / v) is used to separate the tannin.

Phytochemical identification

Phytochemical test was performed on the ethanol extract of Piper crocatum which produce the best of total fenol using Harborne **[13, 14].**

Standard Solution

Creating a standard solution of gallic acid (5 mg / ml) with a weight of 5 g of gallic acid which is then added to 500 ml of distilled water, to obtain a concentration of 1000 mg / ml. Standard solution is taken using a pipette numbers 5, 10, 20, 30, 40, 50, 60 ml and diluted with distilled water to a volume of 100 ml, so that the resulting solution with a concentration of 50, 100, 150, and 200 mg / l gallic acid[7].

Absorbance measurement Total Phenol

The determination of the total phenolic content in the isolated phenolic extracts from sunflower oil and olive oil, as previously mentioned, has been made following the standard method using Folin-Ciocalteu (F-C) reagent with slight modification **[15, 16, 17]**. Weighed amount of 0.3 grams extract and then diluted to 10 ml with methanol to water ratio is 1: 1. The extract solution of 0.2 ml were taken and added 45 ml of distilled water and add 1 ml of Folin-Ciocalteu reagent and then stirred , Let stand for 8 minutes and then add 3 ml of 20% Na2CO3, let the solution for 2 hours at room temperature. Measuring the absorbance is using UV-Vis spectrophotometer at a wavelength of 760 nm maximum absorption which gives a blue color complex. Total content of phenolic in the plant extracts were expressed as gallic acid equivalents (mg of GAE/g sample) and were calculated by the formula Parikh and Kothari **[18].**

$T = (C \times V) / M$ Where,

T= total content of phenolic compounds (mg of GAE/g sample)
C= the concentration of gallic acid established from the calibration curve (mg/ml)
V= volume of extract (ml)
M= weight of methanolic plant extract (gram)

Thin layer chromatography (TLC)

To separate the tannins of red betel leaf is done by thin layer chromatography with methanol: ethyl acetate (1:8, v/v) to separate the tannin class of compounds. Prior to separation by column chromatography tannin test was conducted Thin Layer Chromatography (TLC) to determine the value of retardation factor (Rf) which serves to identify parts of tannin on silica gel. Extract methanol of *P. crocatum* were spotted separately on a TLC plate and developed in a solvent system as stated above **[19]**.

The Rf value of the separate components were calculated
Rf value = Distance travelled by solute
Distance travelled by solvent

Spketrofotometer tannin compound identification by UV-Vis and FTIR

To identify the class of tannins of red betel leaves is done by using UV-Vis spectrophotometer and FTIR. Scanning spectrophotometer is at a wavelength of 200 nm to 550 nm and calibrated using methanol. Cuvet containing tannin filtrate is put into a scanning spectrophotometer and run at a wavelength of 200-550 nm. Total tannin (ug / g) is calculated by the equation E1% 1cm.

Data analysis

Data were analyzed using analysis of variance (ANOVA) with Minitab 14 for Windows.

Results

Phytochemical screening

The results of the screening of secondary metabolites in red betel leaf by using methanol can be seen in Table 1 below:

Table 1. Results of phytochemical screening of red betel leaf (P. cruentum)

Phytochemical Test	Results	Note
Alkaloids		
- Alkaloids Meyer	-	Formed a yellowish color
- Alkaloids Dragendorff	+	precipitation of Orange
Flavonoids	+	Green color changes to orange
Steroids	-	Formed a bluish green color
Tannin	+	Formed blackish green
Saponin	+	formed foam
Triperten	-	Formed brown ring

Note; (+) Detected, (-) Not detected

Total Phenol

The total yield of phenol based on the type of fresh leaves, wilted and dry red betel (Piper crocatum) can be shown in Table 2.

Table 2. Data Total Phenol extract of red betel leaves with various conditions

Treatment	Total Phenols average (mg GAE/g)	5% LSD
Dried leaves	33.33±1.09 a	1.62
Withered leaves	37.86±0.71 b	
Fresh leaves	41.29±0.52 c	

Note: Figures followed by the same letter are not significantly different at LSD test level of 95%.

Tannin Compounds Isolation Using Thin Layer Chromatography (TLC)

TLC results from betel leaf extract of dried red under UV light with a wavelength of 256 nm and 366 nm is shown in Figure 1 below.

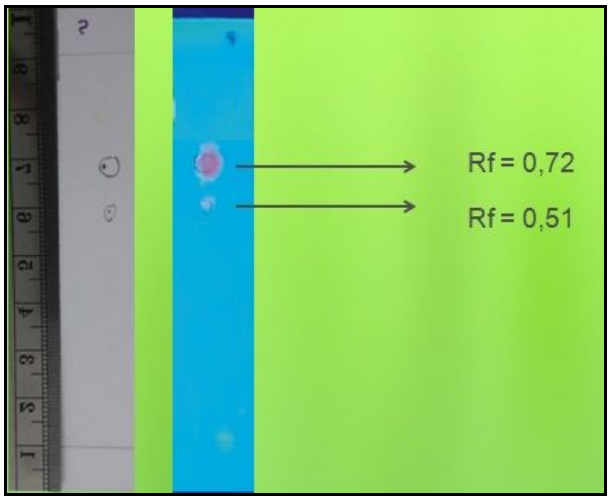

Figure 1. Results of TLC from the extract of red betel leaf (*P. crocatum*)

Results Identification of compounds Tannin using Uv-Vis spectrophotometer.

The results of the identification of classes of compounds in the tannin fraction of one (1) compared with the standard of tannin using UV -Vis is shown in Figure 2 below.

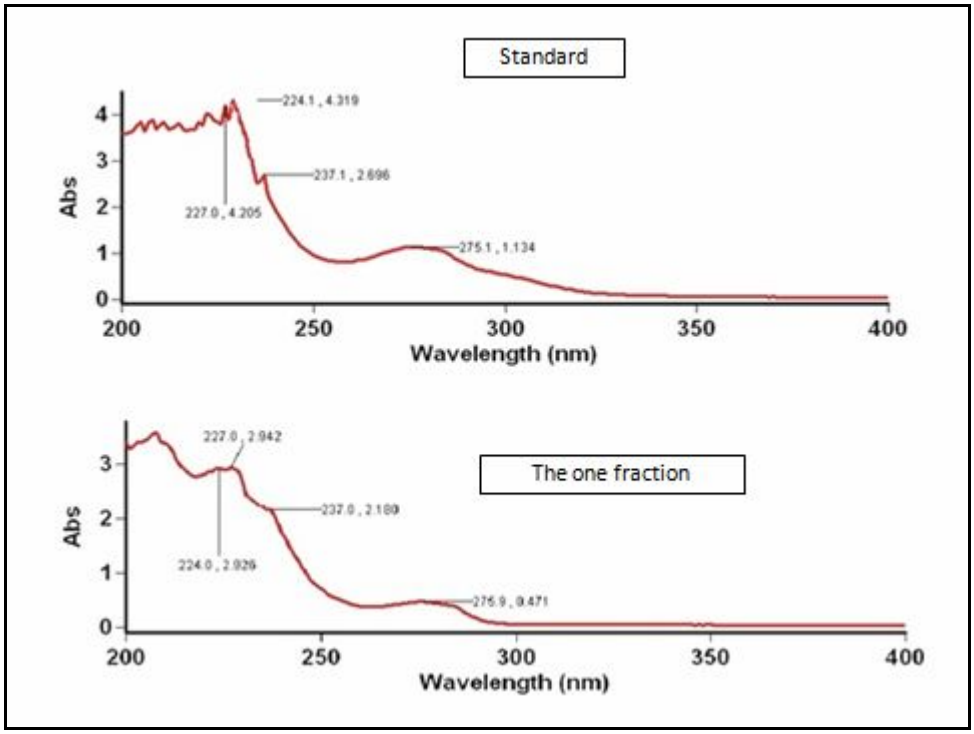

Figure 2. Identification of tannin on the isolate two (2) KLT compared with tannin standards using a UV spectrophotometer –Vis

Results Identification of compounds Tannin using FTIR spectrophotometer.

The results of the identification of classes of compounds in the tannin isolate the two (2) KLT using FTIR spectrophotometer can be seen in Figure 3, below.

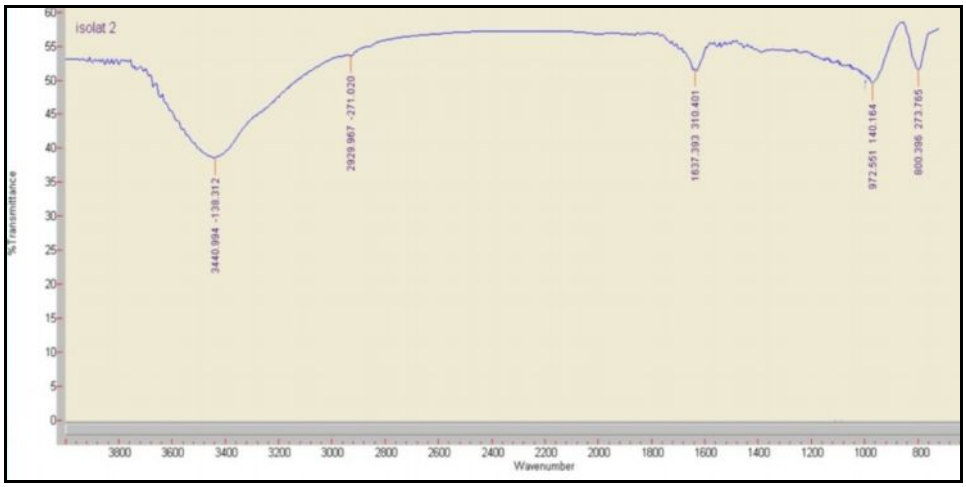

Figure 3. Identification of tannin on the isolate two (2) using FTIR spectrophotometer

Discussion

Phytochemical screening

The study was begun with the screening of secondary metabolites to determine what compounds contained in red betel leaf by using methanol. The results turned out to Red betel leaves are contain secondary metabolites such alkaloids, flavonoids, saponins and tannins compounds. Emrizal et al. **[7]**; Hagerman **[20]**

suggests flavonoids and tannins are polyphenolic compounds, which means a compound which has a section in the form of phenolic. Flavonoids have 15 carbon atoms, consisting of two benzene rings linked together by a linear chain of three carbon atoms, while the tannin compounds are polymers of flavonoid connecting it by means of C8 to C4. Craft et al. **[5]**. state that polevenolad adding flavonoids have antioxidant properties, antidiabetic, anticancer, antiseptic and anti-inflammatory.

Total Phenol

The results mean total phenol extract of red betel leaf of Figure 5 shows the highest value is contained in the treatment of fresh betel leaf is 41.29 ± 0.52 GAE / g. Followed by treatment of betel leaf wilting is 37.86 ± 0.71 mg GAE / g and the treatment of dry leaves total phenol content is a low of 33.33 ± 1.09 GAE / g. Mansour, **[21]** stated fresh leaves of thyme have the highest contents of total phenolics than dry leaves (291.23, 322.12 mg GAE/100 g). Total phenol extract of red betel leaves dry while the lowest value compared with other treatments, but these values are not much different than the value of total phenols other Piper genus. Sundang et al. **[8]** reported a total value of phenol methanol extract of dried leaves of Piper betle amount of 34.29 GAE / g.

The high value of total phenols methanol extract of red betel leaf fresh is due to the high content of water contained in the fresh leaves. Agustini et al. **[22]** stated that phenolic flavonoid compound is attached to a sugar group, which consequently flavonoids can easily soluble in water or other polar solvents. In addition, improper drying is able to cause the release of the water content in the leaves, so that the phenolic compounds contained in the cell membrane in a layer of leaves can also be lost. Other factors that also affect the value of total phenol content are the length of the extraction process. Gao et al. **[23]** found that high levels of phenol is in line with increasing extraction time, because the longer solvent to penetrate the cell walls of the tissue damage and ultimately be more optimal material phenolic compounds dissolved more.

Isolation of Tannin Compounds with TLC

Based on the results of TLC with eluent methanol: ethyl acetate (1: 8 v / v) is has produced 2 isolates on the TLC plate. In Figure 2 TLC plate eluted post is to show the value of Rf 0.72 and 0.51. Tannin compounds are contained in Isolate 2 with Rf value of 0.72, it is in line with the results of standard wavelengths tannin compared to the wavelength isolate the compound class 2. Tannin seen from the color using UV light with a wavelength of 256 is to reveal pink. Determination of the eluent is critical related small-scale isolation of pure compounds, the precise mix-ups between solvent based kepolaranya is the better pure compound can be isolated. Tyson **[13]** stated that the process of determining the eluent is an alternative that can be used in isolation of pure compounds.

Results Identification of compounds Tannin Uv-Vis spectrophotometer

Qualitatively, the absorbance fraction of the P. crocatum is to show the four wavelengths which can be seen in Figure 3. The wavelength at a second isolate is to have the same value with standard wavelengths tannin. Wavelengths in the UV-Vis spectra are 224 nm, 227 nm, 237 nm and 275 nm. Harborne **[24]** states that polyphenols were detected in the UV-Vis spectrum are contained between 200-400 nm.

Results Identification of compounds Tannin using FT IR spectrophotometer

Figure 4 shows that isolate the two (2) methanol extract of P. crocatum is having several functional groups in a specific catchment area, namely the hydroxyl group (OH) is characterized by absorption 3440 cm-1 and CH stretch Aliphatic hydroxy group at 2929 cm- catchment area 1. Absorption at 1637 cm-1 region is the presence of a carbonyl group C = O. Uptake in the region of 800 cm-1 is the meta and para (very sharp) and the absorption area 972 cm-1 is a CH group.

Class of phenolic compounds is covering a variety of compounds derived from plants and have the same chemical structure, which contains one or two hydroxyl group (OH) **[25]**. Based on the structure, tannin is divided into two kinds of condensed tannin and hydrolysable tannins**[26]**. Hagerman**[20]** adds gallotannin chain is formed from Meta-or involving the phenolic hydroxyl of the aliphatic hydroxy group, which is a combination of a carbohydrate compound with gallic acid. Carbohydrates are a crucial component of the microstructural fish **[27].**

Conclusion

Based on the research results of phytochemical screening, red betel leaves are secondary metabolites containing alkaloids, flavonoids, tannins and saponins. Testing the content of total phenols in red betel leaf treatment that produces the best value is in treatment of fresh betel leaf with a total value of phenol is 41.29 ± 0.52 GAE / g. Treatment factor is the condition of red betel leaves significant effect on the value of total phenol content. Insulation class compound tannin red betel leaves dry with the characteristics of UV-Vis spectrophotometer and FTIR showed the isolated compound is compounds tannins.

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