Antioxidant Extraction of Dragon Scale Ferns (*Pyrrosia piloselloides*) With Ultrasound – Assisted Extraction Method

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Abstract. Dragon scales plant or usually called as dragon's scale fern (*Pyrrosia piloselloides*) is an epiphytic plant that lives on trees. This plant is often used as herbal medicine because it contains compounds such as flavonoids, saponins, tannins and polyphenols. This study aims to extract the phenolic compounds to be used as natural antioxidants by using ultrasound – assisted extraction. The extraction was carried out on dragon's scale ferns with the ratio of 1:10 of solid-to-solvent ratio. This extraction was carried out at 40^{o}C and frequency of 40 kHz using ethanol. The results of this study indicate the presence of these compounds in dragon's scale ferns leaf extract which was extracted using the ultrasound – assisted extraction method. The maximum extraction yield was obtained in 45 minutes with a solvent concentration of 50%, namely 2.596 grams with total phenolic content of 5.8977 mg GAE/g of dragon's scale ferns.

Keywords: Dragon's scale fern, *Ultrasound – assisted extraction*, Ethanol solvent, Phenolic compounds, Antioxidant

1 Introduction

One of the well-known herbal medicine in Indonesia is Pyrrosia piloselloides or dragon's scale fern leaves. There is many health benefit of dragon scale fern leaves such as for cough, dysentery, inflammation, and bleeding. Furthermore, dragon scale fern leaves have potential as herbal medicine for cancer since it has high phenolic content. From previous research, phenolic content has high antioxidant activity. Because its high antioxidant activity, phenolic can prevent oxidation from free radical that can have bad effect for human health [1-2]. The research that conducted by Uy & Garcia et.al (2015) prove that dragon's scale fern leaves contain high amount of antioxidant among other herbal medicine in South East Asia [3].

Maceration is one of the processes to extract phenolic compound from dragon scale fern leaves. Maceration process is quite simple however it is time consuming [4-8]. One of the modern extraction techniques that have fast extraction time and higher yield is ultrasound assisted

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extraction. Extraction technique by using ultrasound assisted extraction has been proven successfully extracting phytochemical compound from plant due to its thermosensitive behaviour. Using 20 kHz ultrasound frequency led to shorten extraction time since it can degrade the plant cell wall. When the plant cell wall is broken, then ability of solvent to penetrate the cell wall increase and resulting in higher yield.

In this research, ultrasound assisted extraction has been applied for extracting phenolic compound from Pyrrosia piloselloides or dragon scale fern leaves. Ethanol has been utilized as solvent in this research with the variety of ethanol concentration, following previous research [9]. In this research, we did variations of ethanol concentration and extraction time.

2 Materials and methods

2.1 Chemical Materials and Apparatus

In this research, chemical materials such as: dragon scale fern leaves, aquadest, ethanol 96% (Merck), Gallic acid (P.A, Merck), Folin Ciocalteau reagent (P.A, Merck), Na_2CO_3 (P.A, Pudak) Mg (P.A, Merck), HCl 1M (Merck), $FeCl_3$ 1% (Merck) have been used.

In this research, several apparatuses such as: ultrasonic water bath (Ovan Corp.), erlenmeyer, assay tube, digital scales, measuring flask, volumetric pipette, micro-pipette, 40 mesh sieves, buchner tube, thermometer, vortex mixer (ThermoScientific), vacuum oven (MTI corp.), vacuum pump (Gast) have been used.

2.2 Sample preparation

Dragon fern scale leaves from Bogor, Indonesia were cleaned by using water and then dried by using drying oven at 40 ^oC. The dried leaves then chopped until the size of 40 mesh.

2.3 Extraction process

For the extraction process, chopped leaves were placed in an erlenmeyer flask and then ethanol was added as the extraction solvent. The concentration of ethanol has been varied with 50%, 80% and 96% concentration. The ratio of solid (chopped leaves) and solvent is fixed with the ratio 1: 1, for 200 grams of chopped leaves then 200 ml of ethanol is needed. The Table 1 shows the sample variation during this process.

The extraction processes used ultrasonic water bath with the ultrasound frequency of 40 kHz. The extraction time is also varied as tabulated in Table 1. The extract of leaves was filtered using vacuum filter set then the solvent was evaporated. The evaporation process used vacuum oven with temperature of 50 ^oC and 0.2 atm pressure.

Table 1. Variation of samples

Sample	Sample Variation

number	Ethanol concentration	Time (min)
1		30
2	96%	45
3		60
4		30
5	80%	45
6		60
7		30
8	50%	45
9		60

2.4 Characterization

In this process, the yield is calculated by using the equation (1) with Me is mass of the extract (mg) and Mo is the mass of chopped sample (mg)

$$\%Yield = \frac{M_e}{M_0} x100\% \quad (1)$$

The extract was analyzed by using Fourier Transform Infra-Red (FTIR) Spectroscopy (FTIR, Thermo Scientific iS 5) to identify functional groups of extract. To analyze the total phenolic content in the extract, UV Vis analysis has been conducted by using UV Vis spectrophotometer (Thermo Fisher Scientific Genesys 10s UV – Vis *Spectrophotometer*). The mass transfer coefficient is calculated based on the best sample that has highest yield and total phenolic content.

3 Results and Discussion

Figure 1 shows the yield result from extraction of dragon fern scale leaves via ultrasound assisted extraction method. From **Figure 1**, solvent for extraction with ethanol 50% shows the highest yield with maximum yield of 12.97% for extraction time at 45 minute. Phenolic compound contains a lot of OH functional group. Higher polarity of phenolic compound yield content by using lower concentration of ethanol. Based on previous research, dragon fern scale is rich on flavonoid with majority component is Gallic Acid [3].

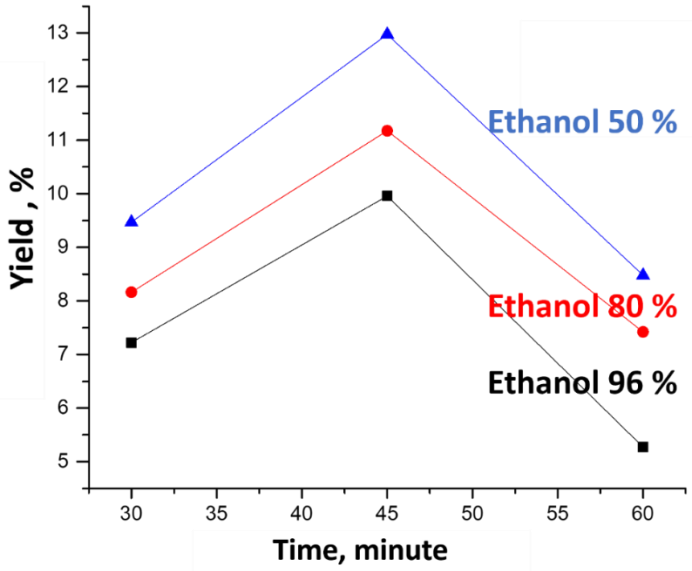

Fig 1. Correlation of dragon scale fern leaves extraction yield (%) with solvent concentration and exctraction time

Table 2. FTIR Result of Chemical Bonding

Wavenumbers (cm^{-1})	Bonding	Identification
3333	O-H	Phenol
2945	$O\text{-}H/CH_2$	Ethanol
2834	$O\text{-}H/CH_2$	Ethanol
1652	C=C/C=O	Aromatic or flavonoid
1417	C-H	Phenol or flavonoid
1112	C-O	Alcohol
1018	C-O	Aromatic
661	C=C	Aromatic

From the yield result of extraction, qualitative analysis of sample has been investigated. The best sample which is sample that extracted with ethanol 50% and extraction time for 45 minutes has been choosen. Fourier Transform Infra Red (FTIR) is conducted for qualitative analysis to analyze chemical bonding. **Figure 2** and Table 2 show FTIR result of dragon scale fern leave extract. For comparison, analyzed gallic acid has been analyzed by using FTIR. Gallic acid is a common phenolic compound that can be found in herbal life. Furthermore, gallic acid for totallic have been used phenolic compound test. **Figure 2** also shows FTIR analysis result of gallic acid and chosen sample. Several chemical bonding that represent active compound for extract paste of dragon scale fern leaves can be observed in **Figure 2** [10]. The wavenumbers of 3333 and 1417 (cm^{-1}) show the chemical bonding of phenol. Phenol is wellknown for itsantioxidant acticvity and it can represent the existence of gallic acid in extract paste.

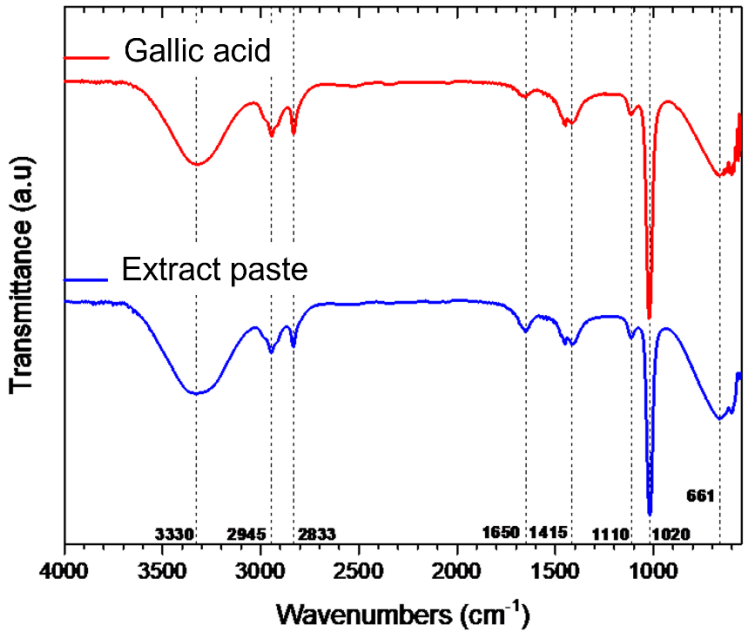

Fig 2. FTIR Analysis of Gallic Acid and Extract Paste

To analyze the total phenolic content in the extract, UV Vis analysis has been conducted by using UV Vis spectrophotometer. The sample was reacted with Folin-Ciocalteu reagent. After reacted with Folin-Ciocalteu reagent, blue solution $(PMoW_{11}O_{40})^{4-}$ is formed and can be detected at wavelength of 765 nm [11]. For the detection at UV Vis Spectroscopy, Gallic Acid (analytical grade) is used for the standard solution and to represent phenolic compound. From the standard curve, we got 3 standard curves for different solvent concentration. Table 3 shows the standard curve equations to estimates the phenolic content that is represented by gallic acid equivalent (GAE). Based on equations at Table 3, the extract sample is analysed, and the total phenolic content (TPC) result is summarized in **Figure 3** and Table 4.

From **Figure 3** and Table 4, the sample that extracted with ethanol 50% and the extraction time 90 minutes show the highest TPC with 5.90 mg GAE/g of dragon scale fern leave. The amount of TPC has similar trends with the yield result in **Figure 1**. The result proves that the yield is rich in phenolic compound. Our research is comparable with another research, the extraction in this study is using maceration method [3]. From the ppm of GAE, best sample show the 591.51 ppm GAE meanwhile from other research shows the value of 142.8 ppm GAE [3].

Table 3. Standard Curve equation

Solvent concentration	Equations	R^2
50%	y = 0.0006x + 0.0194	0.99
80%	y = 0.0006x + 0.0125	0.99
96%	y = 0,0006x + 0,0104	0.99

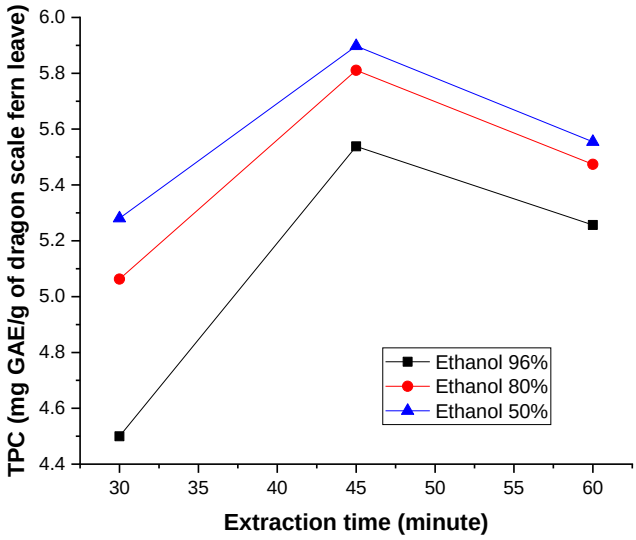

Fig 3. Total Phenolic Content (TPC) of Extract

Table 4. Total Phenolic Content of Extract Dragon Scale Fern Leave

Ethanol Concentration (%)	Extraction time (minute)	Totap phenolic content (ppm Gallic Acid Equivalent or GAE)	Totap phenolic content (ppm Gallic Acid Equivalent or GAE)
	30	528.76	5.28
50	45	591.51	5.90
	60	556.32	5.55
	30	506.97	5.06
80	45	582.50	5.81
	60	548.67	5.47
	30	450.95	4.50
96	45	555.51	5.54
	60	526.88	5.26

The best sample that has highest yield and TPC yield is sample that extracted with ethanol 50% and extraction time of 45 minute. The experiment to calculate mass transfer coefficient for this sample has been conducted. The mass transfer equation for mass transfer coefficient is following the equation (2).

$$k_L a \,.\, t = \ln \frac{c_S}{(c_S - c_L)} \qquad (2)$$

where:

C_S = phenol concentration at equilibrium condition (ppm or kg/m^3)

C_L = phenol concentration at solution (ppm atau kg/m^3)
t = time (minute)
V = solvent volume (m^3)
k_La = volumetric mass transfer coefficient ($minute^{-1}$)

Table 5. Analysis Results for Mass Transfer Coefficient

Ethanol Concentration (%)	Extraction time (minute)	Totap phenolic content (ppm Gallic Acid Equivalent or GAE)	Totap phenolic content (ppm Gallic Acid Equivalent or GAE)
50	0	0	0
	5	422.46	4.22
	15	476.43	4.76
	30	528.76	5.29
	45	591.51	5.90
	60	556.32	5.56
	75	531.582	5.32
	90	497.184	4.67

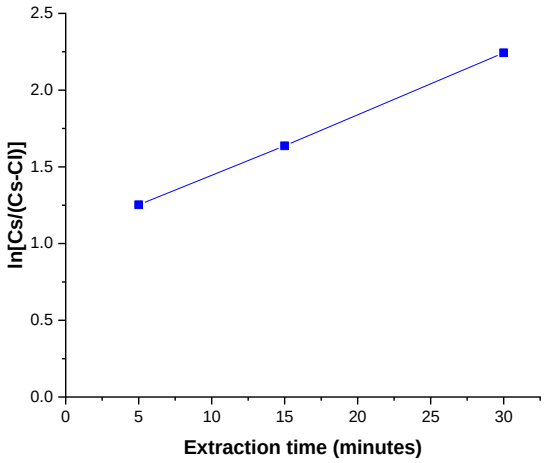

Fig 4. Mass transfer coefficient result

Table 5 shows the analysis result for calculate mass transfer coefficient during extraction of dragon scale fern leave. From the Table 5 and equation (2), **Figure 5** has been arranged. In **Figure 4**., x axis represent extraction time, slope represent kL.a and y axis represent $\ln \frac{C_S}{(C_S - C_L)}$. From the **Figure 5**, the equation for mass transfer coefficient is y = 0.0397.t + 1.04931. From this equation, the kL.a value is 0.0397 ($minute^{-1}$).

4 Conclusion

In this research, the phenolic component from dragon scale fern leaves is succesfully extracted via ultrasound assisted extraction method. The highest yield and total phenolic content yield is achieved for the sample that extracted with 50% ethanol and 45 minute extraction time. The highest yield and total pehneolic content yield for this sample are 12.97% and 5.90 mg GAE/g of dragon scale fern leave. The mass transfer coefficient for the best sample is also investigated with the equation of $y = 0.0397.t + 1.04931$ and kL.a value is 0.0397 (minute $^{-1}$).

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