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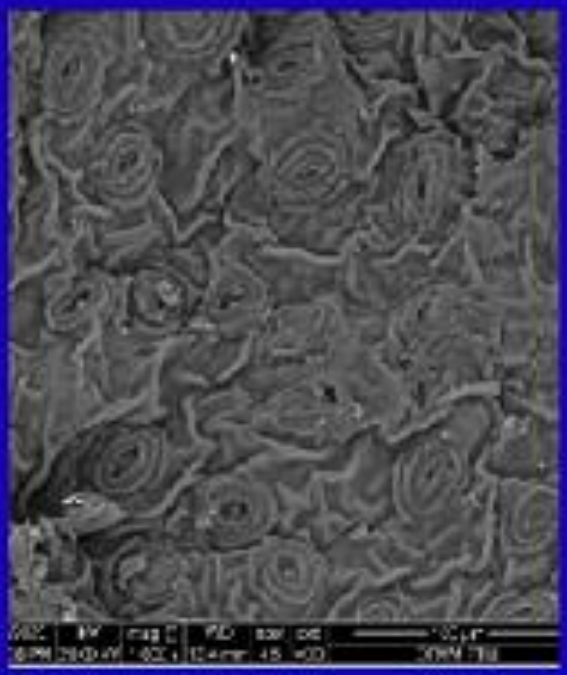

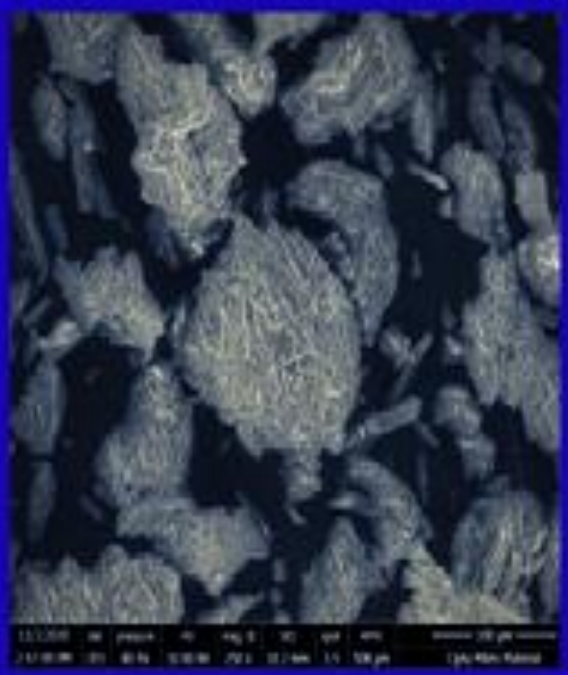

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Tannins Extraction of Tea Leaves by Ultrasonic Method: Comparison with The Conventional Method

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Abstract

Tannin is a compound of polyphenols derived from plants found in tea leaves (Camellia sinensis). Generally, the tannins are separated from the tea leaves using conventional methods of solvent extraction. Unfortunately, the said method resulted in an unsatisfactory yield because High risk of degradation of compounds, so it needs to carry out another way to overcome this matter, namely the ultrasonic solvent extraction method. This research aims to learn the effect of ultrasonic on the increase of tannins yield of extraction and to searched constant kinetic. This research has studied some of the parameters that affect the ultrasonic extraction result. These include the effect of extraction time and the type of tea leaves used from three different altitude areas from the sea level, such as Ciwidey Bandung, Pagar Alam, and Bogor Peak. The extraction time used is 4, 6, and 8 hours where the ultrasonic frequency at 75 kHz with a 70% ethanol solvent. Products containing tannins are analyzed qualitatively and quantitatively. Qualitative tannins content is tested with $FeCl_3$ and Gelatin, while quantitative analysis uses spectrophotometer UV-vis Shimadzu UV 1800. Besides that, the Scanning Electron Microscopy (SEM) characterization confirmed that the ultrasonic method effect on increasing yield. The highest result is on the type of Pagar Alam tea at 8 hours extraction time, 23.1% of yield, which is 1% higher than the conventional method. The constant kinetic of ultrasonic extraction tea from Bandung was 0,0567 kg/m^3.h.

Abstrak

Tanin merupakan senyawa polifenol yang berasal dari tumbuhan yang terdapat pada daun teh (Camellia sinensis). Umumnya tanin dipisahkan dari daun teh menggunakan ekstraksi pelarut. Sayangnya metode tersebut memberikan hasil yang kurang memuaskan karena beresiko tinggi terdegradasi, sehingga perlu dilakukan cara lain untuk mengatasi hal tersebut yaitu dengan metode ekstraksi ultrasonik. Penelitian ini bertujuan untuk mempelajari pengaruh ultrasonik terhadap peningkatan yield tanin hasil ekstraksi dan mencari kontanta kinetika. Parameter yang diamati pada penelitian ini diantaranya waktu ekstraksi dan jenis daun teh yang digunakan dari tiga daerah dengan ketinggian dari permukaan laut yang berbeda, yaitu Ciwidey Bandung, Pagar Alam, dan Puncak Bogor. Variasi waktu ekstraksi yang digunakan adalah 4, 6, dan 8 jam dimana frekuensi ultrasonik pada 75 kHz dengan pelarut ethanol 70%. Produk yang mengandung tanin dianalisis secara kualitatif dan kuantitatif. Kadar tanin secara kualitatif diuji menggunakan $FeCl_3$ dan Gelatin sedangkan analisis kuantitatif menggunakan spektrofotometer UV-vis Shimadzu UV 1800. Selain itu, karakterisasi Scanning Electron Microscopy (SEM) memberikan validasi bahwa metode ultrasonik berpengaruh terhadap peningkatan rendemen tanin. Hasil tertinggi diperoleh pada teh jenis Pagar Alam dengan waktu ekstraksi 8 jam, yaitu 23,1% yield dimana lebih tinggi 1% dibandingkan dengan metode konvensional. Konstanta kinetika ekstraksi ultrasonic teh dari bandung sebesar *was 0,0567 kg/m^3.h.*

Keywords*: polyphenol, SEM, spectrophotometer, ultrasonic method*

INTRODUCTION

Tea is a non-alcoholic beverage loved by almost all the world consumed since ancient times [1]. The tea plant (Camellia sinensis) can grow at an altitude of 200 - 2300 meters above sea level [2]. Tea is known to have benefits such as anti-antioxidants and artificial coloring. It may because tea leaves contain active chemicals such as tannins [3]. There are four major chemical compounds in tea leaves, such as phenol, non-phenol, aromatic, and enzyme [1] [4]. Every 1 gram of dried tea leaves has 12.66 mg of tannins [5]. Tannin with chemical formula $C_{76}H_{52}O_{46}$ is a polyphenol compound that is colorless to yellowish, which can affect the tea itself taste, color and aroma. Tannins give a distinctive taste of bitter on tea leaves [6].

Today, several extraction methods are commonly used to extract active components in plants such as tea leaves, namely soxhlet distillation and solid-liquid extraction. However, those methods have some weaknesses, such as producing low yields [7][8]. Based on these problems, this research aims to study the effect of ultrasonic tannin yields. The method to be used is called the ultrasonic extraction method. The main advantages of ultrasonic wave extraction compared to conventional soxhlet are higher efficiency, shorter operating time, and high solute mass transfer rate [8].

Solvents commonly used for the extraction process of plants are ethanol and acetone[]. In this study, the solvent was ethanol because of several advantages: higher selectivity in extracting solutes, non-toxic, quickly evaporated, and relatively low prices [9]. Besides, from previous studies, ethanol showed better activity as a solvent than acetone [10].

Previous researchers have observed ultrasonic wave frequencies influences in the frequency range of 20 - 50 kHz [7]. It reported that ultrasonic waves have a significant effect on the extraction yield compared to without ultrasonic. This study investigates ultrasonic waves at a fixed frequency of 75 kHz with various kinds of tea. to equipment scale-up, constant kinetic was find with second-order kinetic

RESEARCH METHODOLOGY

Materials and Tools

The materials used in this experiment were tea leaves from three sources: Bandung, Pagar Alam, Puncak Bogor, Ethanol 70% (C_2H_5OH), and distilled water (H_2O). While for analysis, the Merck company supplies for tannins standard.

The equipment used in this study consisted of a set of ultrasonic devices equipped with a heater and also a predetermined frequency and time. Other tools are measuring cups, Erlenmeyer, containers, stirrers, a set of vacuum filters and evaporators, and UV-vis spectrophotometer analyzer Shimadzu 1800. Scanning Electron Microscopy (SEM) was used to characterize solid residues to validate the experimental results.

Experimental Procedures

Before extraction, the tea leaves were crushed to expand the surface area, increasing the contact area between the solvent and the tea leaves during the extraction process. Tea leaves (Camellia Sinesis) have a tannin composition about 0.5 - 1% (w / w). The crushed tea leaves then weighed as much as 50 grams before it goes for a maceration within an hour. Then it goes for ultrasonic and solvent extraction to attract tannin compounds using 70% ethanol as solvent. The ultrasonic apply 75 kHz frequency mechanical pressure to the cell to produce cavities in the sample [11]. Extraction occurs in a variation of time 4, 6, and 8 hours at a constant temperature of 70^oC [12]. The experiments parameter were the same for the

conventional method and ultrasonic using three different tea. After that, the extracted product is collected and filtered to separate the filtrate containing tannins and solvent. The next step is the evaporation process to separate the tannins from the solvent, using a rotary evaporator (Yamato RE 201-AWV) at temperatures close to ethanol's boiling point and pressure of 350 mmHg. Finally, all 18 samples are analyzed using a spectrophotometer. SEM characterizes the best result of the ultrasonic extraction. Complete steps of the research, as Figure 1 shows.

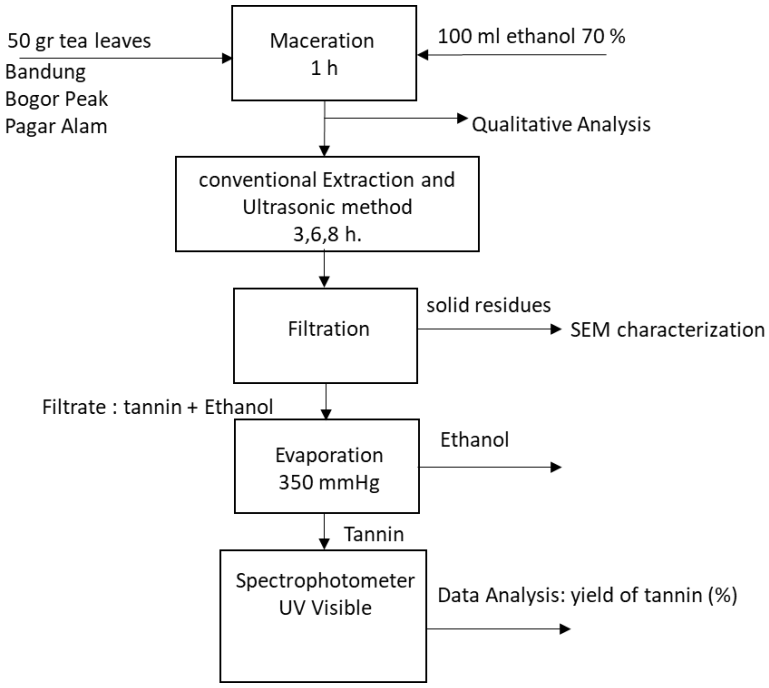

Figure 1. The diagram of research

Determination of the Calibration Curve

First, as much as 50 mg of pure tannin, diluted using 70% ethanol in a 50 mL flask so that the first solution's concentration is 1 g/L. Second, pipette as much as 1 mL of the first solution and dilute it with 70% ethanol in a 10 mL flask to get a 10^{-1} g/L stock solution. Third, that to make a concentration of 4 x 10^{-3} g/L; 6 x 10^{-3} g/L; 8 x 10-3 g/L; 10 x 10^{-3} g/L; 12 x 10^{-3} g/L, and 14 x 10^{-3} g/L, pipette from the stock solution as much as 0.4 mL; 0.6 mL; 0.8 mL; 1 mL; 1.2 mL; and 1.4 mL then diluted with 70% ethanol solvent in a 10 mL flask to have six samples test.

Then the wavelength measurements of each sample are carried out using a UV visible spectrophotometer Shimadzu UV 1800. The principle is a light source from UV light and visible light with a beam arrangement using a monochromator. Beams then enter the sample, rays that are not absorbed and spread over the pieces will enter the detector and processed so that the absorbance value appears on the screen [13], as shown in Figure 2.

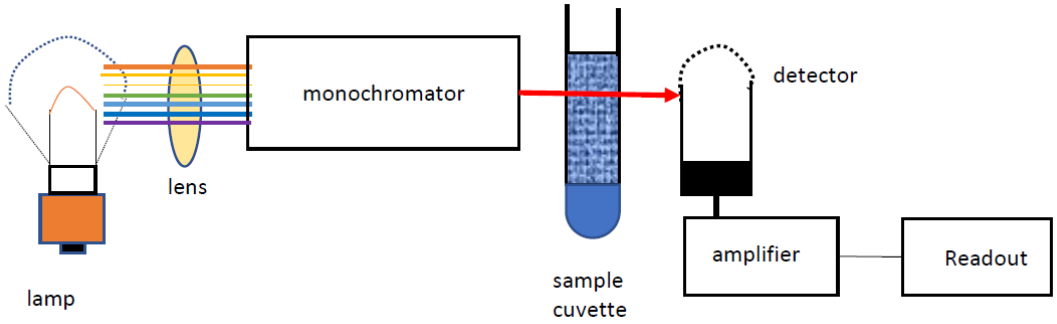

Figure. 2. The Principle of UV visible spectrophotometer

Furthermore, the yield calculation expressed by Eq. (1) [14] as follow:

$$\% \text{ Yield} = \frac{W_{ext} \, x \, V_{tot}}{W_0} x100 \tag{1}$$

where W_{ext} is the concentration of tannin in 1 ml of sample (mg/ml); V_{tot} and W_0 are the total extract volume (ml) and the weight of the tannins found in the feed tea leaves (mg), respectively.

Mathematical modeling

Mathematical modeling was implemented to analyzethekinetics of the solid–liquid extraction process. Thekinetic parameters of three models were determinedfrom the extraction data of TPC for each individualvariable and its respective level. The models of interestfor this study include the second-order rate law. The general rate law for the second-order is expressed in Eq. (2):

$$\frac{dCt}{dt} = k1(Ct - Ce) \tag{2}$$

where k1 is the second-order extraction rate constant (L/g min), Ct is the tannin concentration in the extract at a given extraction time t (g/L) and Ce is equilibrium concentration of the tannin compounds in the liquid extract (g/L). Integrating the second-order law with the boundary conditions, t = 0 to t and Ct = 0 to Ct, it can be expressed in the form of Eq. (3):

$$Ct = \frac{Ce^2 k1 \, t}{1 + Cek1t} \tag{3}$$

The linearized form of Eq. (3) that describes the rate of dissolution is shown in Eq. (4):

$$\frac{t}{Ct} = \frac{t}{Ct} + \frac{1}{k1Ce^2}$$

Staristical Analysis

The kinetic coefficients were determined by linear regression where Microsoft excel 2016 (Microsoft., USA) was used in performing the graphical plots of the experimental and predicted data. The predicted coefficients with 95% confidence boundary levels were analyzed based on the two statistical criteria, coefficient of determination (R^2) and root mean square error (RMSE).

RESULTS AND DISCUSSIONS

Qualitative Tests

Qualitative tests of tannin content were carried out through color appearance tests, addition of $FeCl_3$ solution, and addition of Gelatin. The color appearance test showed different thickness with different sources of tea leaves. Figure 3 shows the tannin contained in tea originating from the South Sumatra Pagar Alam is higher than the two other because the more concentrated the color of the resulting tea water shows, the more the tannin content [15]. These results are consistent with both addition of $FeCl_3$ and Gelatin, as shown in Figures 4 and 5.

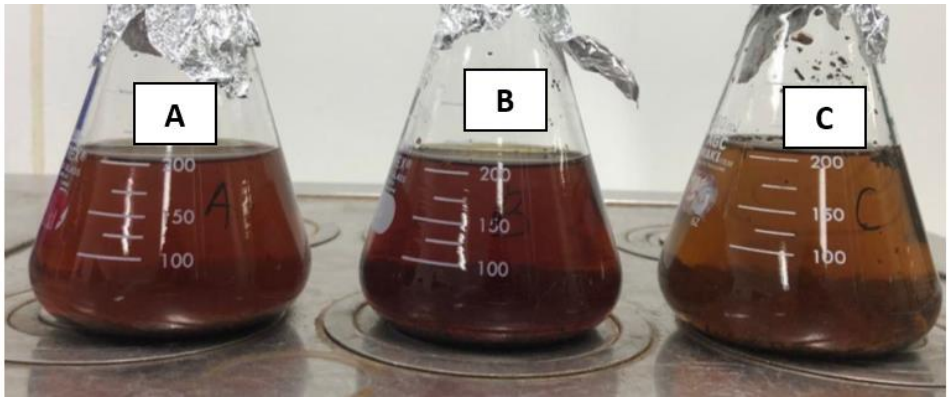

Figure 3. Tea thickness test (A) Bandung, (B) Pagar Alam, (C) Bogor

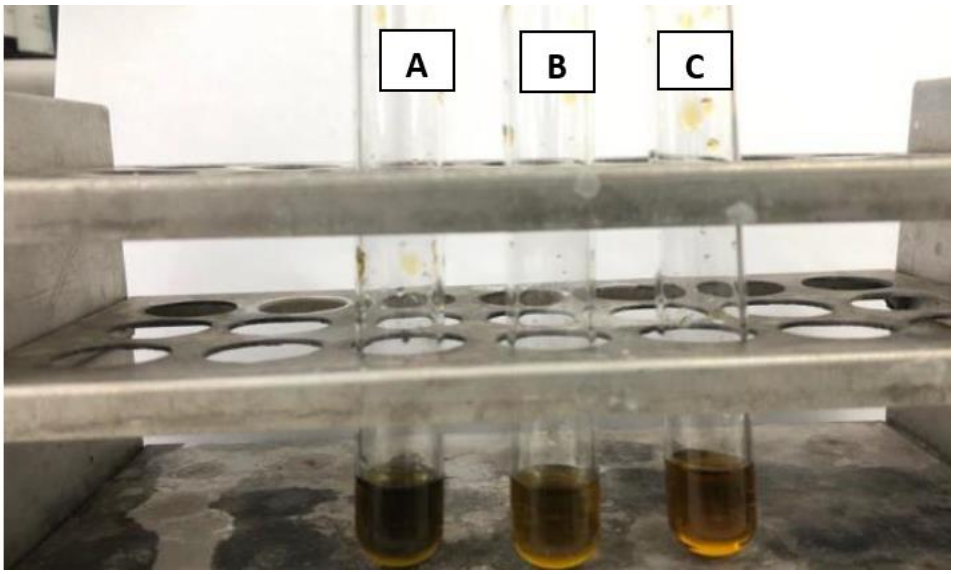

Figure. 4. Test the tannin content with the addition of $FeCl_3$. (A) Bandung, (B) Pagar Alam, (C) Bogor

Qualitatively identify tannin compounds in tea with iron (III) chloride (FeCl3) positive results for all three tea types. Because the phenol group in tannin will bind with iron (III) chloride to form a complex that produces green color, the more concentrated green color produced indicates higher tannin content in tea [1, 16]

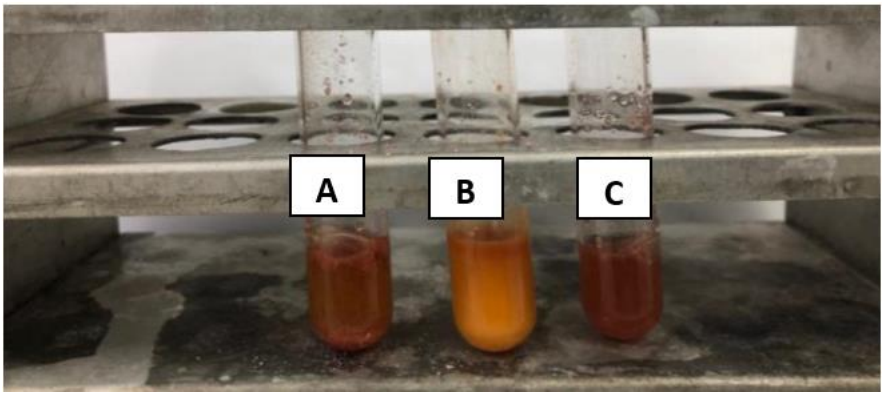

Figure 5. Test results of tannin content with the addition of Gelatin**.** (A) Bandung, (B) Pagar Alam, (C) Bogor.

Test results of tannin content with Gelatin addition also showed positive results marked by the formation of deposits. This because the nature of tannins can precipitate proteins. The more sediment produced indicates the tannin content in it is also higher [17].

Effect of Ultrasonic on Tannin Yield (%)

Observation of the extraction results carried out at 75 kHz frequency power with a variation of time 4, 6, and 8 hours at temperature 70^{o}C. This temperature is the best for extraction using ethanol solvents because it is below ethanol's boiling point. All the samples were then analyzed by using UV-Vis [18, 15]. The understanding of % yield in this study is the amount of tannin received due to extraction compared to the tea leaves' tannin content before extracting as in the equation (1) [14].

Table 1. Tannins content proudeced by convencional extraction and ultrasonic extraction

Type of Tea	10^3 mg/L, extracted tannin					
	Conventional			Ultrasonic		
	4 h	6 h	8 h	4 h	6 h	8 h
Bandung	4.9191	4.9191	5.0143	5.0143	5.1094	5.2046
Pagar Alam	5.2997	5.3949	5.5852	5.4900	5.4900	5.7755
Bogor Peak	5.2046	5.2997	5.2997	5.2997	5.3949	5.6803

Table 1 shows the tannins content proudeced by convencional extraction and ultrasonic extraction Table 2 shows that the yield produced increased by the ultrasonic method compared to the conventional process at each additional time, approximately at 1%. Ultrasonic power causes damage to the tea leaf tissue in the sample used due to mechanical pressure caused by ultrasonic frequency [19]. Figure 6 shows the best results for the Pagar Alam tea species using 75 kHz ultrasonic frequency and 8 hours of extraction time. This

result is consistent with the statement that ultrasonic waves can damage tea leaf cells and increase yield [20, 21, 22].

Table 2**.** Analysis Results: Yield of tannin in the product

Type of Tea	Yield (%)					
	Conventional			Ultrasonic		
	4 h	6 h	8 h	4 h	6 h	8 h
Bandung	19.6765	19.6765	20.0571	20.0571	20.4377	20.8183
Pagar Alam	21.1989	21.5794	22.3406	21.9600	21.9600	23.1018
Bogor Peak	20.8183	21.1989	21.1989	21.1989	21.5794	22.7212

Also, to elaborate on the ultrasonic effect, the researchers used SEM to analyze tea leaf residues' surface morphology. Figure 7 shows the images obtained by SEM on tea leaf using conventional extraction and ultrasonic extraction. Based on Fig. 7c and 7d, it is seen that samples with frequency power added presented cracks or pores inside the tissue cells. These facts indicate that the ultrasonic effect on extraction results. The previous researchers, Kimthet et al., also reported that cell damage could increase the solubility of tannin compounds in the solvent used, ultimately growing the extraction yield [14].

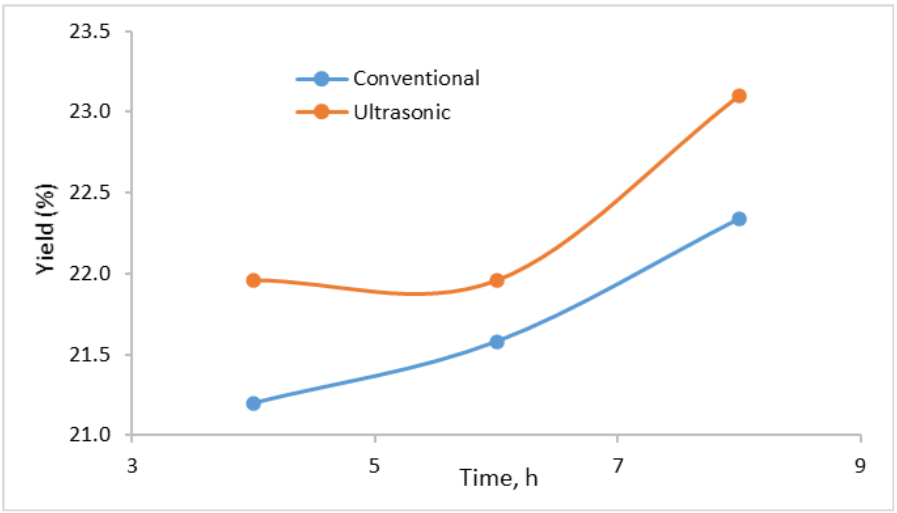

Figure 6. Comparison of conventional and ultrasonic methods in Pagar Alam tea leaves.

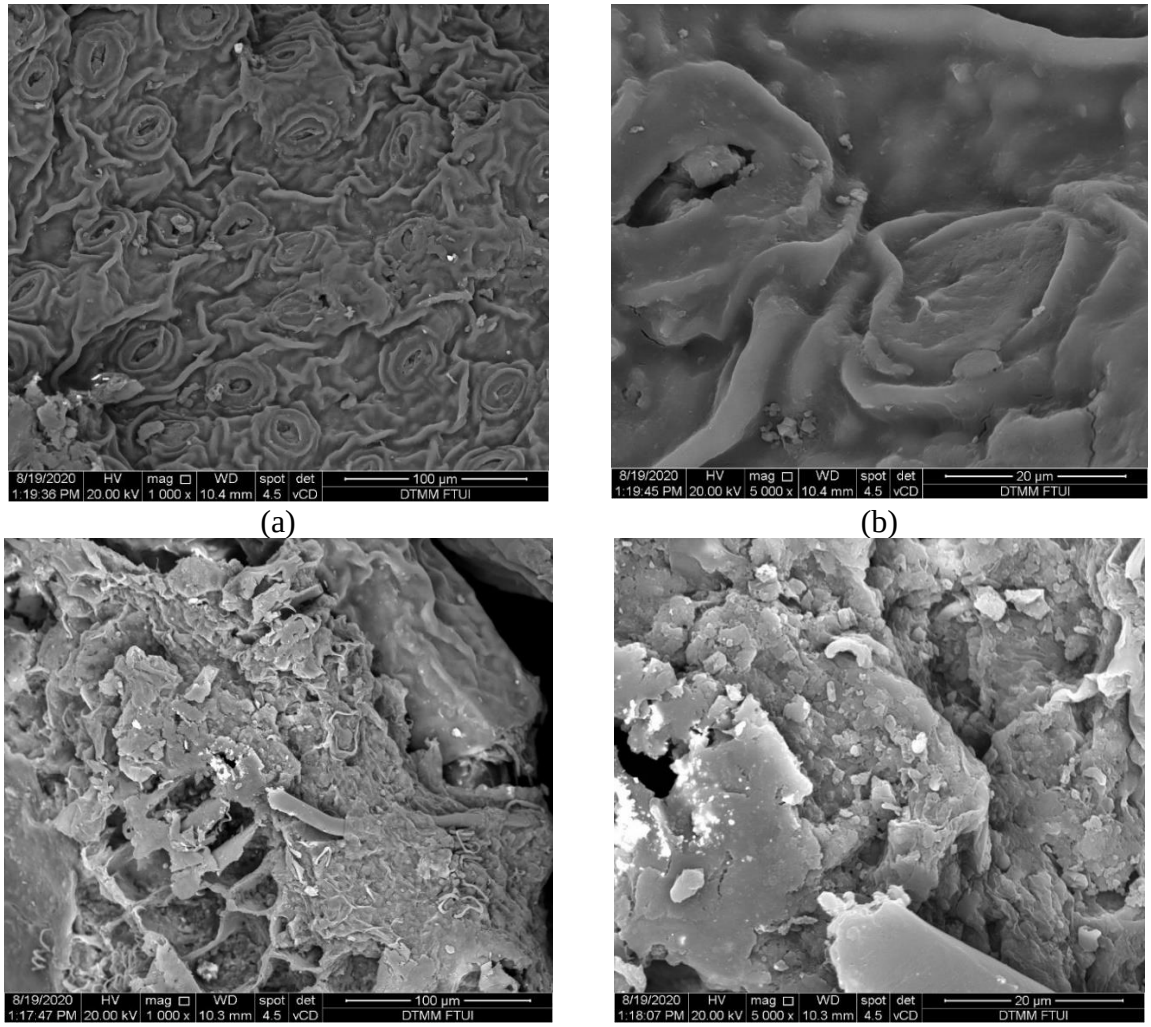

Figure 7. SEM image of tea leaf residues**.** (a) Conventional Method magnification 1000 x (b) Conventional Method magnification 5000 x (c) Ultrasonic Method magnification 1000 x and (d) Ultrasonic Method magnification 5000 x

The Effect Of Sources Of Tea On The Tannin

In this study also observed the effect of regional tea sources on extraction results. Figure 8 shows the highest tannin extract obtained in tea originating from Pagar Alam compared with Bandung and Puncak. Compared to Bandung tea, Pagar Alam tea is 9.3% bigger than compared to Puncak tea, which is 2.3%. This result is consistent with the color concentration test results, where tea originating from the South Sumatra, Pagar Alam, was the highest. The resulting tea water's color showed that the tannin content was also higher based on the qualitative analysis. Compared with Bandung and Bogor Peak, tea from Pagar Alam is planted at an altitude of 1500 meters above sea level while tea from Bandung and Bogor is 1000 - 1200 meters above sea level.

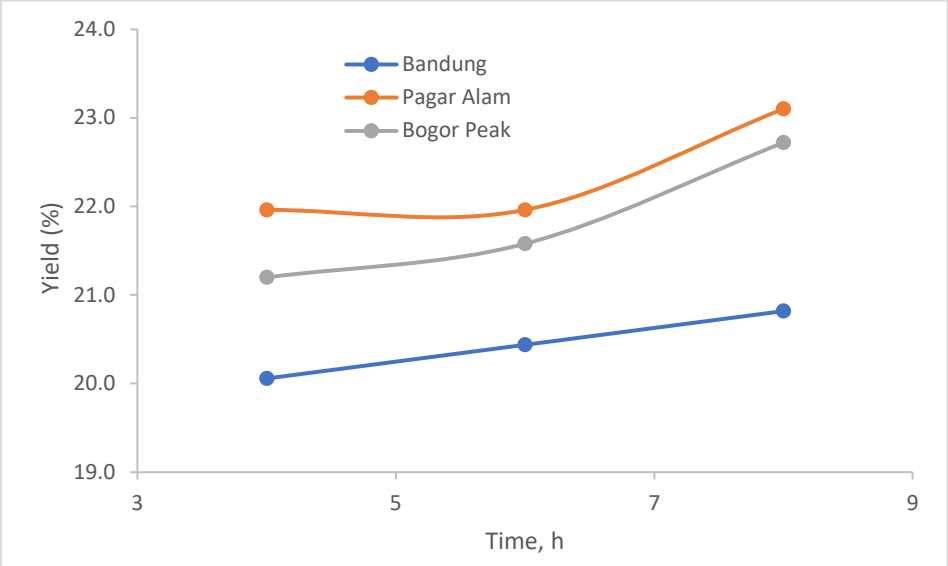

Figure 8. Effect of tea sources on tannins yield (based on ultrasonic method).

Kinetic Modelling

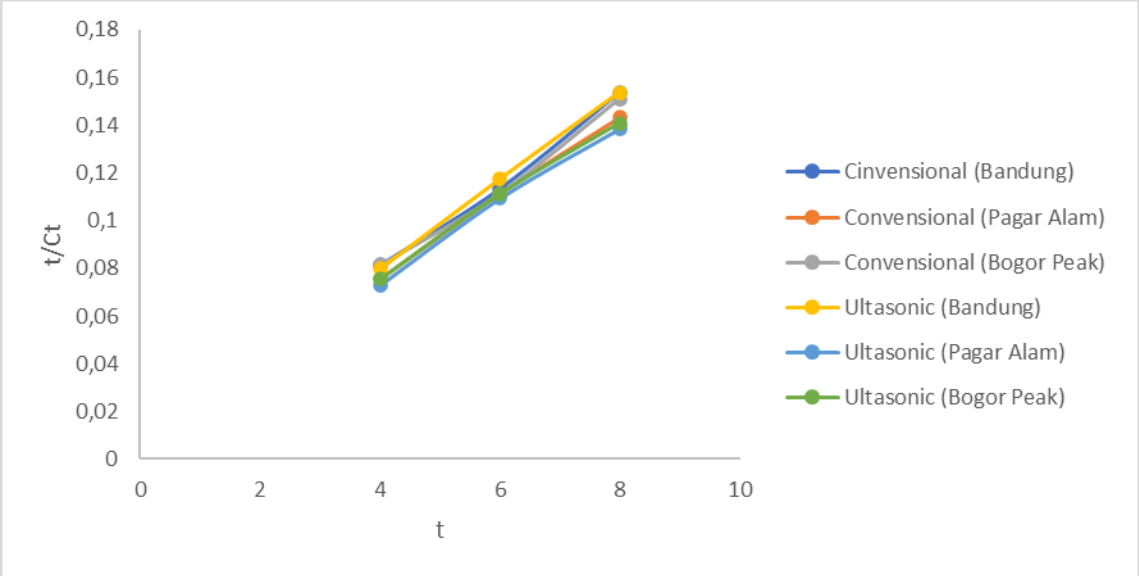

Figure 9. Linear Regression Tanin Extraction

Table 3. Constanta of Kinetic Tanin Extraction

Type of Tea	Conventional				Ultrasonic			
	RMSE	R^2	qe	k1	R^2	RMSE	qe	k1
Bandung	0,004	0,995	55,279	0,043	0,006	0,999	54,112	0,056
Pagar Alam	0,002	0,999	59,031	0,034	0,003	0,996	60,938	0,032
Bogor Peak	0,004	0,993	57,438	0,030	0,002	0,997	61,199	0,024

The value of kinetic tannin extraction can be found by Linear Regression kinetic second order, which showed in Figure 9. The modeling kinetic second-order perfect represents data tannin extraction because value R^2 comes near one and RMSE comes to near zero. Several studies have proposed that the second-order rate law is ideal for quantifying the extraction of antioxidants[23]. The fastest rate of tannin extraction was ultrasonic extraction tea from Bandung, where the constant rate reaction was 0,0567 kg/m^3.h.

CONCLUSION

This study has carried out several experiments to extract tannins in tea leaves from three different Indonesia areas. The operation conditions were at 70^{o}C, 75 kHz, ethanol 70 %, and extraction time of 4, 6, and 8 h. Spectrophotometer's results, the absorbance of the sample test increased, indicating that some tannins in tea leaf separated during the extraction process. With a 75 kHz frequency, the ultrasonic method significantly influences the tannin yield compared to the conventional way based on analysis results. The best extraction results obtained with 8 hour extraction time for Pagar Alam tea leaves with a gain of 23.1018%. Also, the observations show that the type of tea affects the yield in the extraction products obtained. Therefore, it recommended conducting further experiments by observing tea from other regions with different sea levels. The fastest rate of tannin extraction was ultrasonic extraction tea from Bandung, where the constant rate extraction was 0,0567 kg/m^3.h.

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