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Tannin Concentration of Gyrinops Tea Taken Form Different Agarwood Plantation and Different Processing Method

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Abstract. Tannin is one of the essential components that determine the quality of herbal tea products. This research aims to conduct a quantitative tannin assay of Gyrinops tea with different leaf sampling locations and processing methods. Factorial Completely Randomized Design was used as an experimental design with two treatment factors. The first factor is sampling location (Kekait, Lingsar, and Pejaring). The second factor is the processing method (Fresh leaves and Dried leaves). A titrimetric method with KMnO₄ and Indigo carmine reagent was used for quantitative tannin measurement. ANOVA and DMRT at a 5% significant level were used for tannin concentration analysis. The result indicated that the first, second, and interaction factors significantly affected tannin concentration. Drying the *G. versteegii* leaves could increase the tannin concentration of the Gyrinops tea product. *G. versteegii* leaves taken from Kekait have shown highest tannin concentration among other regions. It could be concluded that dried *G. versteegii* leaves Kekait could produce Gyrinops tea with high quality based on tannin concentration measurement.

1. Introduction

Agarwood tea is one herbal tea product that is produced from agarwood leaves [1]. The utilization of agarwood leaves as the raw material of agarwood tea is a good diversification product on agarwood cultivation [2]. Agarwood leaves could be harvested from 3 years old agarwood trees [3]. Harvesting agarwood leaves could give farmers additional income while waiting to harvest agarwood resin as the main product of agarwood cultivation that needs a ten-year investing period [4]. However, agarwood leaves were commonly treated as a waste product from pruning activity on agarwood cultivation [5]. Research and development of agarwood tea are needed to make this product more popular among agarwood farmers [6].

Study about the characteristics of agarwood leaves as the raw material of agarwood tea is an essential topic for the development of agarwood tea [7]. Agarwood products in several countries in Asia are commonly made from agarwood leaves of the *Aquilaria* genus [8]. This genus is also famous as an agarwood tea raw material in Indonesia with “Aqila Tea” product [3]. Mostly agarwood tea products from the *Aquilaria* genus distributed in the west region of Indonesia as the natural habitat of this genus [9]. Agarwood tea from the east region of Indonesia is made from the *Gyrinops* genus instead of the *Aquilaria* genus [6]. Lombok Island is one place from the east region of Indonesia that has become the centre of development of agarwood tea from the *Gyrinops* genus, especially *Gyrinops versteegii* [10].



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This species is endemic to Lombok Island and well distributed in all island regions [11]. Agarwood tea made from *G. versteegii* is called “Gyrinops tea” [12].

Gyrinops tea from Lombok Island needs to be further developed into a national agarwood tea product like Aqila tea. Raw material standardization study is one of the essential things that could support the development of herbal tea products like Gyrinops tea [10]. Tannin is one molecule that has an essential role in the raw material standardization of tea products [13]. Several studies about tannin concentration have been conducted for the standardization of agarwood tea products from *Aquilaria* [14], [15], and Gyrinops [16], [17]. Factors that determine tannin concentration on *G. versteegii* leaves should be the focus of study to develop Gyrinops tea products.

Several studies on the *Aquilaria* genus have revealed that sampling location and leaves processing method are essential factors determining the tannin concentration of *Aquilaria* leaves. Agarwood tea made from *A. malaccensis* leaves taken from different sampling locations on Sumatra Island has shown different tannin concentrations [15], [18], [19]. Different processing methods of *A. malaccensis* leaves also produce agarwood tea with varying phytochemical profiles and tannin concentrations [20], [21]. Different sampling locations of *G. versteegii* leaves, including West Lombok, East Lombok, and North Lombok, have shown variation in qualitative phytochemical profiles [10]. However, quantitative tannin measurement of Gyrinops tea was only reported from agarwood plantation in West Lombok Region [16]. Quantitative tannin concentration assay of Gyrinops tea should represent a variant of this species in a different region of Lombok Island [22]. This research aims to conduct a quantitative tannin assay of Gyrinops tea with different leaf sampling locations and processing methods.

2. Material and methods

2.1. Experiment Design

This research used a Completely Randomized Design with two treatment factors. All treatments were investigated under three replications. The treatment factors are as follows:

Gyrinops versteegii leaves processing method

P₁ : Fresh *Gyrinops versteegii* leaves

P₂ : Dried *Gyrinops versteegii* leaves

Gyrinops versteegii leaves sampling location

S₁ : Sampling location at Kekait

S₂ : Sampling location at Lingsar

S₃ : Sampling location at Pejaring

2.2. Sample collection

Leaves of *G. versteegii* were taken from 3 agarwood plantations on Lombok Island according to the experiment design. Detailed coordinates of sampling locations are Kekait (8°31'26''S, 116°07'03'' E), Lingsar (8°33'32'' S, 116°09'25'' E), Pejaring (8°35'49'' S, 116°26'44'' E). Leaves selection was conducted based on the size, shape, and condition of the *G. versteegii* tree. Ideal *G. versteegii* leaves for Gyrinops tea production should have a length from 5 cm – 15 cm. Leaves samples should not have chlorosis and necrosis symptoms. Last but not least, the sample should be free from pests and disease [12].

2.3. Raw material preparation

G. versteegii leaves samples were cleaned from dust and dirt by washing treatment using flowing water. The washing treatment was repeated three times. Clean leaves samples that were dried at 27°C for three days using a drying rack. The drying process was carried until sample leaves lost 10% of water content. Then, dried leaves were chopped to form 1 – 2 mm particle size using a grinding machine [2].

2.4. Preparation and standardization of the reagent

Indigo carmine and KMnO_4 were the primary solutions for quantitative tannin measurement. Indigo carmine solution was made by dissolving 6 grams of indigo carmine powder (Merck) into 500 ml distilled water with heating treatment. Fifty ml of 95% H_2SO_4 solution were added after the mixture was cooling down. The mixture was diluted by adding distilled water until the volume reached 1 L and then was filtered using qualitative filter paper [23]. KMnO_4 solution was made by diluting 3.3 grams of KMnO_4 powder in 1 L distilled water. The solution was then standardized with oxalic acid by titration. The result of standardization was 1 ml of 0.1 N KMnO_4 equal to 0.0067-gram oxalic acid [14].

2.5. Gyrinops tea extraction

Gyrinops tea samples were made from dried *G. versteegii* leaves particle extraction process using distilled water. One gram particle was extracted on 50 ml distilled water with a heating process at 70°C for 5 minutes. The mixtures were filtered through qualitative filter paper. The filtrates were then centrifuged at 4000 rpm for 15 minutes. The supernatants from this process were Gyrinops tea products [16].

2.6. Qualitative estimation of tannin

Qualitative tannin assay was a preliminary assay for early screening of tannin compound in the Gyrinops tea sample. FeCl_3 was the reagent for this assay. Three drops of FeCl_3 with 5% (w/v) concentration were added to 1 ml of Gyrinops tea. Greenish precipitate formation from the sample indicates the presence of tannin [24].

2.7. Quantitative estimation of tannin

The titrimetric method by titrating the sample with standardized KMnO_4 was carried for quantitative tannin estimation. Twenty-five ml of Gyrinops tea samples were mixed with 25 ml indigo carmine solution in a 1000 ml Erlenmeyer flask. The mixtures were then diluted by adding 750 ml distilled water and were titrated with standardized KMnO_4 . Titrations were carried until the blue color of the mixture changed into green color. After that, a few drops of KMnO_4 were added until the color of the mixture became golden yellow. The blank assays were performed by titration of 25 ml indigo carmine solution without Gyrinops tea sample. All of Gyrinops tea samples were analyzed in triplicate. The tannin concentrations (%T) were calculated using the equation as follows [23]:

$$\%T = \frac{(V - V_0) \times 0.004157 \times 50}{g \times 25} \times 100\%$$

V is the volume of 0.1 N KMnO_4 for sample titration (ml), V_0 is the volume of 0.1 N KMnO_4 for blank test titration (ml), 0.004157 is tannin equivalent in 1 ml of 0.1 N KMnO_4 , g is the mass of the sample taken for analysis (gr), 25 is the volume of Gyrinops sample, 50 is the volume of extraction solvent for Gyrinops sample.

2.8. Data analysis

Tannin concentrations of Gyrinops tea from each treatment were analyzed using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) at 5% level significance ($\alpha=0.05$). Tannin concentration data were also analyzed for their means, and standard errors were then presented as bar charts with error bars to interpret interaction factors between two treatment factors.

3. Result and Discussion

Tannin is an essential molecule that has been approved as one of the quality standard compounds for conventional tea products [25]. Different types of conventional tea, including green tea, oolong tea, and black tea, have different tannin concentrations. This different tannin concentration has determined

the different tastes of each conventional tea product [24]. Standardization of agarwood tea products could adopt standardization of conventional tea products by using tannin as the quality standard. Several studies have revealed different tannin concentrations of agarwood tea from *Aquilaria malaccensis* with different processing methods and different sampling locations [15], [18], [19], [21]. However, very few reports compare the tannin concentration of Gyrinops agarwood tea with different leaves processing methods and different sampling locations.

Table 1. DMRT Analysis of tannin concentration

Factors	Treatment	Tannin Concentration (%)	Significance
Gyrinops versteegii leaves processing method	P ₂ : Dried Gyrinops versteegii leaves	5.68 ± 0.22	a
	P ₁ : Fresh Gyrinops versteegii leaves	1 ± 0.06	b
	LSD 0.05	0.56	
Gyrinops versteegii leaves sampling location	S ₁ : Sampling location at Kekait	4.01 ± 0.60	a
	S ₂ : Sampling location at Lingsar	3.28 ± 0.37	b
	S ₃ : Sampling location at Pejaring	2.73 ± 0.33	b
	LSD 0.05	0.68	

Remarks: Mean values followed by the same letters are significantly different ($p < 0.05$) between levels of each treatment factor

ANOVA has shown that leaves processing method and sampling location significantly affect different tannin concentrations of Gyrinops tea samples. DMRT has confirmed which treatment of each factor is responsible for different tannin concentrations of the sample (Table 1). It could be interpreted that Gyrinops tea from dried leaves has a much higher tannin concentration than Gyrinops tea from fresh leaves. Drying leaves treatment on leaves has an impact on higher secondary metabolite content, including tannin. Dried tea leaves (*Camellia sinensis* L) have a higher tannin concentration than fresh tea leaves [26]. A similar pattern had occurred when *G. versteegii* leaves were treated by drying treatment on this research. A study about agarwood tea with leaves drying treatment has shown that dried agarwood leaves have a bitter taste than fresh agarwood leaves [27]. Tannin is the main compound responsible for the bitter taste of tea products [28]. Thus, this research has confirmed that the bitterer taste of agarwood tea from dried leaves was caused by its higher tannin concentration.

Gyrinops tea samples from different sampling locations have variation in tannin concentrations (Table 1). The highest tannin concentration was the Gyrinops tea from Kekait and was significantly different from other sampling locations. This result confirmed other research revealing tannin concentration variation of Aqila agarwood tea (*A. malaccensis*) from different sampling locations in the North Sumatera Region (Table 2). Tannin concentrations of Gyrinops agarwood tea were similar to those of Aqila agarwood tea, ranging from 2% to 5%. However, this different tannin concentration result implies the importance of sampling location on a raw material selection of Gyrinops tea products. The development of this product should be using *G. versteegii* raw material from the highest tannin concentration location.

Table 2. Tannin concentration variation of Aqila agarwood tea from different sampling location

Location	Region	Agarwood Species	Tannin concentration (%)	Reference
Laru	North Sumatera	<i>A. malaccensis</i>	5.62 ± 0.42	[18]
Hutanbolon	North Sumatera	<i>A. malaccensis</i>	3.08 ± 0.23	
Langkat	North Sumatera	<i>A. malaccensis</i>	2.34 ± 0.16	[14]
Sigiring – giring	North Sumatera	<i>A. malaccensis</i>	3.13 ± 0.20	[15]
S. Kalangan II	North Sumatera	<i>A. malaccensis</i>	3.19 ± 0.21	
Mandailing Natal	North Sumatera	<i>A. malaccensis</i>	5.62 ± 0.41	[19]

Standard error analysis has shown unique interaction between factor treatments in this research (Figure 1). Different tannin concentration from different sampling locations only occurs on dried

leaves of *G. versteegii*. Fresh *G. versteegii* leaves from different sampling locations have no significant difference of tannin concentration. Thus, Gyrinops tea raw material selection from different sampling locations should focus on dried *G. versteegii* leaves since dried leaves have much higher tannin concentration than fresh ones.

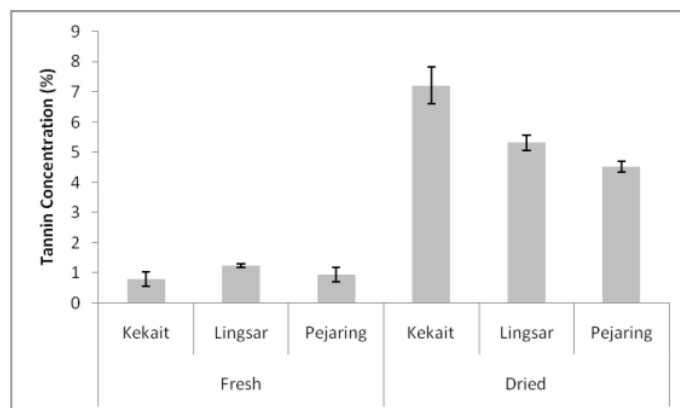


Figure 1. Standard error analysis of leaves processing factor and sampling location factor.

The previous study of tannin concentration on Gyrinops tea only focuses on raw material from agarwood plantation located at Lingsar, representing the West Lombok Region of *G. versteegii* distribution [17]. However, this research has revealed that raw material from agarwood plantation located at Kekait represents North Lombok Region of *G. versteegii* has higher tannin concentration than Lingsar. Based on previous research, the study of *G. versteegii* from the Kekait plantation only was focused on seedling development [29]. The high tannin concentration of *G. versteegii* on this plantation implies its potency as a source of Gyrinops tea raw material.

4. Conclusion

Gyrinops tea from dried leaves has a higher tannin concentration than Gyrinops tea from fresh leaves. Gyrinops tea from Kekait has the highest tannin concentration among the other sampling locations. Thus, the combination of dried *G. versteegii* leaves taken from Kekait could produce high-quality Gyrinops tea based on tannin concentration measurement.

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10

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PAGE 2

PAGE 3

PAGE 4

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