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Research Article

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Tannin Concentration of Gyrinops Tea from Leaves of Juvenile and Mature Agarwood Trees (*Gyrinops versteegii* Gilg (Domke)) with Different Processing Methods

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ABSTRACT

Gyrinops tea made from leaves of agarwood (G. versteegii) trees endemic of Lombok Island can be an alternative product to optimize cultivation of G. versteegii in Lombok, Indonesia, because it could give continuing income for agarwood farmers during the long waiting period for the tree to produce agarwood. As new source of tea, G. versteegii leaves need to be investigated for it tannin content as a standard quality of tea. This study aimed to investigate tannin concentration of Gyrinops tea made from leaves of G. versteegii trees of different ages with different processing methods, by examining the effects of two different ages of the trees (1 or 5 years) and 4 methods of leaf processing (fresh leaves, dry leaves with no oxidation, dry leaves with 2 and 4 weeks of oxidation). The results indicated that Gyrinops tea made from leaves of older G. versteegii trees contains higher percentages of tannin than the tea made from leaves of the younger ones, and dry leaves contain higher percentages of tannin than fresh leaves, with a tendency of higher tannin concentration in leaves of longer duration of oxidation process, but only in leaves from older G. versteegii trees, while in the younger ones, there was no effects of oxidation duration.

Keywords: Gyrinops tea; Agarwood tree; Tannin concentration

INTRODUCTION

Gyrinops versteegii is one of agarwood species endemic of Lombok Island in Indonesia that has a high economic value as non-timber forest commodity [1]. Agarwood from *G. versteegii* is mainly used for production of fragrant product including: perfume, incense and many other aromatic products that have significant influences on social and economic aspects of human history especially in Indonesia [2,3]. Those facts lead to the high interest of farmers on Lombok Island to develop an agarwood commodity by cultivating *G. versteegii* tree species. However, agarwood development in the *G. versteegii* trees in Lombok has several problems especially in terms of the very long duration of time needed.

The aromatic fragrance of agarwood is mainly caused by resin produced by *G. versteegii* stem [4]. To produce that resin, *G. versteegii* needs to be induced physically, chemically or biologically, and it requires at least 12 years of growth from seedling period to fully mature [5]. Because of its long investing period of time needed, it is necessary

to find an alternative usage of the agarwood trees to make sure that agarwood farmers can get some incomes during the long waiting period of time in agarwood production. One of the alternative usages is to produce a herbal tea from the agarwood tree leaves [6].

Using agarwood tree leaves to produce herbal tea not only could give continuing incomes for agarwood farmers but also could give a promising new healthy product for the market [7]. Agarwood tree leaf has a lot of good medical effects including: antioxidant [8], antibacterial [9], laxative [10] and antihyperglycemic [11]. The safety of agarwood tea from *Aquilaria malaccensis* leaves in North Sumatera, Indonesia, has already been confirmed based on toxicology assay [12]. That is why this agarwood tea with product name "Aqila" becomes a promising new product in Sumatera Island in Indonesia [13]. Although agarwood tea from *Aquilaria* spp. is a famous new product of herbal tea, this tree species is not an endemic species on Lombok Island. Thus, to develop an agarwood tea on Lombok Island could be possible by utilizing *Gyrinops versteegii* leaves, which is here called "Gyrinops tea".

As a new source of agarwood tea, *G. versteegii* leaf, i.e. Gyrinops tea, should be preliminarily investigated to study its feasibility. A preliminary study revealed that different methods of leaf processing will produce different characteristics of Gyrinops tea from Lombok Island [14]. This study should be supported with a pharmacological study about the chemical compounds of Gyrinops tea just like what has already been done for the agarwood tea from *Aquilaria* spp. as main source. Since *G. versteegii* is a new source of Gyrinops tea, pharmacological study should be focused on the main component that is responsible for quality determination of tea.

Tannins are phenolic compounds with molecular weight from 500-3000 that are commonly found in plant organs [15]. This compound is considered as the main property that determines the quality of industrial tea beverage [16] [17]. Tannin is also an important compound that is responsible for measuring quality of other beverages made from fruits [18]. For all of that reasons, the aim of this study was to investigate tannin concentration of Gyrinops tea made from agarwood (*G. versteegii*) leaves of different plant ages with different leaf processing methods.

EXPERIMENTAL SECTION

Experimental Design

This study used Completely Randomized Design with factorial arrangement of the two treatment factors investigated under three replications each. The treatment factors are as follows.

Age of the *G. versteegii* tree:

A1 : *G. versteegii* 5 years old

A2 : *G. versteegii* 1 year old

Processing methods of the *G. versteegii* leaves:

P1 : Fresh leaves (without processing)

P2 : Dry leaves without oxidation

P3 : Dry leaves with 2 weeks of oxidation

P4 : Dry leaves with 4 weeks of oxidation

Collecting the Samples

Gyrinops versteegii leaves were taken from agarwood plantation at West Lombok District of NTB Province in Indonesia. Leaves were taken from the branch at the top of *G. versteegii* tree and were selected based on several criteria including: leaf size, leaf color and they should be free from pest and disease. Then the leaves were washed 3 times with aquadest and then treated according to the experiment design.

Leaf Treatments

Basically, there were 3 treatments for the *G. versteegii* leaves, i.e. fresh, dry without oxidation and dry with oxidation. These treatments are standard methods to make different types of tea including green tea, oolong tea, and black tea [19]. For fresh leaf processing method, *G. versteegii* leaves were chopped into 1 mm length with cutter than its tannin concentration was measured by titrimetric method. For dry leaf without oxidation, *G. versteegii* leaves were air dried for 3 days until it lost 70% of its water content, then were grinded using grinding machine Miyako BL-211 PLY. The dry leaf processing with oxidation was similar to dry leaf processing without oxidation except for the additional oxidation process in oxidation chamber for 2 weeks and 4 weeks. All the dry leaves were measured for their tannin concentrations by titrimetric method.

Preparation and Standardization of the Reagent

Indigo carmine and $KMnO_4$ were two main reagents used for tannin measurement. Indigo carmine solution was made based on Atanassova and Christova-Bagdassarian [18], with some modifications. Six grams of Indigo carmine was dissolved in 500 mL distilled water by heating. After the mixture was cooling, 50 ml of 95-97 % H_2SO_4 was added. The mixture was then diluted until to reach 1 L volume by adding distilled water. Indigo carmine solution then was filtered with qualitative filter paper. Preparation and standardization of $KMnO_4$ were done based on Adrianar et al. [20] with some modifications. $KMnO_4$ solution was made by diluting 3.3 grams $KMnO_4$ in 1 liter distilled water. $KMnO_4$ solution was then standardized with oxalic acid by titration method. The standardization result is 0.0067 gram oxalic acid equal to 1 mL of 0.1 N $KMnO_4$.

Preparation of Tea Extract

Preparation of tea extract was done based on Khasnabis et al. [16] with some modifications. One gram of *G. versteegii* leaf sample was added to 50 mL of distilled water and heated at 70^oC for 5 minute. The decoction was cooled and filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 4000 rpm for 15 minutes. The supernatant was stored at 4^oC for later analysis.

Qualitative Estimation of Tannin

Qualitative estimation of tannin was the preliminary assay to make sure that agarwood tea sample contains tannin. Three drop of 5% (w/v) aqueous solution of ferric chloride was added to 1 ml of agarwood tea extract to observe formation of greenish precipitate indicating the presence of tannins in the sample. Positive test result was then continued with quantitative estimation of tannin.

Quantitative Estimation of Tannin

Quantitative estimation was performed by titrating agarwood tea extract with standard $KMnO_4$ based on Atanassova and Christova-Bagdassarian [18] with some modifications. Twenty five ml of agarwood tea extract were mixed with 25 ml indigo carmine solution in 1 L conical flask. Then 750 ml distilled water was added to the mixture. The

mixtures were titrated with standardized $KMnO_4$ until the blue color of mixture change into green color. Then, few drops were added until the solution becomes golden yellow. The blank test carried by titration of mixture of 25 ml indigo carmine solution and 750 ml distilled water. All samples were analyzed by triplicates. The tannin concentration (T%) in the sample was calculated as follows:

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

V is the volume of 0.1 N $KMnO_4$ for titration of the sample (ml), V0 is the volume of 0.1 N $KMnO_4$ for titration of the blank sample (ml), 0.004157 is tannins equivalent in 1 ml of 0.1 N $KMnO_4$, g is mass of the sample taken for analysis (gram), 25 is the volume of sample, 50 volume of extraction solvent for sample.

Data Analysis

Tannin percentages for each sample were analyzed with analysis of variance (ANOVA) and Tukey's Honesty Significant Different (HSD) at 5% level of significance ($\alpha = 0.05$) using the statistical software CoStat for Windows. The data were also analyzed for their means and standard error and were presented as bar chart with error bars to interprete the patterns of interactions between the two treatment factors.

RESULTS AND DISCUSSION

Preliminary study about Gyrinops tea from *G. versteegii* leaves suggested that different processing methods lead to different taste of the tea and needs to be further analyzed chemically by measuring their tannin concentrations. Tannins play an important role in the difference taste of different types of tea. That is why tannin measurement is an important process to be done in order to develop a Gyrinops tea from leaves of agarwood trees endemic in Lombok, Indonesia. Based on the results of statistical analysis (ANOVA & HSD), the percentages of tannins in the Gyrinops tea made from *G. versteegii* leaves were significantly different between ages of the agarwood trees and between methods of processing the leaves in making the Gyrinops tea (Table 1). In addition, there was a significant interaction ($p<0.05$) between the two treatment factors on the percentage of tannins in the Gyrinops tea (Figure 1).

Tea from leaves of 5 years old *G. versteegii* trees have higher percentages of tannins than those from 1 years old *G. versteegii* trees. Tannins are phenolic compounds, which are parts of secondary metabolites in plants and are distributed differently depending on parts and ages of the plants [21]. These compounds tend to be higher in old leaves compared with young leaves as they are responsible for plant defensive mechanism against environmental pressures [22]. The results that older leaves of *G. versteegii* contain higher amounts of tannin than the younger leaves are possibly because the old leaves produce more secondary metabolites for defensive purposes. It also indicates that producing Gyrinops tea using leaves from 5 years old *G. versteegii* trees could result in better quality tea compared with using leaves from 1 year old *G. versteegii* trees due to its much higher content of tannins.

Table 1. Tannin concentration (%) in the Gyrinops tea from leaves of agarwood trees of different ages and different processing methods

Factors	Treatments	Tannin conc. (%)	Significance
Age of *G. versteegii* tree	A1: plant age of 5 years	7.70	a

	A2: plant age of 1 year	4.24	b
	Tukey's HSD (α=0.05)	0.59	
Leaf Processing Methods	P4: Dry with 4 weeks ox.	7.78	a
	P3: Dry with 2 weeks ox.	7.01	ab
	P2: Dry with 0 weeks ox.	6.03	b
	P1: Fresh leaves	3.08	c
	Tukey's HSD (α=0.05)	1.13	

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

There was also a significant effect of leaf processing methods on tannin concentration in the Gyrinops tea, in which the percentage was higher in tea from leaves of older agarwood trees. Tannin is the main compound that responsible for the bitter taste in the tea infusion [23]. Based on our preliminary research about Gyrinops tea from leaves of agarwood trees it was concluded that Gyrinops teas from dry oxidation leaves tastes much bitter than those from fresh leaves. This study confirms that different taste was mainly caused by tannin content of the dry oxidation leaves that is much higher in the dry oxidation leaves than in the fresh leaves or dry leaves without oxidation. Hedonic test is needed for further analysis to examine optimum tannin percentage of dry oxidation *G. versteegii* leaves that taste good for the market [24].

Based on the interaction effects between ages of agarwood trees and leaf processing methods in producing the Gyrinops tea, it can be seen from Figure 1 that tannin content in the tea increases with increasing duration of dry oxidation for the leaves taken from the older agarwood trees. In contrast, tannin concentration was not affected by the duration of dry oxidation of leaves taken from the younger agarwood trees. Dry leaves of *G. versteegii* also show significantly higher tannin content than the fresh leaves. Leaf processing by drying method increases antioxidant capacity as well as total phenolic, total flavonoid and total condensed tannin in leaves [25]. Overall, Gyrinops tea made by drying agarwood leaves tastes more like common tea than the tea from the fresh leaves. This is important information for developing Gyrinops tea made from leaves of agarwood trees in Lombok, Indonesia.

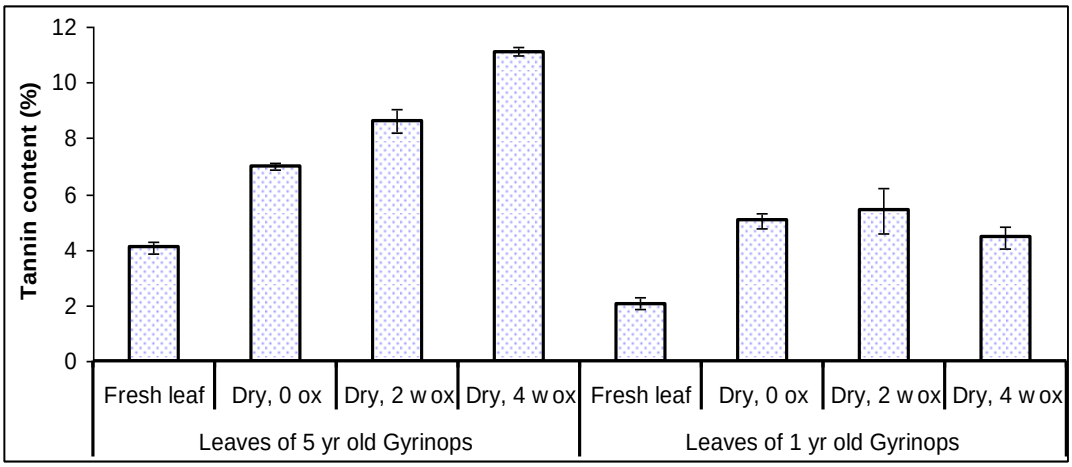

Figure 1. Tannin Concentration (%) in Gyrinops Tea as the Results of the Significant Interaction between Different Ages and Leaf Processing Methods

The different duration of oxidation time in this study was based on the standard oxidation time applied in making different types of tea, including: green tea, oolong tea, and black tea [19]. Khasnabis et al. [16] reported that among the types of tea measured, green tea showed the lowest contents of tannin. Based on that the data obtained in this study, the Gyrinops tea made from fresh leaves could approximately be standardized as green tea, while Gyrinops tea made without oxidation could be standardized as oolong tea, and Gyrinops tea with oxidation of up to 4 weeks could be standardized as black tea.

CONCLUSION

It can be concluded that Gyrinops tea made from leaves of older *G. versteegii* contains higher percentage of tannin than the tea made from leaves of the younger ones, and dry leaves contain higher percentage of tannin than fresh leaves, with a tendency of higher tannin concentration in leaves of longer duration of oxidation process, but only in leaves from older *G. versteegii* trees, while for those from the younger trees, there was no effects of the duration of oxidation process.

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